Supplemental Information for

Light-Triggered Release of Bioactive Molecules from DNA Nanostructures

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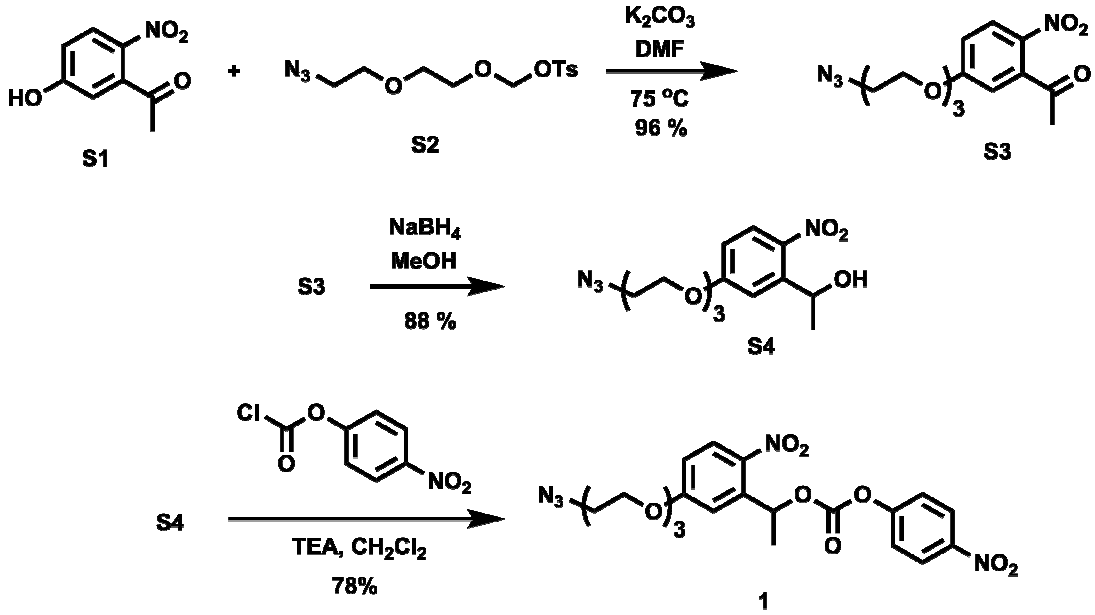
Experimental Section

Cross-linker 1 synthesis (Scheme 1):

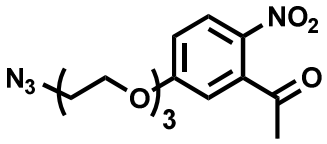
Reactions were monitored by TLC using glass-backed silica gel 60 F254 plates. Flash chromatography was performed in a quartz column with a fluorescent indicator (green 254 nm) added to the silica gel. TLC bands were visualized by UV. Solvent ratios used as elutants are reported in v/v. The purity of the final products was obtained through ^{1}H NMR and ^{13}C NMR.

^{1}H NMR data were obtained on a 500 MHz Varian VMNRS spectrophotometer at the Chemical Instrumentation Center at Boston University. Chemical shifts are reported in parts per million (ppm) and coupling constants were reported in Hertz (Hz). ^{1}H NMR spectra obtained in $CDCl_3$ were referenced to 7.26 ppm and those obtained in DMSO-d6 were referenced to 2.50 ppm. ESIMS data were collected on an Agilent Single-Quad LC/MSD VL instrument at the Chemical Instrumentation Center at Boston University.

The following compounds were synthesized according to literature procedures: 5-hydroxy-2-nitroacetophenone (S1)[1] and ethylene glycol 2-azidoethyl ether tosylate (S2)[2].

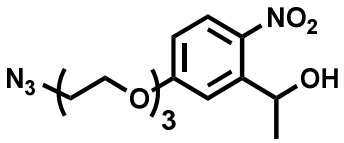


Scheme S1

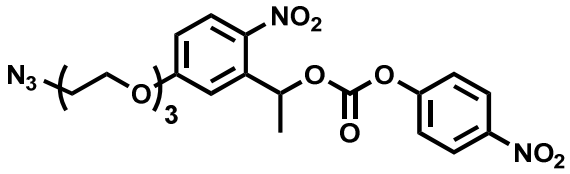


1-(5-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-2-nitrophenyl)ethan-1-one (S3). To a solution of 5-hydroxy-2-nitroacetophenone (2.46 g, 13.6 mmol) and ethylene glycol 2-azidoethyl ether tosylate (4.36 g, 13.2 mmol) in DMF (15 mL) was added potassium carbonate (3.77 g, 27.3 mmol), and the suspension was heated to 75˚C. After 18 hours, the solution was concentrated in vacuo and partitioned between CH_2Cl_2 (40 mL) and $NaHCO_3$ (20 mL). The organic layer was washed with $NaHCO_3$ (3 x 10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to produce 4.30 g (96% crude) of **S3** as a dark brown oil that was taken on without further purification: ^{1}H NMR (500 MHz,

DMSO-d6) δ 8.14 (d, *J* = 9.1 Hz, 1H), 7.21 (dd, *J* = 2.8 Hz, 9.1 Hz, 1H), 7.19 (d, *J* = 2.8 Hz, 1H), 4.29 (m, 2H), 3.79 (m, 2H), 3.59 (m, 6H), 3.38 (m, 2H), 2.53 (s, 3H).



1-(5-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-2-nitrophenyl)ethan-1-ol (S4). To a solution of **S3** (4.12 g, 12.2 mmol) in MeOH (30 mL) stirring in an ice bath was added sodium borohydride (723 mg, 18.7 mmol) in portions. After 2 hours, the solution was concentrated in vacuo and partitioned between CH_2Cl_2 (30 mL) and brine (20 mL). The organic layer was washed with $NaHCO_3$ (3 x 10 mL), dried over Na_2SO_4, filtered, concentrated in vacuo, and purified via flash chromatography (2:1 ethyl acetate : petroleum ether) to afford 3.63 g (88 %) of **S4** as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 9.1 Hz, 1H), 7.37 (d, *J* = 2.8 Hz, 1H), 6.88 (dd, *J* = 2.8 Hz, 9.1 Hz, 1H), 5.55 (dq, *J* = 4.0 Hz, 6.3 Hz, 1H), 4.24 (m, 2H), 3.90 (m, 2H), 3.74 (m, 2H), 3.68 (m, 4H), 3.38 (m, 2H), 2.40 (d, *J* = 4.0 Hz, 1H), 1.54 (d, *J* = 6.3 Hz, 3H).



