Supporting Information

Enhancing biocompatible stability of DNA nanostructures using dendritic oligonucleotides and brick motifs

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Materials design and preparation

Oligonucleotide synthesis and preparation

Oligonucleotide strands were either purchased from Integrated DNA Technologies, inc. (www.idtdna.com) or chemically synthesized in lab using an Expedite 8900 DNA synthesizer (Perseptive Biosystems) or a Mermade 6 DNA synthesizer (BioAutomation). In a typical procedure, controlled porosity glass beads were placed in a 1 μmol synthesis column and phosphoramidites were added from the 3' direction via standard 1 μmol oligonucleotide synthesis protocol. 4,5-dicyanoimidazole (DCI, Glen Research) was used as an activator for improved yield, while other reagents (oxidizing reagent, CapA, CapB, acetonitrile) were chosen in accordance with literature procedures[REF]. Dendritic oligonucleotide strands included a branching phosphoramidite (long trebler phosphoramidite or trebler phosphoramidite, Glen Research), which was introduced during regular synthesis using a 15 minute coupling protocol. After employing the branching phosphoramidite within the dendritic oligonucleotide sequence, the DNA synthesis protocol was then adjusted to a 3 μmol scale to synthesize multiple arms simultaneously after the branching point. On a Mermade 6 DNA synthesizer, this change in protocol was adjusted in the script itself by including additional coupling steps within the protocol script. On an Expedite 8900 DNA synthesizer, the protocol per branching point was adjusted manually. For a 9x dendritic oligonucleotide, two branching phosphoramidites were added consecutively, each using a 15 minute coupling protocol. After coupling the first branching phosphoramidite, the second branching phosphoramidite was added in three times excess (e.g. 3 μmol scale) to accommodate successful coupling of multiple branching phosphoramidites on each existing branching phosphoramidite. After employing these two branching phosphoramidites, the DNA synthesis protocol was changed to a 9 μmol scale to synthesize nine arms of single-stranded DNA (with regular phosphoramidite bases) simultaneously.

Post synthesis, the CPG beads were placed in an AMA deprotecting solution (1:1 volume mixture of 30% aqueous NH3 and 40% methyl amine) at 65°C for 15 minutes in order to remove the synthesized DNA strands from the CPG beads and also deprotect the strands. Then, ammonia and methyl amine were removed by drying the samples with nitrogen gas for approximately 1-2 hours. The remaining solution was filtered through a 0.2 μm syringe filter to separate the DNA strands remaining in solution and the CPG beads that were no longer needed. Synthesized DNA strands were purified with reverse-phase high performance liquid chromatography on an Agilent 1100 HPLC equipped with a Terra MS C18 column (2.5 μm, 4.6 x 50 mm). Note that all oligonucleotide strands that were synthesized in-house used reagents from Glen Research Corp. (Sterling, VA). For reverse phase HPLC, two different types of buffer solutions were used – buffer A: 10% acetonitrile in ultrapure water, and buffer B: 80% acetonitrile with 20% ultrapure water. Ultrapure water was obtained from a Millipore system (Milli-Q Biocel).

Formation of brick nanostructures

All brick strands were adjusted to concentrations of 100 nM per strand in 0.5× TE buffer (5 mM Tris, pH 8, 1 mM EDTA) containing 10 mM (or 40 mM) $MgCl_2$. Dendritic oligonucleotide strands were also added to this mixture in excess (typically, 1:200 molar ratio between a brick

strand and a dendritic oligomer). To anneal the brick nanostructures, samples were subjected to a thermal ramp annealing protocol. Samples were heated at 80°C for 15 minutes, then slowly cooled over approximately three days from 65°C to 25°C at a linear rate of 0.1°C/10 min. Annealed samples were either immediately used or stored at 4°C.

Formation of DNA origami

3D DNA origami used in this work was designed as a three-layered, square-lattice, estimated in the size of approximately 59 nm in length (approximately 176 bp x 0.34 nm = 59.84 nm), 41 nm in height (approximately 14 helices, therefore 14 x 2 + 13 x 1 = 41 nm), and 8 nm in depth (approximately 3 helices in depth, therefore 3 x 2 + 2 x 1 = 8 nm), as shown below.

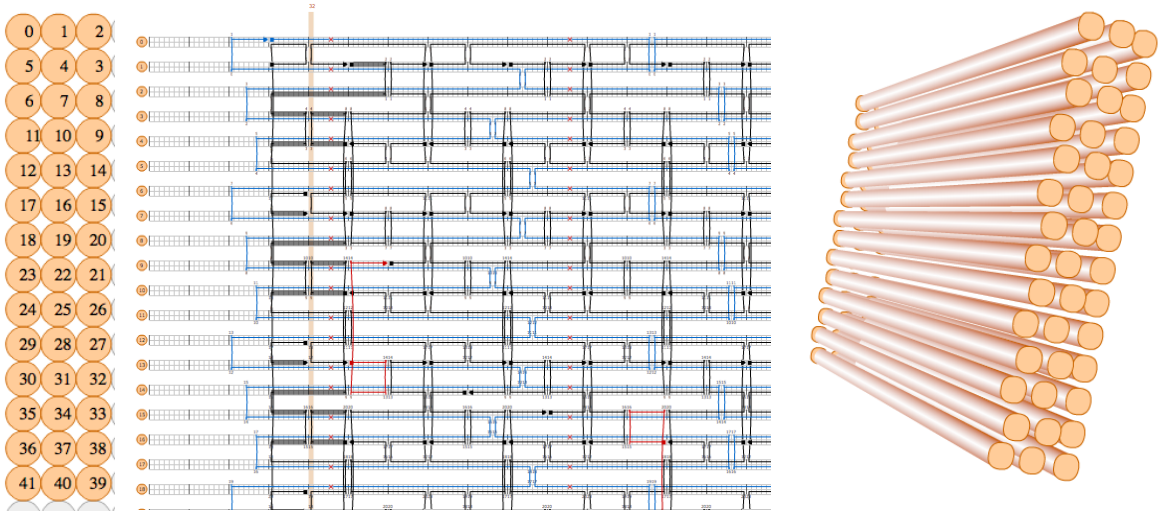

Figure S1: 3D DNA origami design used in this work

To assemble the 3D DNA origami used in this work, M13mp18 scaffold (#N4040S, New England BioLabs inc.) and staple strands (see table S2) were mixed together in a final concentration of 5 nM scaffold and 100 nM staples at 10 mM $MgCl_2$, 1 mM Tris, 0.01 mM EDTA (pH 8). All origami samples were annealed by heating to 90°C, then cooling from 90°C to 60°C at 1°C/min, slower cooling from 60°C to 50°C at 1°C/10 min, and faster cooling from 50°C to 20°C at 1°C/min. Annealed samples were either immediately used or stored at 4°C.

Characterization of structures

Agarose gel electrophoresis

Samples were subjected to a 2% agarose gel electrophoresis at 80V for 2 hours in an ice water bath. Gels were prepared with 0.5× TBE buffer containing 10 mM $MgCl_2$ and a 1:20,000 dilution of SYBR Safe loading dye. First, agarose powder (Bio-Rad or Thermo Fisher Scientific)

was mixed with the buffer solution, then microwaved for approximately 3 minutes to dissolve the powder. SYBR Safe loading dye and 1 M $MgCl_2$ were added appropriately, and the solution was quickly stirred then poured into the agarose gel mold before thickening. Gels were left to solidify at room temperature for approximately 30 minutes to 2 hours before using. Quantification of band intensities were obtained with a Typhoon FLA 9000 gel imager and analysis was performed manually by using the gel analysis option in ImageJ software.

When the gel bands needed to be individually purified, target bands were excised using an X-tracta gel extractor tool (Promega Corp.), then crushed into smaller pieces in a Freeze N' Squeeze column (Bio-Rad Laboratories, inc.) and centrifuged at 12,000 g for approximately 10 minutes. Flow-through was collected and analyzed afterwards.

Transmission electron microscopy imaging

Formvar/carbon-coated grids from Electron Microscopy Sciences were glow-discharged using a PELCO easiGlow glow discharge system (Ted Pella Inc., USA). The current used was 20 mA and grids were glow discharged for approximately 30 seconds. 7 μL of samples were incubated for 5 minutes on the glow-discharged formvar/cabon coated grids. Then, excess sample solution was removed by using a Whatman filter paper. Sample-coated grids were then stained for approximately 15 – 60 seconds with a 1 or 2% uranyl acetate or formate solution containing approximately 25 mM NaOH. Imaging was performed by using a JEOL JEM 1400 TEM operating at 80 kV.

Similar to previous literature (Ong et. al. 2017), we observed dark electron dense cavities and background in some TEM images. This presumably correspond to stain accumulated regions, at which heavy metal salt such as uranyl formate or acetate diffuse across the layers of DNA helices and accumulate within the cavities, causing the brick nanostructures to appear lucent against the dark electron dense cavities.

Removal of divalent salt and buffer exchange

Divalent salt was removed by exchanging the buffer from 10 or 40 mM $MgCl_2$ to 1× PBS by filtration. Samples were spin-filtered (3k Amicon filters) at 14,000 g for 3 rounds. Specifically, approximately 50 uL of sample (in 10 or 40 mM $MgCl_2$) was mixed with 400 uL of 1× PBS, then spun at 14,000 g for 30 minutes in the first round. Second round included approximately 45 uL of remaining solution mixed with 405 uL of 1× PBS at the same spin speed and duration. Last round was repeated the same as the second round. After three rounds of filtration, the filter device was placed upside down in a clean tube to recover the concentrated sample solute. The final magnesium salt concentration, post-filtration, in each 10 or 40 mM $MgCl_2$ samples were calculated as the following:

10 mM $MgCl_2$ × (50 uL/450 uL) × (45 uL/450 uL) × (45 uL/450 uL) ≈ 0.01 mM
40 mM $MgCl_2$ × (50 uL/450 uL) × (45 uL/450 uL) × (45 uL/450 uL) ≈ 0.04 mM

Fluorescence measurement experiment

Complementary strands that are labeled with different fluorophores, Cy3 and Cy5, were designed such that they can hybridize via a complementary overlap with overhang and/or single-stranded region of dendritic oligonucleotide strands.

Varying concentrations of fluorophore-labeled complementary strands were hybiridzed to different aliquots of assembled DNA brick nanostructures. To facilitate hybridization, all samples were kept at a salt concentration of 10 mM $MgCl_2$ and kept at room temperature for at least 2-3 hours. First, assembled brick nanostructures (or assembled brick nanostructures with dendritic oligonucleotides) were spin-filtered to remove excess DNA strands that did not become incorporated in an assembled structure. Then, complementary Cy3- and Cy5- strands were added and incubated for approximately 2 hours. DNA strand sequences for fluorescence measurements were designed such that Cy3-strands can only attach to DNA brick nanostructures in a 1:1 ratio (e.g. 1 Cy3 strand attachment for 1 brick nanostructure). On the other hand, Cy5-labeled strands could hybridize to the single-stranded region of dendritic oligonucleotides so that the total number of oligonucleotides placed at the surface of brick nanostructures could be calculated. To remove the unhybridized strands from the solutions, the fluorescently-labeled DNA brick nanostrucutres were spin-filtered (Amicon filters) for 3 rounds at 14,000 g, and the supernatant was removed.

To quantify the number of strands hybridized to the DNA brick nanostructures, the total fluorescence of the sample was measured using a fluorometer (Synergy Neo2, Biotek) by measuring the fluorescence intensity values, each dye at 540 nm/579 nm (Cy3) and 640 nm/681 nm (Cy5).

Cellular-uptake assay

All uptake studies were performed using HEK293T cells. HEK293T cells were maintained in Dulbecco modified Eagle medium (Gibco, Gaithersburg) and 10% FBS with 2% penicillin-streptococcus. For uptake studies, cells were seeded at a density of approximately 50,000 – 100,000 cells per well into tissue-culture-treated 12-well plates (BD Life Sciences) and allowed to adhere overnight. For sample incubation, samples were prepared by attaching a complementary fluorescent strand that hybridized to the outer sequence (towards the 5' end, within the branched multi-arms) of the dendritic oligonucleotides. Excess Cy5-attached fluorescent DNA strands were removed by applying a spin filter to only collect the DNA brick nanostructures with successfully hybridized strands. Samples were then diluted to a final concentration of 100 nM after addition of the seeded cells (approximately 20 – 50 uL). Cells were then incubated at 37C (5% CO_2) overnight in order to successfully become placed on the plate. All samples were set up in triplicate, and the cellular uptake experiments were repeated on multiple, separate days.

The viability of HEK293T cells with DNA brick nanostructures were measured via manually counting and characterizing cells by staining the cells with commercially available Trypan blue solution (Thermo Fisher Scientific). Cells were incubated at different concentrations of DNA brick nanostructures for approximately 30 minutes (up to 2 hours), then stained with Trypan blue to quickly determine the cell viability. Based upon introducing Trypan blue solution to the cells, viable cells do not take up the dye, but dead cells are permeable and therefore take up the dye. After incubation, cells were checked via a cell counter (Countess II, Invitrogen). It is important to note that no significant difference in the number of cells was noted between cells with and without DNA brick nanostructures.

Additional data and figures

Stability of 52 nt DNA brick nanostructures in 1X PBS for a longer incubation time (24 hr)

52 nt DNA brick nanostructures were incubated at 37 °C for a longer time (24 hours) in different buffer conditions. 5 uL of 100 nM 52 nt DNA brick nanostrucutres, originally assembled in 10 mM $MgCl_2$, were mixed with 25 uL of (i) 10 mM $MgCl_2$ buffer, (ii) 1 mM $MgCl_2$ buffer, (iii) 0.5 mM $MgCl_2$ buffer, (iv) 0.1 mM $MgCl_2$ buffer, and (v) 1× PBS. The final magnesium concentration in each sample, upon incubation, is estimated to be approximately (i) 0.3 mM, (ii) 0.03 mM, (iii) 0.015 mM, (iv) 0.003 mM, and (v) 0 mM. Then, all samples were characterized via agarose gel electrophoresis. Characterization results indicate that all DNA brick nanostructures remain stable in different buffer conditions.

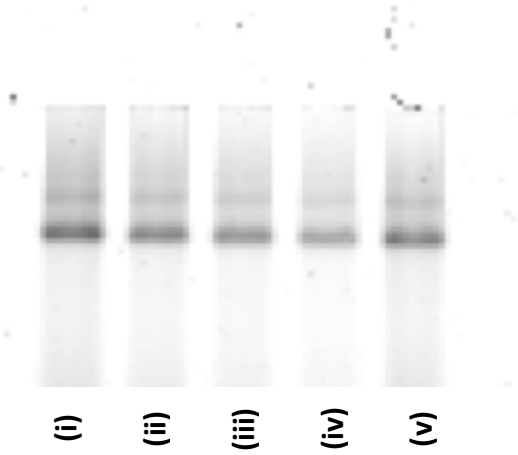

Figure S2: 52 nt DNA brick nanostructures, incubated in five different buffer conditions, for 24 hours

DNA brick nanostructures tested in different conditions

Different DNA brick nanostructures were assembled and tested in 1× PBS for their structural stability. Different lengths of binding domain – 32 nt bricks with 8 nt binding domain, 52 nt bricks with 13 nt binding domain, and 74 nt bricks with 18 or 19 nt binding domain – were tested, as well as different sizes of DNA brick nanostructures (52 nt bricks, 6×6×104 versions 1 and 2, as well as a lager 10×10×156). Versions 1 and 2 of 52 nt brick nanostructures adapted the sequences according to previous literature (Ong et al, 2017). In each colored rectangle, the left lane indicates the listed nanostructure incubated in 10 or 40 mM $MgCl_2$, while the right lane indicates the listed nranostructure incubated in 1× PBS. As a note: 32 nt bricks 6×6×104 used 40 mM $MgCl_2$, 74 nt bricks 6×6×148 used 40 mM $MgCl_2$, 52 nt bricks 6×6×104 ver2 used 40 mM $MgCl_2$, 52 nt bricks 10×10×156 used 40 mM $MgCl_2$, while 52 nt bricks 6×6×104 ver1 used 10 mM $MgCl_2$.

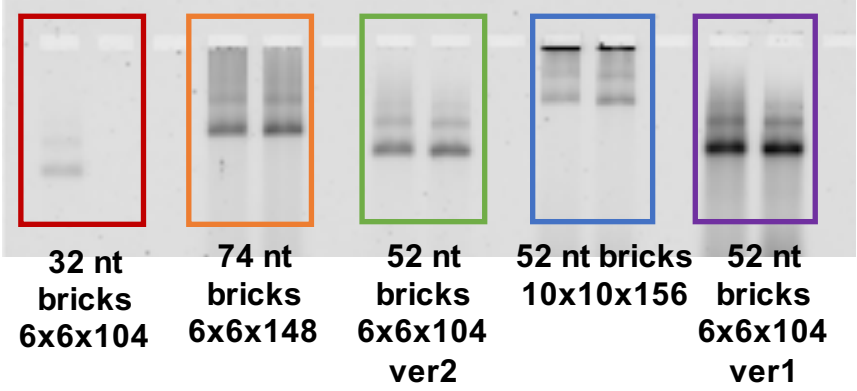

Figure S3: Different DNA brick nanostructures were assembled and tested at 1× PBS in terms of structural stability. Different binding domain lengths and nanostructure sizes were compared, and agarose gel electrophoresis results indicate the importance in binding domain length of brick nanostructures when maintaining their structural stability.

Agarose gel electrophoresis results indicate that all 52 nt brick nanostructures remain stable regardless of their overall size. On the contrary, a distinct difference is found between 32 nt, 52 nt, and 74 nt brick nanostructures. As the binding domain length becomes longer, the overall structural stability of DNA brick nanostructures increases.

Stability of DNA brick nanostructures in 1× PBS

Bare DNA brick nanostructures were found to be stable in 1× PBS (Figure 2, main text). This was further tested by incubating the 52 nt DNA brick nanostructures (13 nt binding domain length) in 1× PBS at 37°C for 1 hour, applying spin-filter to exchange the buffer completely, then leaving the sample at room temperature for approximately 4 days. TEM characterization indicates that the DNA brick nanostructures still remain stable after a prolonged incubation at a low divalent cation salt environment.

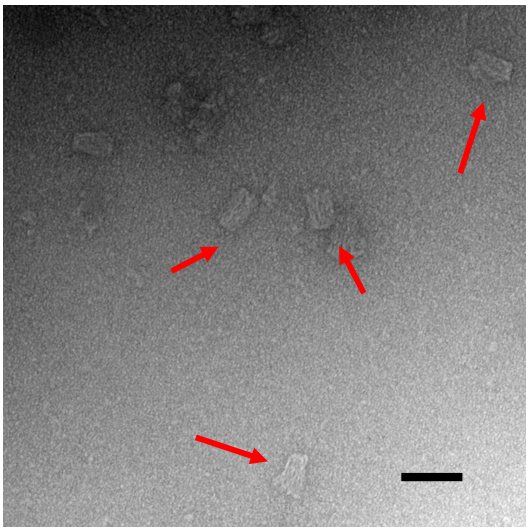

Figure S4: Bare DNA brick nanostructures, incubated in 1× PBS at 37°C for 1 hour, spin-filtered, then kept at room temperature for 4 days, still remain stable as characterized via TEM. Scale bar indicates 50 nm in length.

Testing 52 nt bare brick nanostructures against nuclease digestion

Bare brick nanostructures (52 nt bricks) were tested against nuclease digestion at different DNase I concentrations. All assembled brick nanostructure samples were incubated in DMEM media with different concentrations of DNase I nuclease (control – no DNase I, A – 0.5 U/mL, B – 5 U/mL, C – 50 U/mL, D – 100 U/mL). After incubating the samples with nucleases for approximately 1 hour at 37C, agarose gel electrophoresis characterization was performed to compare the band intensities between different samples.

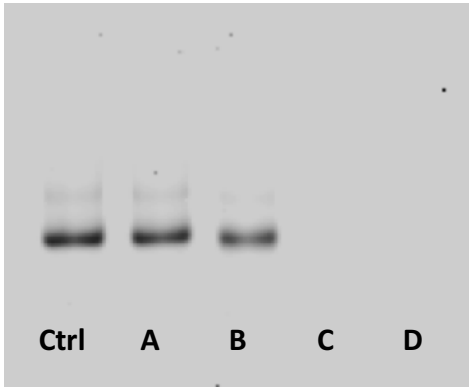

Figure S5: Bare brick nanostructures were tested against nuclease digestion at different DNase I concentrations.