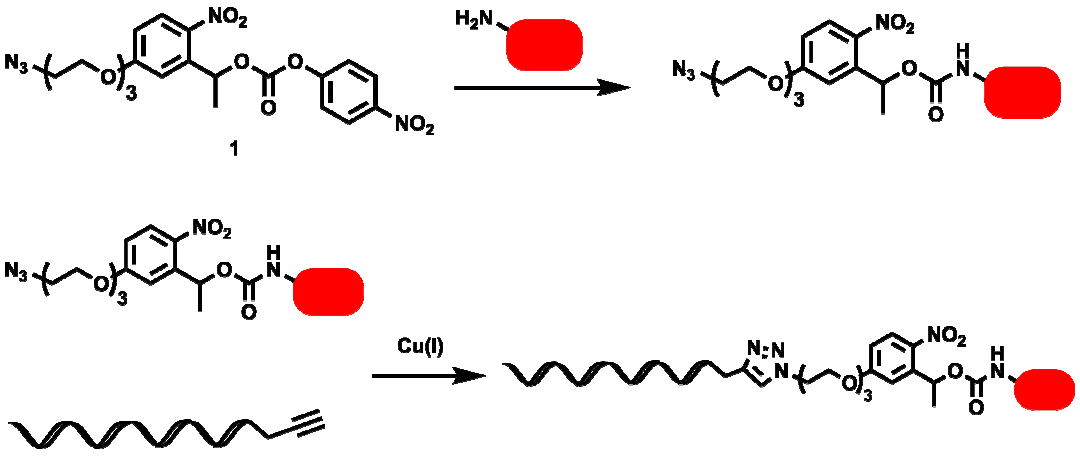
Cross-linker (1). To a solution of **S4** (1.88 g, 5.51 mmol) and 4-nitrophenyl chloroformate (1.65 g, 7.86 mmol) in CH_2Cl_2 (21 mL) was added triethylamine (1.50 mL, 10.8 mmol). After stirring for 24 hours, CH_2Cl_2 (30 mL) was added and the solution was washed with $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered, concentrated in vacuo, and

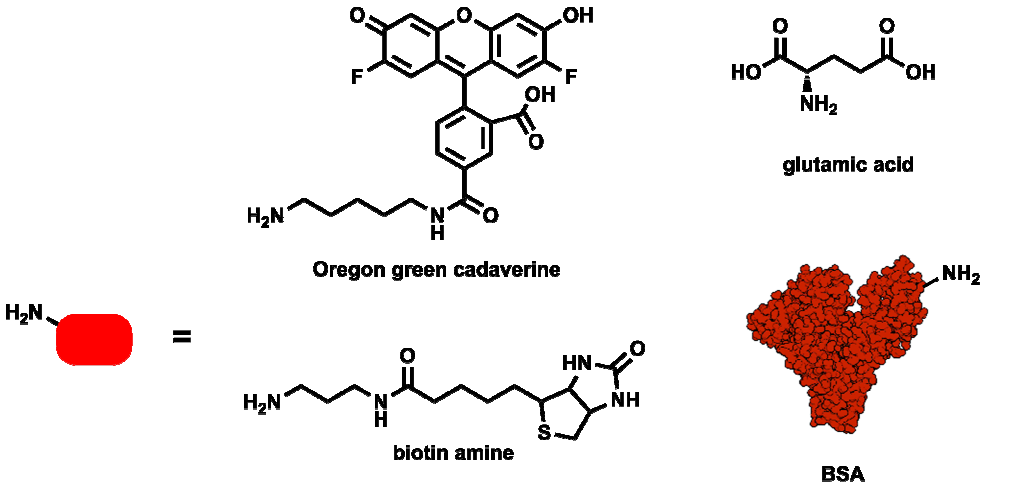
purified via flash chromatography (gradient from 1:3 to 2:3 ethyl acetate : petroleum ether) to afford 2.16 g (78 %) of **S4** as a tan oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, *J* = 9.3 Hz, 2H), 8.12 (d, *J* = 9.1 Hz, 1H), 7.37 (d, *J* = 9.3 Hz, 2H), 7.24 (d, *J* = 2.7 Hz, 1H), 6.95 (dd, *J* = 2.7 Hz, 9.1 Hz, 1H), 6.53 (quart, *J* = 6.3 Hz, 1H), 4.25 (m, 2H), 3.93 (m, 2H), 3.76 (m, 2H), 3.69 (m, 4H), 3.39 (m, 2H), 1.76 (d, *J* = 6.2 Hz, 3H).

General bioconjugate protocol (Scheme 2):

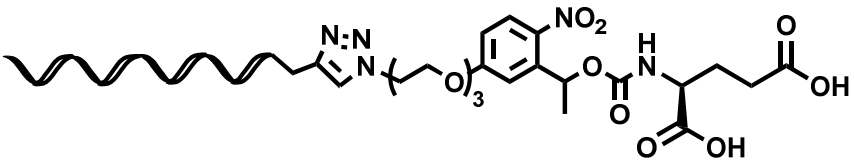
Bioactive, amino-group containing compounds were first reacted in slight excess with Cross-linker 1 in organic solvents such as methylene chloride or dimethylformamide and trimethylamine. In cases where the starting material was insoluble, a dimethylsulfoxide/aqueous buffer mixture was used.

Carbonate intermediates were subsequently reacted with an alkyne functionalized oligonucleotide via the copper catalyzed azide alkyne cycloaddition (CuAAC) reaction using published procedures.[3] In brief, equal volumes of alkyne functionalized oligonucleotide (410 uM in PBS) and activated carbonate (1 mM in DMSO) were mixed. A solution of copper sulfate (10 equivalents, 20 mM in water) and tris(3-hydroxypropyltriazoylmethyl)amine (THPTA) (50 equivalents, 50 mM in water) were separately mixed together and added to the reaction mixture. Lastly, a solution of sodium ascorbate (120 equivalents, 100 mM) was added and the reaction was stirred overnight. The reaction was subsequently purified via HPLC (TSKgel OligoDNA RP column, Tosoh Bioscience) using a gradient from 1:19 to 3:2 acetonitrile : 100 mM ammonium acetate over 30 minutes.