Results indicate that bare DNA brick nanostructures are not stable against nuclease digestion, above a DNase I concentration of approximately 5 U/mL. This result was repeated for multiple times, and also listed in Figure 3 in main text.

Additional experiment on attaching dendritic oligonucleotides on brick nanostructures with less than 100 protruding strands

In order to test whether the type (3x or 9x) of dendritic oligonucleotide or the number of protruding strands from the 52 nt DNA brick nanostructure mattered more in maintaining the overall structural stability, we synthesized a 'hybrid' structure, including 9x dendritic oligonucleotides attached to 52 nt DNA brick nanostructures with 35 protruding strands on the surface. The 35 chosen protruding strands are listed under table S1. By doing so, much less than 100 protruding strands were available on the DNA brick nanostructure surface, and therefore 35 instead of 100 sites were available for dendritic oligonucleotides to become attached. These structures were then conducted through the same DNase I assay experiment to check the stability in the presence of nucleases.

Results indicate that these structures are not as stable as DNA brick nanostructures with triple (3x) or nonuple (9x) dendritic oligonucleotides on 100 protruding strands.

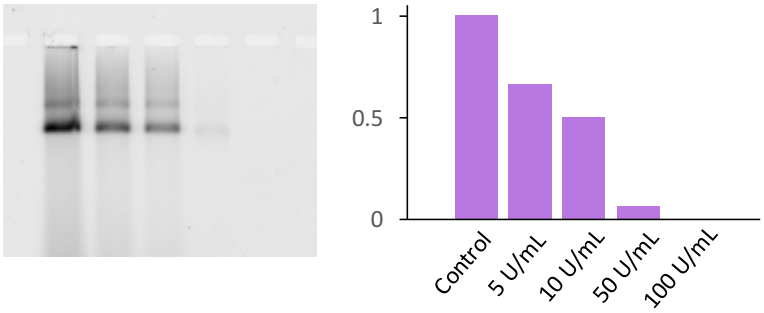

Figure S6: DNase I assay experiment on DNA brick nanostructures (specifically, 9x dendritic oligonucleotides attached to 52 nt DNA brick nanostructures with 35 protruding strands on the surface)

Quantitative analysis of surface oligonucleotide density

52 nt DNA brick nanostructures with 100 protruding strands were designed and assembled (please see table S1 for sequences). Out of the 100 protruding strands, 1 protruding strand (specifically, X_strand2) was modified with a different extended region "CTACCATCTCTCCTAAACTCA" instead of "TTCCTCTCCCTCCTCTCCT". To this modified region, "CTACCATCTCTCCTAAACTCA", a complementary Cy3-strand was attached for characterizing fluorescence intensity of Cy3, which in turn allows us to estimate the number of DNA brick nanostructures. The remaining protruding strands were used the same as listed in table S1. To synthesize and assemble DNA brick nanostructures with dendritic oligonculeotides, dendritic oligos were attached (3x or 9x) only to the unmodified protruding strands. The dendritic oligos were designed to have single-stranded regions, located after the branching phosphoramidite, with a sequence of "ACTCACCACCAC". Cy5-labeled, complementary strands were attached to this region in order to characterize and estimate the number of Cy5 in fluorescence measurements. For DNA brick nanostructures without dendritic oligonucleotides, different Cy5-labeled complementary strands were used such that the complementary region could hybridize to "CCCTCCTCTCCT" of the original protruding sequence "TTCCTCTCCCTCCTCTCCT" instead of having "ACTCACCACCAC" (sequence only present in dendritic oligonucleotides).

As listed under the methods section in this SI, all samples were spin-filtered to remove excess DNA strands (any short oligos or dendritic oligos that did not get used up in assembling DNA brick nanostructures), then complementary Cy3- or Cy5-labeled strands were added sequentially. For example, complementary Cy3-labeled strands were added to the brick nanostructures and incubated at room temperature for approximately 2 hours to allow hybridization between the Cy3-labeled strands and the complementary strand (modified version of X_strand2) on the DNA brick nanostructures. The sample was constantly shaken during this incubation period. Afterwards, Cy5-labeled strands were added and incubated for approximately 2 hours while constantly shaking to allow hybridization between the dendritic oligonucleotides (or protruding strands without dendritic oligos) and the fluorescently labeled strands. After both

rounds of incubation, another spin-filtering step was inserted to remove all unhybridized fluorescently labeled strands from the solutions.

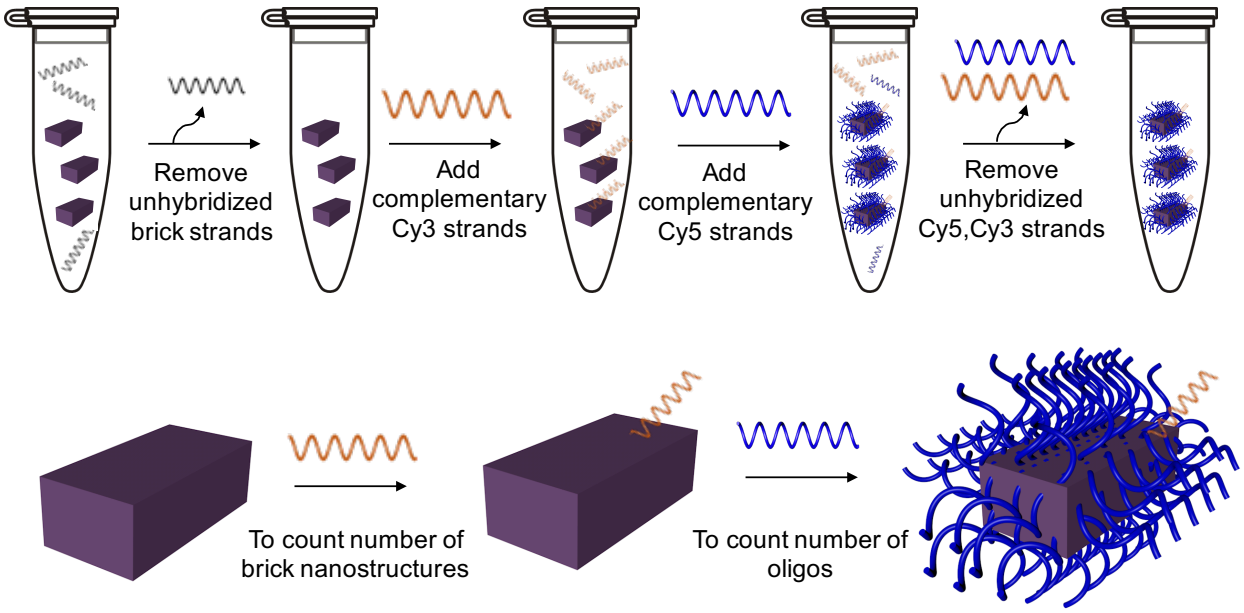

Figure S7: Schematic of quantitative analysis of surface oligonucleotide density

To quantify the number of strands hybridized to the DNA brick nanostructures, each dye (Cy3 and Cy5)-labeled oligonucleotides were measured at different concentrations to get a standard curve for fluorescence values. Then, fluorescence intensities of DNA brick nanostructures with fluorescently labeled strands were measured separately in triplicates and averaged. This averaged value was compared against the standard curve to estimate the concentration of fluorescently-labeled strands. The estimated concentration of Cy5-labeled strands was divided by the estimated concentration of Cy3-labeled strands to calculate the total number of available strands per brick structure (as shown below).

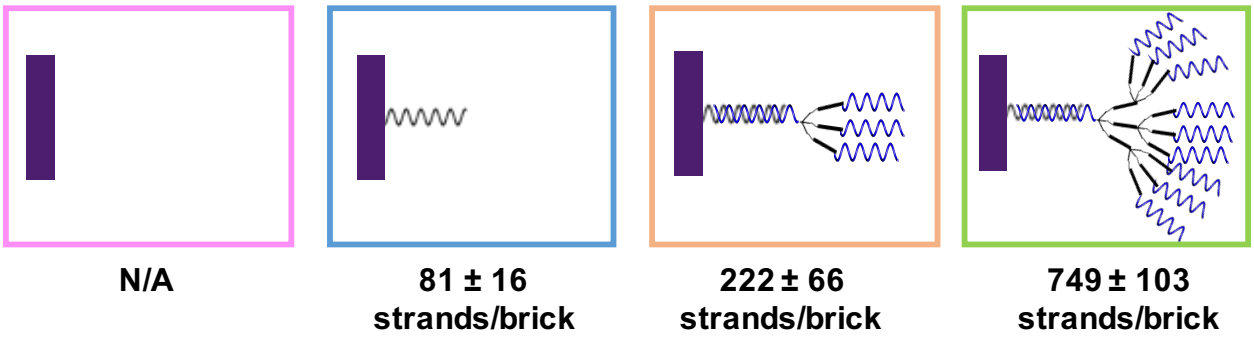

Figure S8: Quantitative data for indirectly measuring and calculating the number of oligonucleotides available on the outer surface of DNA brick nanostructures. Data is not available for left-most, pink-lableled, bare DNA brick nanostructures as fluorescently labeled strands could not hybridize on the outer surface of DNA brick nanostructures without any protruding strands. Second from the left, DNA brick nanostructures with protruding strands but

no dendritic oligonucleotides (single) had on average 81 fluorescently DNA strands attached per brick. Third from the left, DNA brick nanostructures with dendritic oligonucleotides (3x), triple, had on average 222 strands per brick. Last one on the right, DNA brick nanostructures with dendritic oligonucleotides (9x), nonuple, had 749 strands per brick.

Stability of DNA brick nanostructures in 10% FBS

DNA brick nanostructures, with and without dendritic oligonucleotide strands attached, were tested in 10% FBS for different periods of time. Assembled nanostructures were incubated in 10% FBS, DMEM at 37°C, then characterized via agarose gel electrophoresis.

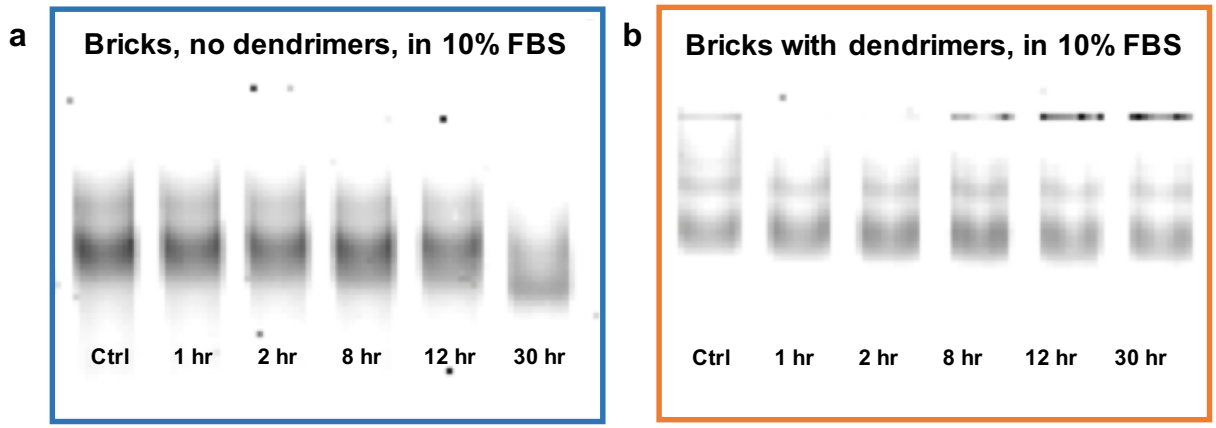

Figure S9: Brick nanostructures, with and without dendritic oligonucleotide strands attached, were tested in 10% FBS up to 30 hours. Results indicate that only dendritic-oligonucleotide-coated brick nanostructures survive up to 30 hours while structures with no dendritic oligomers fall apart at approximately 12 hours.

Results indicate that brick nanostructures without dendritic oligonucleotide coating falls apart at approximately 12 hours of incubation (indicated by a fainter band and shift in band location), brick nanostructures with dendritic oligonucleotide coating remains stable up to 30 hours before significant degradation. The structural stability of dendritic-oligonucleotide-coated DNA brick nanostructures at 24 hour time-point was also confirmed via TEM.

TEM data of nonuple brick nanostructures (bricks with 9× dendrimers) in 10% FBS after 30 hours

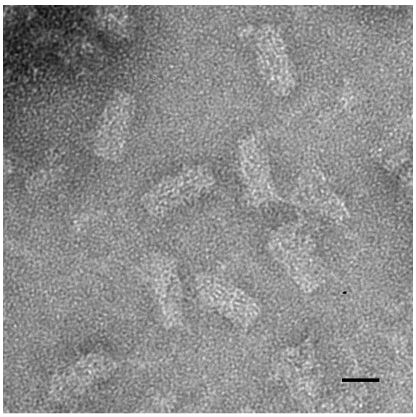

Figure S10: TEM data TEM data verifies that nonuple brick nanostructures (DNA brick nanostructures encoated with 9× dendritic oligonucleotides) remain stable in 10% FBS after 30 hours. Scale bar indicates 20 nm in length.

Additional figures

Additional TEM figures were taken for different samples (as listed).

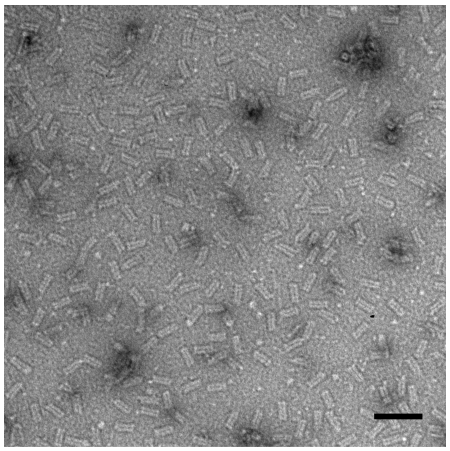

Figure S11: A more zoomed-out view of TEM data confirms synthesis of consistently sized 52 nt DNA brick nanostructures assembled. Scale bar indicates 100 nm in length.

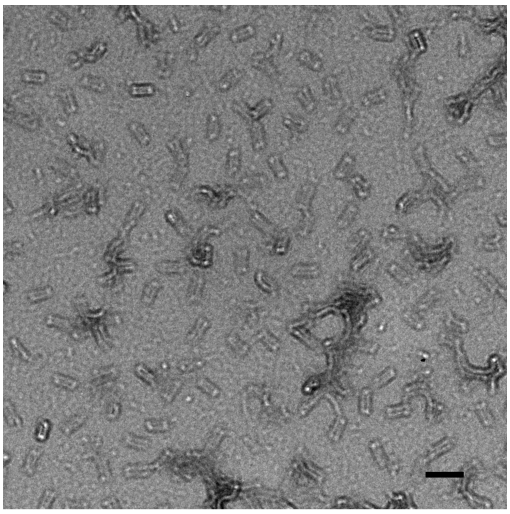

Figure S12: A more zoomed-out view of TEM data confirms synthesis of 52 nt DNA brick nanostructures with nonuple (9x) dendritic oligonucleotides assembled in consistent sizes. Scale bar indicates 50 nm in length.

Additional microscopic images (in situ characterization) were also taken for cells incubated with DNA brick nanostructures (with nonuple dendritic oligonucleotides) for 2 hours.

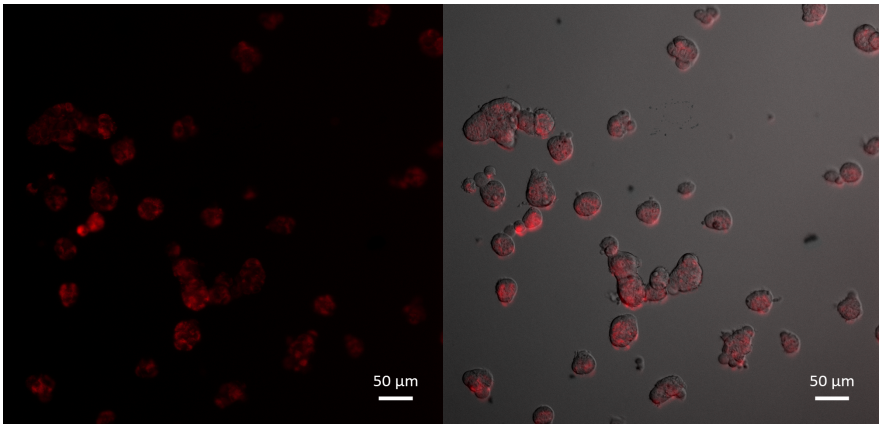

Figure S13: The fluorescence channel as well as brightfield channel confirms successful uptake of DNA brick nanostructures (with nonuple dendritic oligonucleotides) post-2hour-incubation. The images included in the main text were taken after a 30 minutes incubation period.

DNA sequences

Table S1: DNA brick sequences for bare DNA brick nanostructures

All sequences are listed in 5' → 3' direction.

X_strand0	ACGGTTAACTGTTGTGTCCTATGTTT TTCCTCTCCCTCCTCTCCT
X_strand1	ACGCTCGGGTAGTCTCAAGAAGATAG TTCCTCTCCCTCCTCTCCT
X_strand2	AACCGTAGTGGGATCGATCTCCCTTGCCAGGTTAAGTGGCTCAATCATACTC TTCCTCTCCCTCCTCTCCT
X_strand3	TAATATGGTCCAGTGATCTTTAGTCTACTTAGGGATGGTACGAACTCAACGC TTCCTCTCCCTCCTCTCCT
X_strand4	CGGCAAGGTCGAGGTATAGGTGGGAATACCCACTCGCGTGGCACGTCTACGA TTCCTCTCCCTCCTCTCCT
X_strand5	ATACTAACTCCAGTCCATCGAGGCGGAGTGACTTGGACGAGGTTTCACTGGG TTCCTCTCCCTCCTCTCCT
X_strand6	TGAGTAGGCCTAGTACCACAGCGTATACTCCGGCCTCCTTGGTATATTAGCT TTCCTCTCCCTCCTCTCCT
X_strand7	GTCCCACGATACGAAATGAAGCTCTACTCGTCGAACGAGGACTGTTCTACAT TTCCTCTCCCTCCTCTCCT
X_strand8	ACATATAGGGTTACTGCTAGAAGTATAAGCAAACGTGGGAGTGCCCGAGCGT TTCCTCTCCCTCCTCTCCT
X_strand9	CATAATGACGACACTGGGATAGAAGCGTCTCAAGTGCTAACAGTACTAATTA TTCCTCTCCCTCCTCTCCT
X_strand10	TGGTCGGGTAAAGGCGGGGATTACTTTGTGCATTCCACGTGAGTCGTACTTA TTCCTCTCCCTCCTCTCCT
X_strand11	GGTCGCGAGCCGGGATGGGTGTCCATCTGAGGTATGGTTTACCCACTATGTT TTCCTCTCCCTCCTCTCCT
X_strand12	GTGGCCAACCAATGACATGCACCCCG TTCCTCTCCCTCCTCTCCT
X_strand13	GTATATAAAGTGAACGTGTACATTCG TTCCTCTCCCTCCTCTCCT
X_strand14	CGCCAAGTACCAAGTCAAAGAGGTCG
X_strand15	CCACCCCAGCCTCTCGATGCAAGATA
X_strand16	TCATGACGGTTGAAAGGAAGGTAGACCAGTTTTGGCGTTACGAGTCAAATTG TTCCTCTCCCTCCTCTCCT
X_strand17	GCCCTTGAGAATCAACAAAGCCCCCGGCAGCTATCTATATTGACGTCACTTC TTCCTCTCCCTCCTCTCCT
X_strand18	GAGTGCCGACATACAACCATAGACTCTGCGTTTCAAGTGGCTTGGACCTCGA
X_strand19	GGCCTGACAGAAAGTGCATACACAGTACAACCATAGTCTTACAGGATCCGAT
X_strand20	CGCAAGGATACTACGCCTGTTCATCTTGACGGATGACTTTTGACCGATAACC
X_strand21	TTGGCACTGTATGAAGTACTAGTGGTTCGGTCTCATTCTGTCAGTTGCTCGC
X_strand22	CCTACAAGGTTAACTCGGCAAGCGGAACGTAATAATGAATTACGTCCGAAAT
X_strand23	ATCGCTCTCTAACTGGAAGCGTTATAGTCTTGATTATTTCTTTTGTTCCCGG
X_strand24	GGTTAGGGAAGGCTAAGCCCTCTTCTTCGGATCGTGGAGCGCCGCCGGAGCG
X_strand25	ATAAACGGTTTATGGGTACTCTAGATCATGCACCGCCTAAGGGTATCGGTAG
X_strand26	TAATCACCTATCCATGGTGTTCATTC TTCCTCTCCCTCCTCTCCT
X_strand27	TCGGGAAACAGGTCCGCGCTGGTCTG TTCCTCTCCCTCCTCTCCT
X_strand28	CCGTTCCAACTACCCTTTAATTGTCG TTCCTCTCCCTCCTCTCCT
X_strand29	GTGATGGTTGGGGATTCGCGACATAC TTCCTCTCCCTCCTCTCCT
X_strand30	AACCTTATGCAAACATCAACCACGAGGTTAGTCTTGCAACAGGCGCCAGTCG TTCCTCTCCCTCCTCTCCT
X_strand31	ATAGCAATTATATACACCTACCCCTTCCATTACACAGGGGCTCCTGGAGTAT TTCCTCTCCCTCCTCTCCT
X_strand32	AATAGTCGTCTATCTCGAAACGTGGCCTTGCCATACGCACTGGCGCAAGTGC