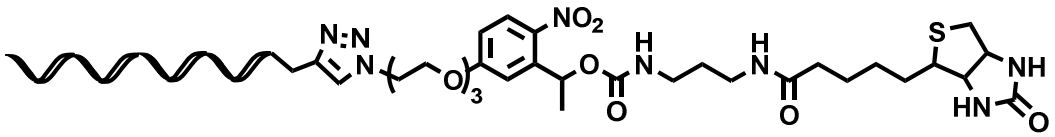

1
Cu(I)


Oregon green cadaverine
glutamic acid
=
biotin amine
BSA

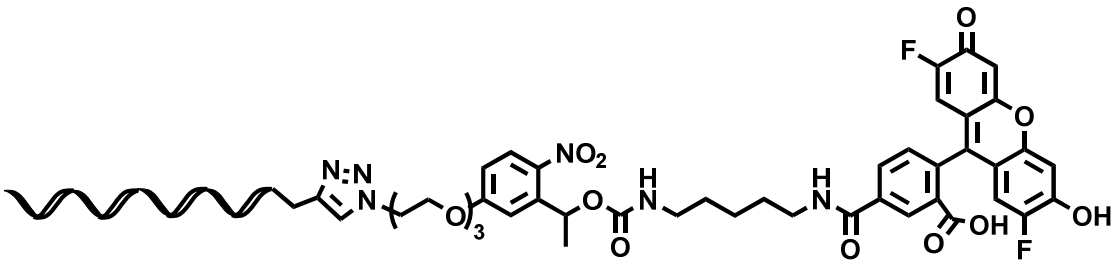
Scheme S2



Glutamic Acid Conjugate. To a solution of cross-linker **1** (322 mg, 637 μmol) and L-glutamic acid di-tert-butyl ester hydrochloride (217 mg, 734 μmol) in CH_2Cl_2 (6 mL) was added triethylamine (570 μL, 4.09 mmol). After stirring for 48 hours, CH_2Cl_2 (25 mL) was added and the solution was washed with $NaHCO_3$ (2 x 15 mL). The combined aqueous layers were washed with CH_2Cl_2 (2 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, concentrated in vacuo, and purified via flash chromatography (1:2 ethyl acetate : petroleum ether) to afford 383 mg of a yellow oil. To a solution of this intermediate in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (700 μL, 9.15 mmol). The solution was concentrated in vacuo and purified via flash chromatography (5% MeOH in CH_2Cl_2) to afford 152 mg (47% over 2 steps) of product: ^{1}H NMR (500 MHz, DMSO-d6) δ 8.1 (dd, J = 2.0 Hz, J = 9.0 Hz, 1H), 7.8 (d, J = 8 Hz, 1H), 7.2 (dd, J = 2.8 Hz, J = 17.3 Hz, 1H), 7.1 (m, 1H), 6.1 (m, 1H), 4.3 (m, 2H), 3.9 (m, 1H), 3.8 (quart, J = 3.2 Hz, 2H), 3.6 (m, 4H), 3.4 (m, 2H), 2.3-2.2 (m, 2H), 2.0-1.7 (m, 2H), 1.5 (t, J = 6.8 Hz, 2H), 1.4 (d, J = 8.5 Hz, 3H). ESI-LRMS m/z 512.1 (M-) Product molecular weight = 513.46. Azido intermediate was reacted with the oligo-alkyne using the general bioconjugate protocol and purified via HPLC to afford a solution of the product.



Biotin bioconjugate. To a solution of cross-linker **1** (103 mg, 204 μmol) and biotin-amine (100 mg, 234 μmol) in CH_2Cl_2 (1 mL) and DMF (1 mL) was added triethylamine (150 μL, 1.08 mmol). After stirring for 2 hours the solvent was removed with a stream of air. CH_2Cl_2 (25 mL) was added and the solution was washed with $NaHCO_3$ (15 mL). The aqueous layer was washed with CH_2Cl_2 (10 mL). The combined organic layers were dried over Na_2SO_4, filtered, concentrated in vacuo, and purified via flash chromatography (gradient 2% to 10% MeOH in CH_2Cl_2) to afford 109 mg (80%) of a solid: ^{1}H NMR (500 MHz, DMSO-d6) δ 8.1 (d, J = 9.0 Hz, 1H), 7.7 (t, J = 5.8 Hz, 1H), 7.4 (t, J = 5.8 Hz, 1H), 7.1 (m, 2H), 6.4 (s, 1H), 6.3 (s, 1H), 6.1 (quart, J = 6.5 Hz, 1H), 4.2 (m, 3H), 4.21 (m, 1H), 3.8 (t, J = 4.5 Hz, 2H), 3.6 (m, 4H), 3.4 (t, J = 5.0 Hz, 2H), 3.1 (m, 2H), 3.0 (m, 2H), 3.0-2.8 (m, 2H), 2.6 (d, J = 12.5 Hz, 1H), 2.0 (t, J = 7.5 Hz, 2H), 1.5 (m, 5H), 1.6-1.2 (m, 8H). Azido intermediate (128 μL, 2 mM in DMSO, 256 nmol) was reacted with the oligo-alkyne (120 μL, 410 μM in 1x PBS, 49.2 nmol) using the general bioconjugate protocol. The product was isolated by ethanol precipitation and purified via HPLC to afford 11.3 nmol as a 100 μL, 113 μM solution of the product.



Oregon Green Conjugate. To a solution of cross-linker **1** (1.25 mg, 2.52 μmol) and Oregon Green cadaverine (1.21 mg, 2.39 μmol) in DMF (300 μL) and water (20 μL) was added triethylamine (20 μL, C). After stirring overnight the solution was purified via HPLC to afford 2.39 mg an orange solid: ESI-LRMS m/z 863.2 (M+) Product molecular weight = 862.80. Azido intermediate (150 μL, 1 mM in DMSO, 150 nmol) was reacted with the oligo-alkyne (150 μL, 410 μM in 1x PBS, 61.5 nmol) using the general bioconjugate protocol and purified via HPLC to afford 38 nmol as a 200 μL, 190 μM solution (62%) of the product.

BSA Conjugate. A solution of BSA (200 μL, 500 μM in 1x PBS, 100 nmol) and cross-linker **1** (20 μL, 5 mM in DMSO, 1000 nmol) in 80 μL DMSO was mixed overnight. The reaction was centrifuged at 17000 rcf for 5 minutes to pellet insoluble materials. The supernatant was dialyzed in 1x PBS against a 25 kDa cutoff to afford 300 μL (333 μM) of product. Azido intermediate (50 μL, 333 μM in PBS, 16.7 nmol) was reacted with the oligo-alkyne (200 μL, 410 μM in 1x PBS, 82 nmol) using the general bioconjugate protocol and purified using Amicon spin filters (3 spins with 30 kDa cutoff tube and 3 spins with 50 kDa cutoff tube) against buffer (5 mM Tris, 1 mM EDTA, and 16 mM $MgCl_2$) to produce 50 uL of product solution.

Design and assembly of DNA nanostructures. Nanostructures were designed using caDNAno.[4] Single stranded M13mp18 bacteriophage DNA was prepared as described previously.[5] All oligonucleotides were purchased from Integrated DNA Technologies (IDT) and used with no additional purification. Creation of nanostructures was performed by first heating a solution containing a final concentration of 40 nM m13 scaffold DNA and 200 nM of each staple in a folding buffer containing 5 mM Tris, 1 mM EDTA, and 20 mM $MgCl_2$ to 80°C, followed by cooling from 80°C to 60°C over 80 minutes, and then from 60°C to 24°C over 48 hours. Removal of excess staple strands was accomplished by three rounds of precipitation with polyethylene glycol solutions.[6] Pellets were re-dissolved in 5 mM Tris, 1 mM EDTA, and 16 mM $MgCl_2$.

Cavity functionalization. Nanostructures were mixed with 70 equivalents of oligo bioconjugates (5 equivalents per handle, with 14 handles in the cavity interior) and incubated overnight at 40°C and subsequently purified by at least two rounds of PEG precipitation[6].

Gel electrophoresis. Reaction solutions were electrophoresed on 1.5% agarose gels containing 0.5x TBE, supplemented with 10 mM $MgCl_2$. DNA dye SybrSafe was mixed with gel solutions before loading onto the gel. The gel box was submerged in an ice water bath to prevent excessive heating.

TEM sample preparation and imaging. TEM samples were prepared by placing 3 μL of sample solution onto a carbon coated grid (FCF400-Cu, Electron Microscopy Sciences)

which was previously charged using a plasma etcher (30 seconds of irradiation). After 2 minutes, the solution was wicked away from the grid with filter paper (Whatman 50 hardened). The grid was immediately treated with stain for 30 seconds and excess solution was wicked away. The remaining solution on the grid was evaporated at room temperate prior to imaging. TEM images were acquired with an FEI Tecnai Spirit Transmission Electron Microscope operated at 80 kV. Saturated uranyl formate (in ddH_2O prepared freshly before usage) was used for protein caging experiments and 2% uranyl acetate (diluted with ddH_2O from 4%, Electron Microscopy Sciences) was used for all other samples.

Kinetics of o-NB cleavage. Samples of the Oregon Green cadaverine oligonucleotide bioconjugate were irradiated with a handheld UV lamp (UVM-57, 6W, 302 nm) for varying lengths of time and analyzed using HPLC (TSKgel OligoDNA RP column, Tosoh Bioscience) using a gradient from 1:19 to 3:2 acetonitrile : 100 mM ammonium acetate over 30 minutes. Irradiation durations used were 5, 10, 15, 20, 25, 30, 35, 40, and 60 seconds. A UV detector monitoring at 490 nm was used to collect traces containing Oregon Green. The degree of o-NB cleavage was obtained by comparing the areas under the peaks corresponding to the Oregon Green-oligo conjugate starting material with the released Oregon Green cadaverine (Figure S1).

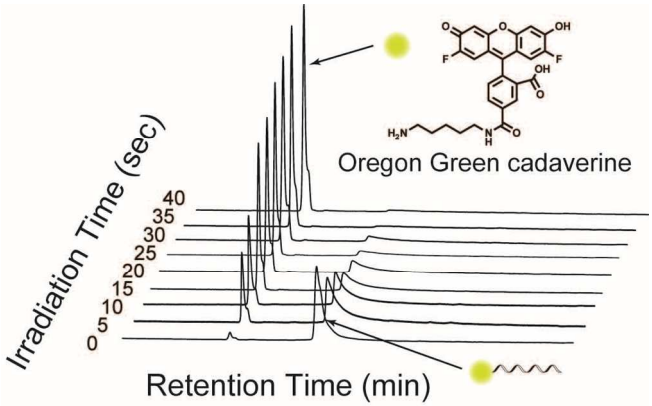


Figure S1. HPLC traces of Oregon Green cadaverine photolysis experiment. Increased irradiation duration results in an increase in the cleavage of Oregon Green/oligonucleotides conjugate. A wavelength of 490 nm was used to monitor traces.

2 dye labeling experiment. The general cavity functionalization protocol was followed but with two different oligos. 5 oligos per binding site were used. The cavity contained 14 binding sites for the activated Oregon green oligo, whereas 1 binding site on the unfolded loop was available for the Alexa Fluor 647 oligo. Reactions were incubated overnight at 40°C and subsequently purified by at least two rounds of PEG precipitation[6]. The final solution was analyzed using the UV setting of a Nanodrop 2000. The ratio of the dyes was obtained by comparing the concentrations of each dye in solution as calculated with Beer's law.

Oregon Green cadaverine uncaging. 25 µL 2 dye labeled nanostructures was irradiated with a handheld UV lamp (UVM-57, 6W, 302 nm) for 60 seconds. The solution was placed in half of a microdialysis chamber and dialyzed against 2 L of buffer (5 mM Tris, 1 mM EDTA, and 16 mM $MgCl_2$) overnight. The resulting solution was analyzed using the UV setting of a Nanodrop 2000.

Protein uncaging. Protein containing nanocages were created following the general cavity functionalization protocol. 5 equivalents per oligo handle were used. For BSA encapsulation, the BSA/oligo bioconjugate was used. For streptavidin, the nanocage was first modified with the biotin-amine/oligo bioconjugate and subsequently with streptavidin (5 equivalents per oligo handle). Each round of modification was purified using two rounds of PEG precipitation[6]. Uncaging experiments were performed by irradiating a PCR tube containing 5 uL of a 0.5 nM solution of protein-containing nanocage for 60 seconds with a handheld UV lamp (UVM-57, 6W, 302 nm). Samples were heated at 40°C for 30 seconds and then imaged by TEM. The extent of uncaging was analyzed using particle counting of the TEM images. The entirety of each TEM image was analyzed to avoid bias.

Glutamate uncaging. Glutamate containing nanocages were created following the general cavity functionalization protocol. 5 equivalents of activated glutamate/ oligo handle were used. Reactions were incubated overnight at 40°C and subsequently purified by three rounds of PEG precipitation[6]. For cell testing, the structures were PEG precipitated and dissolved in a modified Tyrode buffer (25 mM HEPES, 119 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM $MgCl_2$, pH 7.4), at a final concentration of 180 nM. 9 days old primary rat hippocampal neurons were prepared on 12 mm diameter glass coverslides. The calcium dye Fluo-4 AM (life technologies) was dissolved in DMSO to yield a stock concentration of 2.3 mM. Neurons were loaded for 30 min in Fluo-4 AM at 2.3 uM, diluted in the modified Tyrode buffer at room temperature. Neurons were then

rinsed three times with Tyrode buffer, and incubated at 37C for another 30 min to allow complete de-esterification of intracellular AM esters. Glass coverslides were fractured into smaller pieces (approximately 1 mm^2) with a pointed tungsten-carbide glass cutter to limit the use of nanocage reagents. The buffer was wicked off the surface of the fractured glass and replaced with 2 uL glutamate containing nanostructures in Tyrode. Neurons were then placed under a custom microscope with a 10x objective, equipped with a 5W LED (LZ1-00B200, 460 nm; LedEngin, San Jose CA) for excitation, an excitation filter (HQ 470/50), a dichroic mirror (FF506-Di02), an emission filter (FF01-536/40), and imaged with a Hamamatsu camera (C11440-42U) at 20 Hzn After baseline activity was collected for 5 s, the flash lamp (JML-C2, Rapp OptoElectronic GmbH, Hamburg, Germany) was triggered to deliver a light pulse for1 ms at 240 – 400 nm, and the calcium activities of neurons were measured for another 25 s.

Calcium signal processing. All analysis was conducted with MATLAB (MathWorks, Massachusetts, US). Individual neurons were manually identified, and the mean fluorescence intensity averaged for all pixels within each neuron was then further processed to represent individual neuron calcium changes. Due to the saturation effects of the high intensity uncaging flash light that lasted for 6 frames (300 ms) following the light illumination, the fluorescence intensities of these 6 frames were removed and replaced by a linear fit connecting the end values that were not affected by the flash. The fluorescence of each neuron was first baseline subtracted using its linear fit for the 5 second baseline period, and then normalized by the standard deviation of the baseline and smoothed using a built-in function, Smooth, with a moving average filter with the span of

25 frames. The temporal derivative of the signal was calculated and smoothed using the moving average filter with the span of 6 frames. To screen for activated cells, we first calculated the root mean squared error (RMSE) for each 5 second intervals throughout the 30 second recording sessions, and thus 6 RMSE values were calculated. We then used the maximum RMSE of these 6 values to represent the RMSE of each neuron, and obtained the 95% confidence interval of the RMSE for the control group. We then calculated the threshold value for the instantaneous temporal derivative that would correspond to the 95% confidence interval of the RMSE. Cells were deemed as activated when their temporal derivative exceeds the threshold. To determine the onset of calcium responses, we calculated the z score of the fluorescence trace of the activated cells. Onset threshold were set as the first time point of 10 consecutive points in which the temporal derivative values had a z score bigger than 3.