X_strand33	GCAATACAATGGTAGCGGTTGAGCGGTTGGTCATGTAATGGAAAAGGTACCG
X_strand34	TGTCATTGGTAGCCCTGACTAGTCGCGCCAAGTCGCGACCTAGTAGCGCACT
X_strand35	CGACCAGAATCTAGGTTCACGTGACCTCCGTACCACCAGCGGCATATAGGCG
X_strand36	GGCTATAATCGCCCTCAATTCGTCGGCACGGTTCGCGTCGAACTTGTTTTGG
X_strand37	CCTACGCCCTTGCAGGTTGGCCATCCCTTCTGAGGGAAGGGTGAGACTAGGT
X_strand38	AAGAGCCTAAGCGAGGGATTCATAACAATTTTCTATACTTGTTCGCGCTTGA
X_strand39	TAACCTGGCGTTATACCAAGGACCGCCGTTAATGTCGCCAAGGCAAAGAATG
X_strand40	TGCTGTGGCGTGCACGAGTATTAGAC TTCCTCTCCCTCCTCTCCT
X_strand41	AGAATTTCAACTGACCCACGGTTCAA TTCCTCTCCCTCCTCTCCT
X_strand42	TTGACTGTGCAGATAACGAAGACCTT
X_strand43	CGCGTGTTCGGGGGCAAGCCTGACCC
X_strand44	TACGACTCCGTCGCAGCACGCTGCCATCCTACTGGTTGAGGGACTTCGCAAG TTCCTCTCCCTCCTCTCCT
X_strand45	CTTTGGACGGCGAGTCTGAAGTATGATGAATATCCTTAACGGGTTCCGTCAT TTCCTCTCCCTCCTCTCCT
X_strand46	TCTGGGCGTGTATGGGAGTTTAGAAAGACGCTGTCAAGGCCCTTAGCCTGGC
X_strand47	CATATCGGGTCTGCCGTTCCCGATGACTAATGGCCTACTGGAAACTAGCCCT
X_strand48	CGAGCTCATAAGAATGAGCACGTCTTGGTAACACGTAGCTTCGTGCCCATCT
X_strand49	GGGGAAGACCGCGGCATATGGCAATGGGAATTGACCCGGGCCAGACACCGTC
X_strand50	CACTTATGGCTCCAGTTCTGAGCCTATTATGCTAACTATTCCTTTCAAGCGC
X_strand51	GCAGAAATGCTACATCTGGTTAAGCTTAGGTTATTCTCCGCCCTACGTCCGT
X_strand52	AACTTAAAAAGTGTTAAGCAGGCGCCTCTTAGCGTTCCCAGGAAGGGAGTAT
X_strand53	CCCACAATCTGCAAGCCATCGTTACTGGTTAGCCGGGATGTGAACCGAAAGT
X_strand54	TCCGTAAACGTGTGTGAGTCGCCAGT TTCCTCTCCCTCCTCTCCT
X_strand55	CATGGTCAGATATCGATCATCCAGCA TTCCTCTCCCTCCTCTCCT
X_strand56	GTGTATATAAATAGTTCTTATTGACA TTCCTCTCCCTCCTCTCCT
X_strand57	ACGCAGCTAGTAACGCTTTGCATACG TTCCTCTCCCTCCTCTCCT
X_strand58	TGTAGCCTAATGTCGACATACTATTAGATGTCTATCTACCCTTACTATACGG TTCCTCTCCCTCCTCTCCT
X_strand59	AATCGAGAGGACCGAGAGTAAGTCCTATATAGCTAAAGAAGTATTGCGGAGG TTCCTCTCCCTCCTCTCCT
X_strand60	GGCGGGATGGATAGAGTAGTGGGTTTCCCGTGTGTTTAATGATACTGAGCTC
X_strand61	CGGGTGGAGCGCCTACCACCTGGTAACATGCGGGATTAGAGACTGCCTGTAC
X_strand62	GGGTTATCTAAATATCGGGCTCGGCTCCTTAAGGGCACCTAGGGGTCGGGCA
X_strand63	CCGACGATGCTTCAATTAAGTGAACACCCAGTCACGTTTAGAGATTTTGCAG
X_strand64	CGGAACGTGCATGGTGCGGTCGAACCTACATGTATCTGGTTGAGGGTCGCTT
X_strand65	GGCTGCAAAAACTATGGTCGTTTCCTTCGTCTGCTAACACCATGCGCACGCC
X_strand66	ATGTCCACTTATCACATCGCGGGAGCGAAGCTTCGACTGACGTAGGTAGGGT
X_strand67	GGATGCGCGTCCTAGGGTGAGTATGGAGGGCGGATTCCAATGCTGACTGTCA
X_strand68	GTGGCGGACTTGACTATGTCGTTCGC TTCCTCTCCCTCCTCTCCT
X_strand69	TACGGTCTTCGGTTTTATGCTCGAGG TTCCTCTCCCTCCTCTCCT
X_strand70	TTACGCAAGTCTTAGACAGTTACGCT TTCCTCTCCCTCCTCTCCT
X_strand71	GTCGCTTCAGCGCTCTTGAGCAGTAG TTCCTCTCCCTCCTCTCCT
X_strand72	TCAAATCGAGTGCGCGCATTTGGACAGTGACCAACCTTAGCAAAGCGTGTTG TTCCTCTCCCTCCTCTCCT
X_strand73	CCATTGGAGGTTACGTGGTAGAATACGTGAAAATGTCTCAGCTTCCTACGCA TTCCTCTCCCTCCTCTCCT
X_strand74	GCTATGTGATCACGACAGGGCAAAGCTAATTAGATGGAGTTGATGCCTAAAG TTCCTCTCCCTCCTCTCCT

X_strand75	TCTAGAGAGTTAGCCCTCGAAATCCGTTATCTGTTTCGAGGTTTGCACTTTC TTCCTCTCCCTCCTCTCCT
X_strand76	CATGCGGCGCATTTTATGTCCAAGCTAACACCTATGTGCGGGCTTTCCAGAC TTCCTCTCCCTCCTCTCCT
X_strand77	TGGGGCCTAGGGCACGAAATCATGATTAGCTTGAAGCATGCGCAAGTGGGCT TTCCTCTCCCTCCTCTCCT
X_strand78	AGTCACGGCGAGGTAGTCCGGTTTTGCGTTAGATACACCACGTAGCTGTATG TTCCTCTCCCTCCTCTCCT
X_strand79	TACCGTTGCCTAGGGTTTGCTACCGAGCTAAGATAACCAATCAGCAAGCTTA TTCCTCTCCCTCCTCTCCT
X_strand80	TAGATGACAATAGCATCTAACCTGTAGTGCGTTTTAGACACTGTACCAGTTC TTCCTCTCCCTCCTCTCCT
X_strand81	ATCGTCAGGCCGCATCGGGTCTACCTCAAGCCAGGCTTACGTCAATTAACTT TTCCTCTCCCTCCTCTCCT
X_strand82	TCCCCTATGTCGCACAGATCGTGTCC TTCCTCTCCCTCCTCTCCT
X_strand83	GCTGAGGGTAAAATCGGCTGCTACAA TTCCTCTCCCTCCTCTCCT

Y_strand0	CCACTTAACCTGGTTTTTTTT
Y_strand1	ACCATCCCTAAGTAAACATAGGACAC TTCCTCTCCCTCCTCTCCT
Y_strand2	TTTTTTTTCTATCTTCTTGAG TTCCTCTCCCTCCTCTCCT
Y_strand3	TCCCACTACGGTTTTCCCACCTATAC TTCCTCTCCCTCCTCTCCT
Y_strand4	CTGGACCATATTACCGCCTCGATGGA TTCCTCTCCCTCCTCTCCT
Y_strand5	AGGAGGCCGGAGTTTTTTTTT TTCCTCTCCCTCCTCTCCT
Y_strand6	CTCGTTCGACGAGTCGTAGACGTGCC TTCCTCTCCCTCCTCTCCT
Y_strand7	TTTTTTTTCCCAGTGAAACCT TTCCTCTCCCTCCTCTCCT
Y_strand8	CTAGGCCTACTCAATACTTCTAGCAG TTCCTCTCCCTCCTCTCCT
Y_strand9	CGTATCGTGGGACGCTTCTATCCCAG TTCCTCTCCCTCCTCTCCT
Y_strand10	CGTGGAATGCACATTTTTTTT TTCCTCTCCCTCCTCTCCT
Y_strand11	AACCATACCTCAGACGCTCGGGCACT TTCCTCTCCCTCCTCTCCT
Y_strand12	TTTTTTTTTAATTAGTACTGT TTCCTCTCCCTCCTCTCCT
Y_strand13	CTTTACCCGACCACGGGGTGCATGTC TTCCTCTCCCTCCTCTCCT
Y_strand14	CCGGCTCGCGACCCGAATGTACACGT TTCCTCTCCCTCCTCTCCT
Y_strand15	AACAGTTAACCGTGAGTATGATTGAGGGATAGGTGATTAAGAAGAGGGCTTA TTCCTCTCCCTCCTCTCCT
Y_strand16	ACTACCCGAGCGTGCGTTGAGTTCGTACCTGTTTCCCGAATCTAGAGTACCC TTCCTCTCCCTCCTCTCCT
Y_strand17	ACGCGAGTGGGTAAGCTAATATACCATTAACCTTGTAGGAGATGAACAGGCG
Y_strand18	CGTCCAAGTCACTATGTAGAACAGTCGTTAGAGAGCGATACCACTAGTACTT
Y_strand19	CCCACGTTTGCTTTAAGTACGACTCATATGTCGGCACTCGTCTACCTTCCTT
Y_strand20	TAGCACTTGAGACAACATAGTGGGTATTTCTGTCAGGCCCGGGGGCTTTGTT
Y_strand21	TTGGTACTTGGCGTTTTTTTTTTTTTTTTTAAGTAATCCCCGC TTCCTCTCCCTCCTCTCCT
Y_strand22	GAGGCTGGGGTGGCAATTTGACTCGTATTGGTTGGCCACATGGACACCCATC TTCCTCTCCCTCCTCTCCT
Y_strand23	TTTTTTTTGAAGTGACGTCAATCACTTTATATACTTTTTTTT
Y_strand24	CACTTGAAACGCATTTTTTTTTTTTTTTTTATACGCTGTGGTA TTCCTCTCCCTCCTCTCCT
Y_strand25	AGACTATGGTTGTGGTTATCGGTCAATAACCCTATATGTTAGAGCTTCATTT TTCCTCTCCCTCCTCTCCT