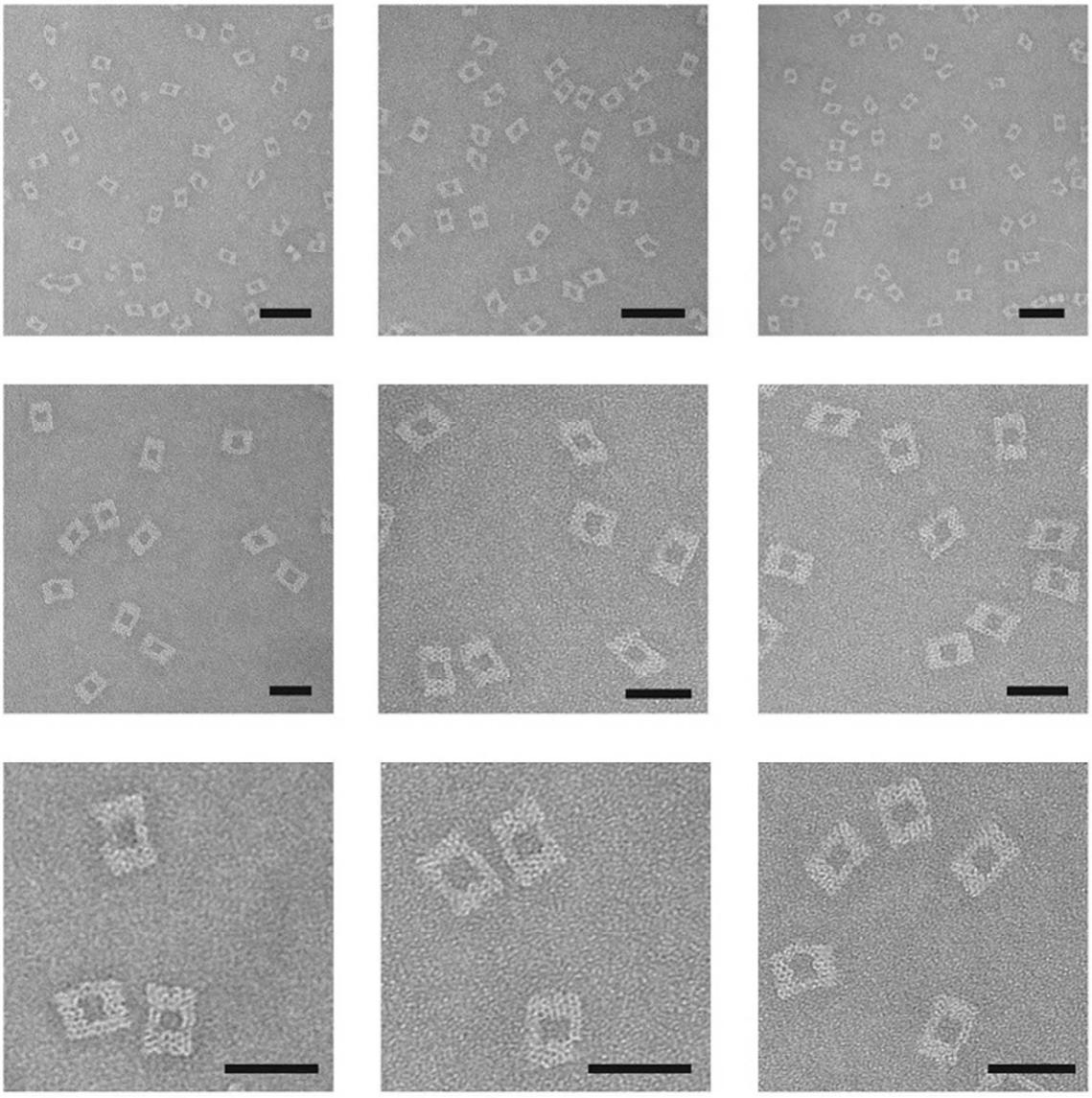


Figure S2. TEM images of unmodified DNA nanocage. Scale bars equal 100 nm (top three images) and 50 nm (remaining six images).

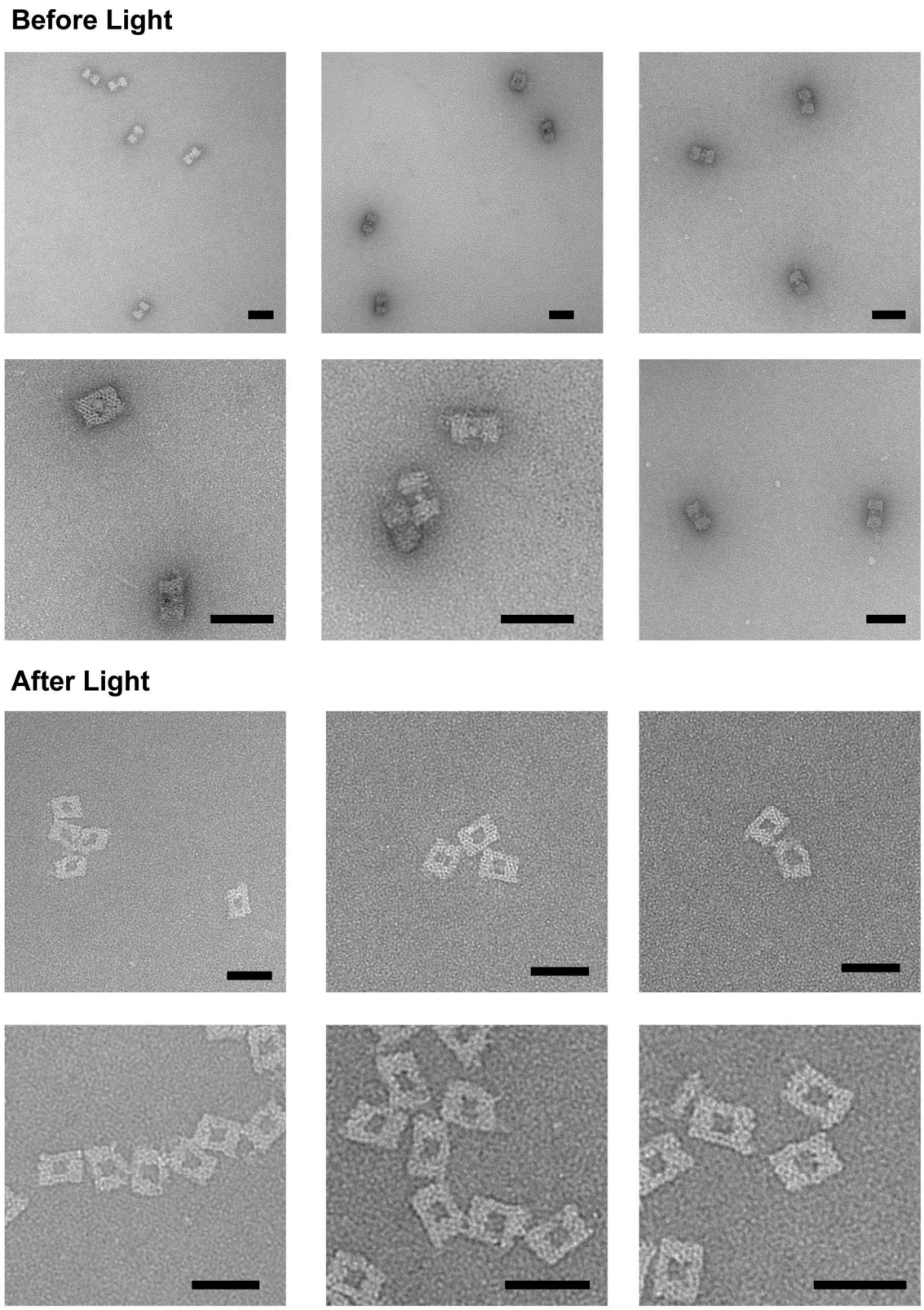


Figure S3. TEM images of streptavidin containing DNA nanocages before (top) and after (bottom) light irradiation. Scale bars equal 50 nm.

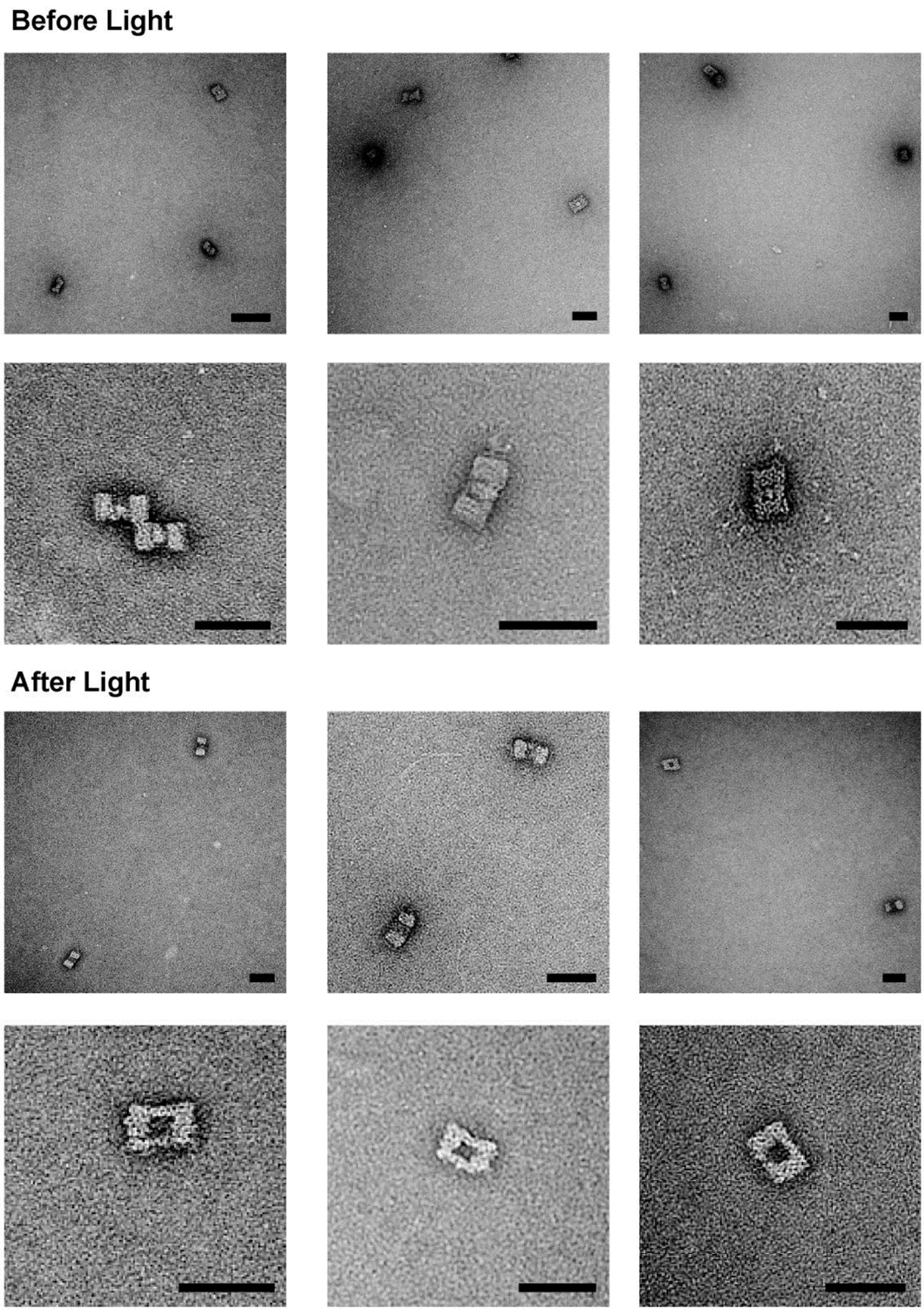


Figure S4. TEM images of bovine serum albumin containing DNA nanocages before (top) and after (bottom) light irradiation. Scale bars equal 50 nm.

Video S1: Neuronal responses evoked by released glutamatic acid from DNA nanocages. A representative video showing intracellular calcium changes in neurons loaded with calcium indicator Fluo 4, before and after flash lamp illumination in the presence of DNA nanocages containing glutamate. Video is speed up 5 times.

Video S2: Control experiments showing lack of neuronal responses upon light illumination in the absence of DNA nanocages. A representative video showing intracellular calcium changes in neurons loaded with calcium indicator Fluo 4, before and after flash lamp illumination, without DNA nanocages. Video is speed up 5 times.

Staple List:

Center staples – those which do not come in contact with the edges of the nanostructure