Y_strand26	TTTTTTTTGCGAGCAACTGACTGTCGTCATTATGTTTTTTTT
Y_strand27	TTCATTATTACGTTTTTTTTTTTTTTTTTCAAGGGAGATCGA TTCCTCTCCCTCCTCTCCT
Y_strand28	AAATAATCAAGACCGCTCCGGCGGCGCTCGACCTTGCCGAGACTAAAGATCA TTCCTCTCCCTCCTCTCCT
Y_strand29	TTTTTTTTCTACCGATACCCTCTGGAGTTAGTATTTTTTTTT
Y_strand30	AACGCCAAAACTGCGACCTCTTTGACCGCTTAGGCTCTTGTCTAATACTCGT TTCCTCTCCCTCCTCTCCT
Y_strand31	TATAGATAGCTGCTATCTTGCATCGATAACGCCAGGTTATTGAACCGTGGGT TTCCTCTCCCTCCTCTCCT
Y_strand32	AAGTCATCCGTCATCGAGGTCCAAGCGCTACCAATGACACCGACGAATTGAG
Y_strand33	AGAATGAGACCGAATCGGATCCTGTATAGATTCTGGTCGGGATGGCCAACCT
Y_strand34	CTCCACGATCCGAATTTCGGACGTAATTTGCATAAGGTTGCCACGTTTCGAG
Y_strand35	TAGGCGGTGCATGCCGGGAACAAAAGATATAATTGCTATCCGCTCAACCGCT
Y_strand36	TTGCAAGACTAACTTTTTTTTTTTTTTTTGAATGAACACCAT TTCCTCTCCCTCCTCTCCT
Y_strand37	CCCTGTGTAATGGCGACAATTAAAGGGCCTTCCCTAACCCAGACCAGCGCGG TTCCTCTCCCTCCTCTCCT
Y_strand38	TTTTTTTTGTATGTCGCGAATATAAACCGTTTATTTTTTTTT
Y_strand39	GTCGCGACTTGGCTTTTTTTTTTTTTTTTTCCGCTTGCCGAG
Y_strand40	CTGGTGGTACGGAGCACTTGCGCCAGTAGTATCCTTGCGTATAACGCTTCCA
Y_strand41	TTTTTTTTCGGTACCTTTTCCCATACAGTGCCAATTTTTTTT
Y_strand42	AGTATAGAAAATTTTTTTTTTTTTTTTTGAGTCTATGGTTG
Y_strand43	GGCGACATTAACGCCAAAACAAGTTCTCAACCGTCATGAACTGTGTATGCAC
Y_strand44	TTTTTTTTACCTAGTCTCACCGATTCTCAAGGGCTTTTTTTT
Y_strand45	GTAGTTGGAACGGCGACTGGCGCCTGACACGTTTACGGAGGCGCCTGCTTAA TTCCTCTCCCTCCTCTCCT
Y_strand46	CCCCAACCATCACATACTCCAGGAGCATATCTGACCATGAGTAACGATGGCT TTCCTCTCCCTCCTCTCCT
Y_strand47	TGCGTATGGCAAGAGTGCGCTACTAGGGAGCCATAAGTGAAGACGTGCTCAT
Y_strand48	ATTACATGACCAACGCCTATATGCCGGTAGCATTTCTGCCATTGCCATATGC
Y_strand49	GACGCGAACCGTGTCAAGCGCGAACAATACACGCCCAGATGGCAGCGTGCTG
Y_strand50	CTTCCCTCAGAAGCATTCTTTGCCTTCAGACCCGATATGTCATACTTCAGAC
Y_strand51	TCTGCACAGTCAATTTTTTTTTTTTTTTTTGTTATGAATCCCT TTCCTCTCCCTCCTCTCCT
Y_strand52	CCCCGAACACGCGCTTGCGAAGTCCCGCACGCCACAGCAGCGGTCCTTGGTA TTCCTCTCCCTCCTCTCCT
Y_strand53	TTTTTTTTATGACGGAACCCGCAGTTGAAATTCTTTTTTTTT
Y_strand54	CCTTGACAGCGTCTTTTTTTTTTTTTTTTTGCGACTAGTCAGG
Y_strand55	AGTAGGCCATTAGAGATGGGCACGAAGGCGATTATAGCCGGTCACGTGAACC
Y_strand56	TTTTTTTTGACGGTGTCTGGCGCAAGGGCGTAGGTTTTTTTT
Y_strand57	ATAGTTAGCATAATTTTTTTTTTTTTTTTCTCGTGGTTGATG
Y_strand58	GGAGAATAACCTAATACTCCCTTCCTATAGACGACTATTAAGGGGTAGGTGT
Y_strand59	TTTTTTTTACTTTCGGTTCACACCATTGTATTGCTTTTTTTT
Y_strand60	TCAACCAGTAGGAAAGGTCTTCGTTAGATAAGTGGACATGCGAACGACATAG TTCCTCTCCCTCCTCTCCT
Y_strand61	TTAAGGATATTCAGGGTCAGGCTTGCAGGACGCGCATCCCCTCGAGCATAAA TTCCTCTCCCTCCTCTCCT

Y_strand62	GCTACGTGTTACCGCCAGGCTAAGGGATTTAGATAACCCGGTTCGACCGCAC
Y_strand63	CCGGGTCAATTCCAGGGCTAGTTTCCGAAGCATCGTCGGAGGAAACGACCAT
Y_strand64	GGGAACGCTAAGAGCGCTTGAAAGGAACATTAGGCTACAAAACCCACTACTC
Y_strand65	ATCCCGGCTAACCACGGACGTAGGGCGGTCCTCTCGATTTTACCAGGTGGTA
Y_strand66	GTAGATAGACATCTTTTTTTTTTTTTTTTTACTGGCGACTCAC TTCCTCTCCCTCCTCTCCT
Y_strand67	TCTTTAGCTATATTGTCAATAAGAACCACTTTTTAAGTTTGCTGGATGATCG TTCCTCTCCCTCCTCTCCT
Y_strand68	TTTTTTTTCGTATGCAAAGCGTGCAGATTGTGGGTTTTTTTT
Y_strand69	GGTGCCCTTAAGGTTTTTTTTTTTTTTTTTTAGGCTCAGAACT
Y_strand70	AAACGTGACTGGGGAGCTCAGTATCATCTTATGAGCTCGAGCTTAACCAGAT
Y_strand71	TTTTTTTTGTACAGGCAGTCTCGCGGTCTTCCCCTTTTTTTT
Y_strand72	CAGTCGAAGCTTCTTTTTTTTTTTTTTTTTTTCTAAACTCCC
Y_strand73	TGGAATCCGCCCTAAGCGACCCTCAACGACGGAGTCGTATCATCGGGAACGG
Y_strand74	TTTTTTTTGGCGTGCGCATGGTCGCCGTCCAAAGTTTTTTTT
Y_strand75	TATTTATATACACCCGTATAGTAAGGGCGACATAGGGGATACAGGTTAGATG TTCCTCTCCCTCCTCTCCT
Y_strand76	TTACTAGCTGCGTCCTCCGCAATACTTTTTACCCTCAGCAGGTAGACCCGAT TTCCTCTCCCTCCTCTCCT
Y_strand77	TTAAACACACGGGTGCCCGACCCCTACCTCGCCGTGACTAGCTTGGACATAA TTCCTCTCCCTCCTCTCCT
Y_strand78	CTAATCCCGCATGCTGCAAAATCTCTCTAGGCAACGGTAATCATGATTTCGT TTCCTCTCCCTCCTCTCCT
Y_strand79	CCAGATACATGTAACCCTACCTACGTGTGATCACATAGCTGTCCAAATGCGC TTCCTCTCCCTCCTCTCCT
Y_strand80	TGTTAGCAGACGATGACAGTCAGCATCTAACTCTCTAGAGTATTCTACCACG TTCCTCTCCCTCCTCTCCT
Y_strand81	AAGACTTGCGTAATTTTTTTTTTTTTTTTTGCTCCCGCGATGT TTCCTCTCCCTCCTCTCCT
Y_strand82	GCGCTGAAGCGACCAACACGCTTTGCTCAAGTCCGCCACCCATACTCACCCT TTCCTCTCCCTCCTCTCCT
Y_strand83	TTTTTTTTTGCGTAGGAAGCTACCGAAGACCGTATTTTTTTT
Y_strand84	CTCCATCTAATTATTTTTTTTTTTTTTTTTAGCCGAGCCCGAT
Y_strand85	TCGAAACAGATAAGTCTGGAAAGCCCCATGCACGTTCCGTGTTCACTTAATT
Y_strand86	TTTTTTTTAGCCCACTTGCGCAGTTTTTGCAGCCTTTTTTTT
Y_strand87	GGTGTATCTAACGTTTTTTTTTTTTTTTTTTAATAGTATGTCG
Y_strand88	TGGTTATCTTAGCGAACTGGTACAGTTATCCATCCCGCCAGGACTTACTCTC
Y_strand89	TTTTTTTTAAGTTAATTGACGGGCGCTCCACCCGTTTTTTTT
Y_strand90	TAAGGTTGGTCACAGCGTAACTGTCT TTCCTCTCCCTCCTCTCCT
Y_strand91	GAGACATTTTCACCTACTGCTCAAGA TTCCTCTCCCTCCTCTCCT
Y_strand92	TTTTTTTTGCTTTGCCCTGTC TTCCTCTCCCTCCTCTCCT
Y_strand93	GCACTCGATTTGACGGATTTCGAGGG TTCCTCTCCCTCCTCTCCT
Y_strand94	TAACCTCCAATGGTTTTTTTT
Y_strand95	GCACATAGGTGTTCTTTAGGCATCAA
Y_strand96	ATGCTTCAAGCTAGAAAGTGCAAACC
Y_strand97	TTTTTTTTCAAAACCGGACTA TTCCTCTCCCTCCTCTCCT

Y_strand98	AATGCGCCGCATGTCGGTAGCAAACC TTCCTCTCCCTCCTCTCCT
Y_strand99	GCCCTAGGCCCCATTTTTTTT
Y_strand100	GTCTAAAACGCACCATACAGCTACGT TTCCTCTCCCTCCTCTCCT
Y_strand101	TAAGCCTGGCTTGTAAGCTTGCTGAT TTCCTCTCCCTCCTCTCCT
Y_strand102	TTTTTTTTGGACACGATCTGT TTCCTCTCCCTCCTCTCCT
Y_strand103	CTATTGTCATCTATTGTAGCAGCCGA TTCCTCTCCCTCCTCTCCT
Y_strand104	GCGGCCTGACGATTTTTTTTT

To note, 52 nt brick nanostructures with less than 100 protruding strands (specifically, 35 used) were assembled using the same set of DNA strands as noted in the table above, with the following 35 strands selected as the protruding strands: X_strands # 1, 2, 4, 7, 10, 11, 26, 27, 54, 55, 59, 69, 74, 75, 79, 82, and 83, and Y_strands # 3, 5, 8, 12, 13, 21, 27, 30, 37, 45, 51, 52, 66, 77, 81, 93, 100, and 101. All other strands that are not listed within the selected 35 did not contain the extended region's sequence "TTCCTCTCCCTCCTCTCCT".

Table S2: DNA origami staple strand sequences

TAA AAG AGA ACA TCA CAG CCG GCG AAC GTG GCG AGA AAG CAG GGC GTT TTT TT
TTT TGA CGA GTA ATA AAG AAT CCT GAG AAG TGG AGC GGG AGG TTG CTT TTT TTT T
GTC CAC TAA TCC CTG GGA GCC CCC GAT TTA GAG CTT GAC GTA TAA TTT TTT T
TAC TTT GCG CTG GAT TAC TCC AAC GTC AAA GCA AGT TTA GGT GCC TTT TTT T
CTA GTT GAA TAG GCA ATG CCG TCG AGA GGG TTG ATT CGG ACT AAA ATA TTT TTT T
ACA ACT TTG GCC CTG ATC AGG GCG ATG CGC CAA GAA CGC CCC GGA ATA TTT TTT T
CCG TAA CAC CCT CAT AGC GGG GTT TTG CTC AGT ACC AGG ACC GTA CTT TTT TT
TAG AAA GGG GAA TTG CCC CTC ATT TTC AGG GAT AAT ACC GTT TAG ACA TTT TTT T
TGA ATT TAG CTG AGA CTC CTC AAG AGA AGG ATC CCC TGC CTT TTT TT
GGC AGG TCA CCA GAA CAG GAG TGT ACT GGT AAC GGG GTC AAA CCT ATT TTT TTT T
GCT ATT AGC GAA CCA CTA TCC AGA ACA ATA TTT TAG TAA TTC TGT CCA
CGT GGT CGT TTG GGC TTT CCT CGT TAG AAT CAT TTT TAT ACC ACC GAG
TGG GCG CGG AAA CCC CGA AAT CGG AAC TAA CTC ACA AAT TAA AGA
AGT ATA GCC ACT ACG TGA ACC ATC ACC CAA ATG GCG AAA AGA ACA AGA
CCG CAA CGT CGG CAT ATT TCT GTA TGG GAT TTA GCC CGA GGT GTT GTT
TCA GGA GGC CAC CCT CAG AAC CGC CAC CCT CCC CTC AGA AGT ACA AA
TTT CGA GGC ATC GCC CTT GTC GTC TTT CCA GAA CAG ACA GCT GAG TTT
TAT TTC GGG TGC CTT GAG TAA CAG TGC CCG TAT AGC AAG CCC CAT GTA
GAG CCA CCT CCT CAT TAA AGC CAG GCA GTC TCG CTT TTG A
GTT TTC ATC AGT AGC GCG CCA CCC TCA GAG CCG AGC CGC CAG ACG ATT CAT ACA TG
CGT ACT ATG CTA AAC AGG AGG CCG ATT AAA GCA GGA ACG AAT TAA CCA TGG AAA T
CGC CGC TAG AAG GGA AGC AAT ACT TCT TTG AAC CGC CAA CCT ACA
TGA ACC TCG AAT TGA GTG AAA GCG TAA GAA TAA CAC GAC CCT CAA TCG
TGA CGA GCA CGG GGA ATT GCC TGA GTA GAA GAT CGG CCT TGG ATT ATT

CTG GTG AGA ATG AGT CTT TTC ACC AGT GAG AGG TCC ACC CCA GCA
GTA AAG CAA CCC TAA ATA TAA ATC AAA AGA ATT GCT TTG ATC CTG TAA
CAG CGA AAG GAA GCA TGC CCT TCA CCG TGA AAC ACG TCC TCA ACA GTT
GGT GTA TCC GGA TAA GCG CCT GTA GCA TTC CCG TTA GTG TGA GAA
AAC GGC TAG AGG ACT AGC TCC AAA AGG AGC CTG CCC TAA AAA CAA AGC
GTT AAT GCT AGG ATT AGT TAG CGT AAC AAA GCA ATG GGA TGA GGT TGA
AGT ATT AGA ATT TTA ACG CCT GCA ACA GTG CCA TAC CGA ATC TTT AAT
ACG TGA GGA TCA GTG GGG CAG ATT CAC CAG TCC GTG GCA CTT TGA ATG
TCA TGG TTG CCA AGC GCT TTC CAG TGA GGA GAG GTC GCA GGG TGG
CCA GTT TGA CCG TCT AGA GAG TTG CAG CAA GCC GGG CAA CTT GCG TAT
ACC ATC CCA GAG GCC CTT TGC GGG ATC GTC ACT TAA TGA ACG CGG GGA
CGT CAC CGC CAC CAG AAT AAT AAT TTT TTA TCT CCA AGC TTG ATA
GCA AAA GAA AAG TAC ACG CTG AGG CTT GCA GGA CAA CAA CTG AAT TTC
TGA AGG AAC CAA TTA CCA CCA CCA GAG CCG CCT TAA TTG TCA GCT TGC
AGC AAA ATT GCC ATC TTT TCA TAA CGG AAC CAG CCA CCC T
GTA AAT ATG CGC CAA ATC ACC AAT GAA ACC ATA CCG TAA TCG GCA TTT TCA GAG CC
TCA CGC AGT ACG CCA AGG GAC ATT CTG GCA GAA CCC TCT GAT AGC AAA GCA TC
GCC ATT GCA CAT CGC CAT TAA AAA CGC TGA ACC TTG C
TCC TGA TTT ACT TCT GTA GAT TAG AGC CGT CAG TTG AAA GAA ATA TCA
TCT GAA ATG CTG GTA ACA GCA GAA GAT AAA ACC GGT CAG TAT CAA TAT
AAG TTG GGC GCT ATT AGT GTG AAA TTG TTA TGG TGC CTC TAA CTC
GGC GAA AAT GGT GGT TTG TCG TGC CAG CTG CAC CTT GCG CTG CGT CAG
GCG CAG ACT GTT GGG AAC AAC ATA CGA TTT CCG GAA GGC CGA CAG CAT
TCA GCG GAA AAT GAA TGT TGC GCC GAC AAT GGA GTT AAG GGT AGC
TGA CCA ACG ACA GAT GTA CGT AAT GCC ACT ACC TTG CTT TCA GAG TAG
ATT GAC AGC TAA AGT TAC GCA TAA CCG ATC ACT CAA AAT ATG TAG CGC
AAT TGC GTC GGG AGA ACA GAA GGA GCG GAA TTA CGT TAT TAC TTT ACA
TTT ATA TTA GAC ATT CAG TTG GCA AAT CAA CAA TAG ATA AGA TTT AGA
AGA TCG TAG ATG AAA CGA CGG CTT TGC ATC ATC AGC ATA GCT G
GAG GCG GTA GCT GAT TAA AGT GTA AAG CCT GGC CGC TCA CAT TCG TAA
AAG TGA GGC AGT TGC TCA TGT TAC TTA GCC GGT CGA CTC TCG GGT ACC
TTA AAC AAA AAA AGA AGA CTT TTT CAT GAA TTA AAC GCA TCT TTG
TCA ACG TAC GAG TAG TGA TAA ATT GTG TCG AAC GCG AAA CAT ACA CTA
CAG TTT ATA TCG GAA CAC AGA ATC AAG TTT GCG AAG GCA CCG AAA GAG
CAA CAT ATC GTC ACC GAC TTG AGC TTA GAG CCC AAG GCC G
CAA AAG AAA AGT TAC CGT CAC AAT CAA TAG AAG TTT ACC ATG ACG GAA ACC ATT AG
GCG CGA ATC TGA CCG AAG GTT ATC TAA AAA GCA CTA ACG ACA ACT AAT TCA TC
GAG CCA GCA AAT CCT TTG CCC GAA TCA TCA AAT ATA A
CAT CAA GAA ATT ACA TGC ACG TAA AAC AGA AAG GGG ATT AGT TTG GAT
AAC CCT CAA TTA ACA CAA GTT TGA GTA ACA TTG GAA CAA AAG GCG ATT
ATT CTC CGG CCA GCT TGC CGG AAA CCA GGC AGC CTC TTT AAC GCC A