GGATTAGCAATATAAAAAGCG

AATCGTCATAAATATTCAGAATTTG

CAATAGAAAGGGCGACATTAACTGT

CGAAAGAAGGCTTTGAGGAGCACAG

GCCCGAAATTGCATTGGAAGTGCGA

TACCTTTTTACATTACAAACATACC

ATTATCAGGAATTATCATCGTTGCCTTA

CAGACGAGCATTGAAGAACCAATGAAAC

GAAAAAGAAATCCAATCGCAGCCAGGTT

GCATGATCAAGAAAATTGAGTAAAATAG

GCTATAATGCAGTACGGATTTGGGCAAT

GGGTTATACCTACCATATCAGAAGTTTG

TCCGCGACATCGCCACCTTATAGGACGT

TTTGTCAGGCAACAACGTAGAGCAACTG

ATACGTTTTAGCGAACCGAACGCCTACGCAT

TTTCCTTGAAAACAGTCAATAGTGAAGAGTGTAAC

TAAATCATCCAGTTTGGAAGCGCCAGGGAGCTGATTAT

CCCTCAAGACGGAATAGGTGTAAAAGAAGGCACCAGTAA

GATTATACATTAAAAATACAACGAACCGTCTATCAATCA

AAAAGCGGTTACCAGAAGGAAAGCAGATACCGAAGTTATCCC

AAGTTTACCAGACGCAAAAGAAGTTTGTGCAGACGGTCGAAA

AATTGGGCTTAGAAACATCAGTGAAAATCAAGACAAGACAAT

ACAAATGAATAACAGCTGCTTGCTACCAGTCGCGATTTCTTT

ACTAAGGAATAACTAATGTTGAGAAATATATATACATTAATA

ATGAAAATAAGGTAACCCACAAGACAATGAAATAGCTGAATT

CATCGTATAGCACCATTACCAAGCCAGCCCGACTTAATACCC

CCAGCTACAAGTCTTTCCATAATGGGATAGGTGCATCTGCAC

CTGTTTACTCAACAGTAGGGCAACAGTATAAAGCCGAAAACT

GATGCCAGAGGGGGAATACTGCGGAAGCACGGTGTATCATAA

GCAAATCACATCATTACCGCGCAATAGATAAGTCCCACGCGC

GCGCGAAATTTGACCCCCAGCAAAAAGGCTCCAAAAGGTTGA

GGCTTAAAATTTATCAAAATCATAATCCTGATTCCAGATATT

TGGGATAAAACACTCATCTCATGATACCGATAGCATAATTTT

TTTAATCATAACCGACCGGTAAAGGGCATTTAAACCAAATCA

TTTTCAAACAAGACAAAGAACAAAACAGAAATAAAAGAAAAT

ATACGAGCCGGGAGTGAGAGGTGAGCACGCTGGAAATTGTACA

AATTCATGAGTACAAACGCCTGGCGGGCAACTGGTTTTGCGGTTT

ATATCAATCAAAAAACATTCGCGTCCAATAGAGCTTTCATAGCAA

CCACCACCACCGGAATCCAAAAAGGGTCTTTACCCTGATCCATAA

GAATACACTAACGCCAAATCATAACCCTCTTTGATAAAATACCAA

TATTTGCAGAAGATAAAACAGCTCGAACGAACCACTTGCATGCCC

TTCTGCCGCCTCCCTCAGCCACCACCCTCATTCAAAGCAGAGGAA

AAATCTCGCGTAACGATCTAAAGACAGCTGAGTTTCGTCACCCTAA

ACCGTCAAAAATCACCAAGCAATAAAGCAAACATTTAGCTATGCTG

CGCCACCCAGGAGGTTGCTCCTTTTGATAATTGCTCATCCAAATTC

CTATCTTAGCCGAACATGAGAGTCTGGAAAACTAGCAACCCGATCA

TGTTTCCAACCTGTCTCACATAATATCACCAGCAGTTGAATATACC

AATAAATACAAACAACCGATTGAGATTAAAGGTGAAGATAATAGTTATT

ACAGTTTGAGGCACTCCAGCCTCCCGACTTGTTGCTATTTTGCCATTAA

AGAGAGCCGCTTGCCTTTAGCGTAAGATAGCAGCACCGATTATTCGGAG

CCGACAATATTCGGTATTAAAATCGGCAAAATCCCCCCAGCATCAGCAG

GTAATATATTTGGTTTGTTAATTGATTTAGGTGAACAATGTAGAAAGAT

TTGGTGAGAAGCTACAGCAGCATCCCACGCTGGTTTGCCTTCACCAATT

CAAGAAAAATCTACTACAGGTTTGCTTCTTAAAAGTTTGACACAACTCGTCCT
AAA

Right edge staples – those which come in contact with the right edge (when visualized in cadnano) of the nanostructure; helix ends contain TT overhangs to prevent nanostructure aggregation

TTGACTACCTTTTTAACCTCC

TTATGAACGGTCCCGGTTGATT

TTTCATTACCCATAAGGCTTTT

TTAACCACCAGAGCCCGAGATT

TTGGGCGCATCCGACAGTATTT

TTCCACGCATAAGTTAAAGGTT

TTGCTCATTATTTCGAGGTGTT

TTTTAAATATGCATATAACATT

TTCGGCCTCAGGCTTCTGGTTT

TTTAATCAGAAATATTTAAATT

TTAATTTCTTATTTCTGTATTT

TTTAATTTCAATAAGAACTGTT

TTAAATCGGTTTGCGGGAGATT

TTTAAGACGCTGAACAAAGATT

TTGCCCTGACGGAGATGGTTTT

TTAACGGAACAAACCATCGCTT

TTGTTGATTCCACCGGATATTT

TTAGCCTTTATCATATATTTTT

TTGGATGGCTTCAACATGTTTT

TTACGGCCAGTAGGATCCCCGGGTT

TTGGGATTTTGAGTACAAACTACTT

TTTGTACCAACTCAGAGCATAAAGCTTT

CGAGTAACCGTCACGTTGGTGTAGATTT

AACACATTATGTTAATAAAACGAACTTT

TACAATCGTAGCAAACAAGAGAATCGTT

TTACAACATGTTCAGAGAACAAGCAATT

TTGAAGCAAAGCGGAGACTTCAAATATT

TTTTGTATCGGTTTACAAAGTACAACTT

TTTTTGAAAGAGGACTGGCTGACCTTTT

TTGGACTCCAACGTATCAGATGATGGTT

TTGCCGTTTTTATTTTGTTAAATCAGTT

TTTAGGGTTGAGTGTTGTAAAGAATAAGG

TTTCAACCGTTCTATTTTGAGAGATCTT

TTCTAAAACATCGCCTTCTGAATAATTT

TTTTTCCAGACGTTAGGAGCCTTTAATT

TTGGAGATTTGTATCCTGCTCCATGTTT

TTCATCAAGAGTAACTATTATAGTCATT

TTAAATGCTGATGCCCTGTTTAGTATTT

TTTACTTAGCCGGAGAACTGACCAACTT

TTTTCGCAAATGGTGCGCGAGCTGAATT

TTAAGGTGGCATCATAAATCATACAGTT

TTTCGCGTTTTAATACTCCAACAGGTTT

TTTAGCCAGCTTTCTCGGATTCTCCGTT

TTCATATGCGTTATCGACGACAATAATT

TTCAATTCATCAATATAGGTCTGAGATT

TTGGAAGGGTTAGAATAACTATATGTTT

TTACGTTATTAATTTGTAAATCGTCGTT

TTGGTGGTTCCGAATCCTTTGCCCGATT

TTCTCATTTTTTAACTGGCCTTCCTGTT

TTAAGATTTAGTTTGACCATTAGATACATTT

AGCTTTTGCGGAGAAGATAGCGATAGCTTAGATTT

TTCCGCTTTTGCGGGATCTGCAGGGACCGATATGAC

TTAACGCCTGTAGCATTCCACAGTTTTGTCGTCTT

TTGCCGGAAACCAGGCCACGGCACCGAAGATCGGGA

TTTTGTAAACGTTAATATTAAGCAAAAGCCCCTATG

TTTACCGAGCTCGAATTCGTAGAACTGATAGCCTT

TTTAAATGCAATGCCTGAGAACCCTTTCAACGATAC

TTCAAGGCGATTAAGTGTGCAGGGGGATGTGCTGTT

TTCATTCAGGCTGCGCAACCAAAGCGCCATTCGCTT

TTGTTTTCCCAGTCACGCACTGGGTAACGCCAGGTT

TACCCCTGTACAAGGATTACACCATCAATATGATATTT

TTCGCCAGCTGGCGAAGAAACCTCTTCGCTATTATT

TTGCGATCGGTGCGGGCGTCAACTGTTGGGAAGGTT

TGGCAGGTCGACTCTAGGCCAAGCCAGACGTTGTAAAACGTT

TTTGGGAACAAACGGCGGATTGACCGGACGAGTAACAAATAG

TTTACAAAGGCTATCAGGTCATTGCCAAGAGAGGGATTTATC

TTGCAAGGCAAAGAATTAGCAAAATTAGGCATTAAGAAGAGC

TTCAGGATTAGAGAGTACCTTTAATTGACAGACCGGAAGCAATCGA

TTCACTAACTTTCATGAGGCTGTCACCCGGCGAAAATCCTGTTTGATTT

TCTTTCATTCCAACTAATGTAGCTAGAGCTTAAGAGGTCATTTTTGCTT

CCTGATTCAAAGGGCGAAATGGGCAAGAGTCCACTATTAAAGAACGTTT

TTCTATTAATTAATTTTCCCTTAGAACAAATAACCAGAAAGAGCTTGCG

TGAACACATATCAGAGAGAAATAAAGGTCATAAAGATTCAAAAGGGTGAGA
AAGTT

Left edge staples – those which come in contact with the left edge (when visualized in cadnano) of the nanostructure; helix ends contain TT overhangs to prevent nanostructure aggregation