ACA TTA ATT CAC TGC CCT TGC ATG CCT GCA GGA ACT CAC GCC CAG GAG
TTA TAC CAT CAA AAA TTC AGG CTG CGC CAT AGA GGC GAA CGG TCA ATC
CGG AAC GAA GGC CGC TAG CGA TTA TAC CAA GAT CCG CGA CCG AAC
CTA CGT TAC TAA CGG ATC AAG AGT AAT CTT GAT CAA AGA GTT TGA ACC
CGT CAG ACT ATT CGG TAC GGA GAT TTG GGG AAC ATT TTA TGA GGG AAG
TGA ATA ACA ACA TAG CAA ATC GCG CAG AGG CGT TTT ACA TAG ATT TTC
TTT TTG AGT ACA TCC TCG CCA GCT GGC GAA AGT AAC ACC GTC CGG AGA
ATT TAA ACA ATC ATA GGT CAC GTT TGC TTC TGA TGG TGC ACT CCA
GAG CTC GAA ATT CCA CAG GGC GAT CGG TGC GGA AGC GCC AGG CCT CAG GA
ATC CCA AAA AGC CAT ATG CGA TTT TAA GAA CTG CAT CTG CGG ACG ACG
AAA CAC TGG TAA AAA ACG GTG TAC AGA CCG CTG GCT GCA GTG AAT
AGG AAT TAA AAA ACC AAT TTC AAC TTT AAT CAC ACC AGA AAC AAA GCT
GAA TAA AAC AAC CAC GGA CAA AAG GGC GAC ATC AAG AAC CTA CCC AAA
AAT AAG AGT TAG CAA ACG TAG AAA AAA GGT GGA ATA AGT T
CAC CCT GAA TAA CAT ACC CTT TTT AAG AAA AGG CCG AAC ACT GGC ATG CAC CAC GG
AAC AAT TCA ACT AAA ATA ATG GAA GGG TTA TAT CAA AAC GTC AGA AGA TGA TG
TAT TCC TGT ACA GTA ACA GTA CCA ATT ATT AAA CAA A
GCT TAG GTA ACT ATA TTA CAT AAA TCA ATA TAT GAA AAT TAA ACA GCG
GTG CTG CAG AAA CCA CAC AAT AAC GGA TTC GCT GAA TAC CCC CGT CGG
GGC TAT CAA TAA ATT AAA ATT TTT GTT AAA TTC CTG TAT GGG AAC
GGG TTT TAC GTT GTA GGC GCA TCG TAA CCG TGG CAC CGT GAT TGT CA
AAT ACT GCC CAT CAA TCA ATA GGA ACG AAG ATG TAG ACC AGT CAG GAC
ATA AGG GAA CCT GCT CTG CCC TGA CGA GAA ATT GTG AAG AAA AAT
AAT CCC CCT AAA CAG TTA GGA ATA CCA CAT TCA ACA CGA AAT AAA GCA
GAT TGA GGC ATC GCC TAA ATT GGG CTT TAC ATA TAC AGA GGG AAT ACC
GAC CTA AAA AAC ACC GAG AAG AGT CAA TAG TGT CCT TGA ACT TGC TTC
GCC AGC TTC TTC TGG TTC ATC AAC ATT AAA TGT GTT AAA ATT TGT GAG
GCA ATG CTT GCG GGA ATC GAT GAA CGG TAT AGC ATG TTT GTA AAC
ACA GTA TCT TCG CCA TAA TTC GCG TCT GGC CTC AGC TCA TAA GCA AAT
ATA AAT TTA TAA ATC GAT GTT TAG ACT GGA TAA ATC AGA AAA CAG GAA
GCT CAT TAC CTT CAA CAA CAT TAT TAC AGT CAT CAG TGC AAC ACT
AAA GAT TAG AAC CAG AAA GAA GTT TTG CCA GAA CGA CGA TCG AGG CAT
TAT TTC ATG GAT ATT TAG AAG GAA ACC GAG GAA ACT AAT GAC GCC AAA
CAT ATT ATA GAG ATA ACC CAC AAG AGC CCA ATC TAT CTT A
CAA TTT TAT TTT GAA GGT CAA AAA TGA AAA TAC AGA GAG AAC AAA GTC AGC AAT AG
AGG TTT AAT TAT TTT TAA CAA TTT CAT TTT TTT AAT GCG TCG CTA CCT TTT TA
CAT TTC AAA ATT TTC CCT TAG AAA ATT TAT ACC TCC G
ATA TTT AAA TAA AGT AGA GAA AAC TTT TTC AAC TAT ATA TTG GGT TTT
AGT AAC AAA AGT TAC AGA TAG CTT AGA AAA ACA TCG TTT ATC TAC AAA
CAG AGA GCA TCT CAG GCA GGT AAA GAT TCA AAA CTA GCT GGG TCA TT
AAA CGG CGA ATG GGA TAT GTA CCC CGG TTG ATG CGG AGC AGT CTG TCC

GAT GGC TTG CAA AGA AAG ACA GTC AAA TAA ATG ACC ATC AGG AAT CGT
GTT GGG AAT TAC CTT AAC CCT CGT TTA CCA GGG GGG TAT TCA TTG
GCT GTA GCT TAA ATA TTA CCC TGA CTA TTA TAT TAT GCT TTC AAA GAC
ATA ATA ACA TGG TTT AAA ATA GCG AGA AGT TAA ATT GGG CTA ACT GAA
CAA TAG ATA CCA ATC ATT ATA CAA ATT CTT ACA TAA GAA TTT TAA TGG
GTT AAT ATT TCG CAT TAT GCC GGA GAG GGT AGA TAT ATT TCA TCT TCT
CTG AAA ATT CCA AGC CCT GTA ATA GCC TGA AAA ACT TCT GAG TAA
GAT TGT ATT TTT TAA CAT GAT ATT CAA CCG TTG GGT GAG ATT TTA AAT
CTG TTT AGA CTC ATC GTT GAT AAG AGG TCA TTA CGC AAG GTT AGA ACC
AGT AAG ATG AGA TTT CAG AAA ACG AGA ATC AAA AAT CGA CTT CAA
CAT TAG ATT TCA TCG TCA GGA TTA GAG AGT ACT TCA AAG CAG AGG AAG
CCG ACA TAC AGA TAA GAA AAC AGG GAA GCG CAG TCA GAA GTG CAT CAA
TAT CCG GTC AAG CAA ACA GAG CCT AAT TTG CCT AAA CAG CAT TTT TTG
AAC GAT TTG AGT AGC GAG GCG TTT TAG CGA ACG CGG GAG GTC CTG AAT AAG AAA CG
TGT AAA TGA AAC AGG TAA ATG CTG ATG CAC AAG ACA ATA CCG ACC TAA TTG AG
CAA AAT CAT AAA TAA GGC GTT AAC AGT ATA AAT CGC C
AAC AAG ACG ACA ATA AAC AAC ATG TTC AGC AAG AGA ATC AAC GCC A
TTG AGA GAA GAC GCT GGA ATC ATA ATT ACT AGT TTA GTA TTT TAG G
CAA GCC TGA ACG CTT CTA CTA ATA GTA GTA GAT AAA GCA AAG CTA
GCC TGA GAA ACA AGA GAG AAG CCT TTA TTT CAT TTA AAA AGT ACC GCG
TCA TTT GGT CCA ATA AAT CAT ACA GGC GGA AGT GTC TAA GAG AGC TTA
CAT AAA TAA TAG TAA ACG TTT TAA TTC GAG CCT TTA ATA TAT AAT
CAA ATG GCA TAT AAC AGT TGA TTC CCA ATT GTT ATG TTT CAA CGC T
GGG AGA ATT TTT GCA ACC GGA AGC AAA CAA AAA GTT ACT CCC CAG CTA
TTT GAA AAG AAC GCC CGA CAA AAG GTA AAC CAG ACG AAA AAT AAT TTT TTT T
AAG CCA ACT CCT AAT TTA CGA GCA TGT AGA AAA GTC CTG TTT TTT T
TGT AAT TTT AGT TGT ATT TTC GAG CCA GTA ATT AAT GCA GTT TAT CAA TTT TTT T
ACA TGT AAC ATA TGC GAT AAT CGG CTG TCT TTC CTT ATC AGG TGG CAT TTT TTT T
CTC ATA TAA AGG CCG GTT AGC AAA ATT AAG CAC ATT AAC AGG CGC GAG TTT TTT T
AAT CGG TTC ATT ATG AAA CGG GTA TTA AAC CAA GTA CCG CCT ATA TTT TTT TTT T
CCC GAA AAG GTC TTG CAA CTA AAG TAC GGT TTC ATT CTC AAT AAC TTT TTT T
ATT GCT GAT GCT CCT TAG AAC AAG CAA GCC GTT TTT ATT ACA TTT CGT TTT TTT
TTT CGG ATC AAA GAA CCC TTA AAT CAA GAT TAC TGC GAA CAG TTT GAC TTT TTT T
ATT TTG CAC AAC AGG TAG GAA TCA TTA CCG CGC CCA ATA GAT TCT AAG TTT TTT T
TAA AAG AGA ACA TCA CAG CCG GCG AAC GTG GCG AGA AAG CAG GGC G
TTT TGA CGA GTA ATA AAG AAT CCT GAG AAG TGG AGC GGG AGG TTG CTT
TTT GAA AAG AAC GCC CGA CAA AAG GTA AAC CAG ACG AAA AAT AAT
AAG CCA ACT CCT AAT TTA CGA GCA TGT AGA AAA GTC CTG
ACA TTA ATT CAC TGC CCT TGC ATG
CCT GCA GGA ACT CAC GCC CAG GAG

Sequence for dendritic oligonucleotides

[different sequences of ssDNA] – $[trebler]_n$ – AGGAGAGGAGGGAGAGGA

Above, "n" represents the number of incorporated trebler moieties. Trimer dendritic oligonucleotides are formed when one trebler moiety is used (n=1), and nonuple dendritic oligonucleotides are synthesized by incorporating two trebler moieties consecutively (n=3). Sequence "AGGAGAGGAGGGAGAGGA" hybridizes to the overhang sequences from the brick strands (see Figure 1b in main text for a detailed schematic).