TTACCACATTCTACGAGGCATT

TTATTAAACGGACCTAAAACTT

TTAGGAGGTTTAGTACCGCCA

TTGTAGCAACGTAGAAAGGATT

TTTTCTAAGAAATAACATAAAAATT

TTGCATTTTCGGTCATAATCAAATT

TTACCAACGCTTTACAAAATAAATT

TTCTAACAACTTGAGGATTTAGATT

TTCAGTGAGACCCTGAGAGAGTTTT

TTAATCAACAGTTGTTAGGAGCATT

TTCAGCCATATTATCCCTTTTTATT

TTCTTAAATCAATTTTTTGTTTATT

TTCCGCCTGCAAAAATCTAAAGCTT

TTAGTACATAAATCTTTAGGAATTT

TTGCAGCAAGCGGTGGAACGAGGTT

TTACAGAATCAAGTCACCCTCAGTT

TTAGACTGGATTCGGAACCTATTTT

TTTTGACGGAATAATCAGTAGCGTT

TTCCGCCGCCATTGGCCTTGATATT

TTTAGTAAGAGATAAGTGCCGTCTT

TTCAATAATAACGGGAGCCATTTTT

TTCAAATGCTTGCCTTGAGTAACTT

TTGAAACGTCACCACCACCAGAGTT

TTAACCGCCACGAAAGCGCAGTCTT

TTTTACGCAGTATGAGGTAAATATT

TTTAGCGAGAGCTCAAGAGAAGGTT

TTGGGTAATTGGAAACGCAAAGATT

TTACAACGCCAAGTAATAAGAGATT

TTCAGGGAAGCGCAAAAGTCAGATT

TTGAAAGAGGCATCACCGTACTCTT

TTCCAGCGCCAGCGTTTTCATCGTT

TTAGTATTAGACTTGAAGTTTCCTT

TTCACCACGGAATATATGGTTTATT

TTTCCTAATTTGTCTTTCCTTATTT

TTGCGCGGGGAGAGTCTTTTCACTT

TTAGAAAAGTAACCGAGGAAACGTT

TTCAAAATTAATTTAATGGAAACTT

TTTAACGTCAGGGAGAAACAATATT

TTATTCATTTCAATTCAAGAAAATT

TTGGGAATTAGTTAGCAAGGCCGTT

TTACGTCAAAAATGTAAGCCCAATT

TTGCCTGGGGTGTTGCGCTCACTTT

TTATCACCGGATTTTGATGATACTT

TTACAACTAAACTCAGAACCGCCTT

TTTCTGACCTAAAATAAGGCGTTTT

TTTAATAAGAGTAAGACTCCTTATT

TTACGGATTCGCCTCAGAGGCGAATTTT

TTGCCCGCTTTCCAGAATCGGCCAACTT

TTATCACCTTGCTGGGTCAGTTGGCATT

GCCTATTAGCGTCCTAATAGTAAAATGTTTTT

GGTCAGTTAAACAGTTCATTGAATCCCCCTTT

GAGACTCGCTTTTGACGATAAAAACCAAAATT

TTTTATCCGCTCACAATTCCACACAATGTCATAGC

TTATTCTGAAACATGAAAGTATTAAGCAACCCCCT

TTTTCACAAACAAATAAATCCTCATTACGGCAGGT

TTAGGAGTGTACTGGTAATAAGTTTTCAATGTCAT

TTATTAGGATTAGCGGGGTTTTGCTCGTGTAGGCT

TTAGTGCCCGTATAAACAGTTAATGCAGATAACGG

TTTGTAACACCCTCATAGTTTCAGGGATAGCAAGCCTT

TTCAATAGGAACCCATGTACCCAGCGGACGAATAACTAC

GATAATATCTAAAGGAACATTAATGTCGGGATGTGTGAAATTGTT

CAGCCCATGAAATAAGAAACGAGATTAGCGGGAGGTTTTGAAGCTT

AATCCAGGCCTAATTTGCCAGAACGAGCTTTTATCCTGAATCTTTT

AATCGCGGATTGCTCAAATGAACAGTGCGCGGTCAGTATTAACATT

TTACCCTCAGAGCCACCACCCTCATTTACAAGAACCGCCACCGGAA

TTTCTGAATTTACCGTTCCAGTAAGCTAGAAAAGCCAGAATGCCTC

TTGAGAGGGTTGATATAAGTATAGCCTTTTAGTACCAGGCGGCAAC

TGAAATATCTAACCTCATAATTGCGCCTAATAAGCATAAAGTGTAAATT

ATAGGAGAATATTTTACAGAGAGACGCGAGGGAAGGCTTATCCGGTATT

CTACCTGAACTTAGACGATCGGCTACGAGCAGAAAAATAATATCCCATT

TTAAATAAGAATAAACACCGGTACATCGATGAATACGTAGATTTTCAGGTTTT

TTATATAAAGTACCGACAAAAGTGTGATAATTTAATTAGTTAATTTCATCTTT

TTCATTCCAAGAACGGGTATTTCGAGCCACATGTAAGAATCGCCATATTTATT

Cavity staples – those which protrude into the cavity of the nanostructure exposing AAAAAAAAAAAAAA handles

AACATTTTTAGTAATGTGTAGGATGAAAAAAAAAAAAAAA

CGAATAGATAGTGAGTGTTTGAATGAAAAAAAAAAAAAAA

CTAATAGAGCCTGATGAATAACAATGAAAAAAAAAAAAAAA

ACATGGCACCAGAGTCTTTTCATAGCCCGAAAAAAAAAAAAAAA

GACTTTTACGTAATTTCATCAGCAGATAGAAAAAAAAAAAAAAA

GGGAACCACGAGGCAGTAAATCATTGTGGAAAAAAAAAAAAAAA

ACTCATCGCTAATGCAGAAGATAATTCTCAAAAAAAAAAAAAAA

GTCCAGAACAAATTCTTACATATTACTACAAAAAAAAAAAAAAA

TAAATTTTTCATCGTAGGACAGTACCGCGAAAAAAAAAAAAAAA

ACGAGTAGCGAACGAGAATGACTTCGTAACAGAAAAAAAAAAAAAAA

GCGTATTCCAGCTGTTGAGGACTCAATCGCAAAAGGTTACAAGAAAAAAAAA
AAAAAA

ATCAAAAACCAGGCGCATAGGCAGATGATGCTCATCCAGAACCAAAAAAAA
AAAAAAA

CCTTATTGCTCAGACTGTAGCAAGACAAAAATTCAAGTTTATGAAAAAAAAA
AAAAAA

TTGCCGGAGACAGTCAAATCAGATTGTATTTGTTAAAATTACGAAAAAAAAA
AAAAAA

Cavity binding oligo

/5Hexynyl/TTTTTTTTTTTTTTT

Loop binding oligo

/5Alex647N/TGAGTAGAAGAACTCAAACTATCGGCCTTGCTGGTAATAT

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