Light Sensitization of DNA Nanostructures via Incorporation of Photo-Cleavable Spacers

Richie E. Kohman & Xue Han

Biomedical Engineering Department, Boston University, Boston, MA, USA.

**Experimental Methods**

**Design and assembly of DNA nanostructures.** Nanostructures were designed using caDNAno.1 The sphere’s northern hemisphere was unaltered from that in reference 2 while its southern hemisphere and equator contained a different set of staples strands. Single stranded M13mp18 bacteriophage DNA was prepared as described previously.3 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 20 nM m13 scaffold DNA and 200 nM of each staple in a folding buffer containing 5 mM Tris, 1 mM EDTA, and 16 mM MgCl2 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.

**Gel electrophoresis.** Reaction solutions were electrophoresed on 1.5 or 1.8% agarose gels containing 0.5x TBE, supplemented with 10 mM MgCl2. DNA dyes ethidium bromide or SybrSafe were mixed with reaction solutions before loading onto the gel. The more sensitive dye Ethidium bromide was used when visualizing faint bands as in Figure S1 lane 1. The gel box was submerged in an ice water bath to prevent excessive heating.

**Purification of DNA nanostructures.** *o*-nb containing product was purified by first running the reaction mixture through a 1.5% agarose gel. The product band containing the correct nanostructures was excised from the gel and centrifuged at 13,000 rcf for 3 minutes at room temperature in a Freeze ‘N Squeeze DNA gel extraction spin column (Bio-Rad). Samples were then concentrated by diluting with folding buffer containing 5 mM Tris, 1 mM EDTA, and 16 mM MgCl2, and then centrifuged in Amicon Ultra 0.5 centrifugal filter devices (Millipore) at 14,000 rcf for 5 minutes at room temperature.

**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). After 2 minutes, the solution was wicked away from the grid with filter paper (Whatman 50 hardened). The grid was immediately treated with 2% uranyl acetate (diluted with ddH2O from 4%, Electron Microscopy Sciences) for 30 seconds and excess solution was wicked away. Finally the grid was washed with ddH2O 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. Images were used directly without any additional manipulation.**

**UV light irradiation.** Approximately 10 µL of a 1 nM solution of purified nanostructures in a closed PCR tube was irradiated with handheld UV lamps from UVP LCC for a given period of time and then directly characterized by gel electrophoresis or TEM. For irradiation with 302 nm light, we used lamp model UVM-57 at 6W. For irradtiation with 365 nm light, we used lamp model UVGL-58 at 6W at the long wavelength setting.

**TEM Particle counting.** For each condition, several hundred structures were counted from TEM images (at a magnification of 18500x). The entirety of each TEM image was analyzed to avoid bias. Particles were only counted if they unambiguously resembled a spherical DNA nanostructure and the location of the unpaired scaffold was used to help determine this. Aggregates of structures and structures that were located at the edge of the image were not counted. Particles were only considered “open” if spherical objects were seen separated by the scaffold spacer. Partially open structures were not counted as “open” therefore a percent opening of 100% is unlikely because many open structures adhere to the TEM grid in an orientation where the hemispheres lay next to each other. An example of the counting process is shown in Figure S8.

**References:**

(1) Douglas, S. M.; Marblestone, A. H.; Teerapittayanon, S.; Vazquez, A.; Church, G. M.; Shih, W. M. *Nucleic Acids Research* 2009, *37*, 5001.

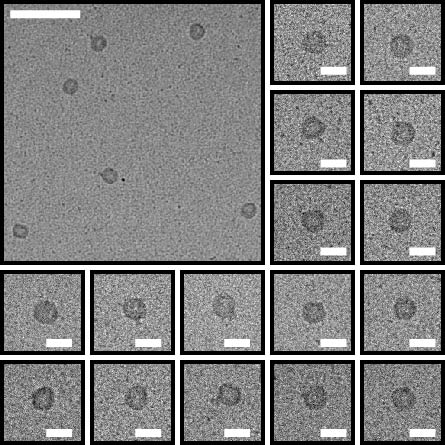
(2) Han, D.; Pal, S.; Nangreave, J.; Deng, Z.; Liu, Y.; Yan, H. Science 2011, *332*, 342.

(3) Sambrook, J. Molecular Cloning : A Laboratory Manual; 3rd ed. ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y. :, 2001.

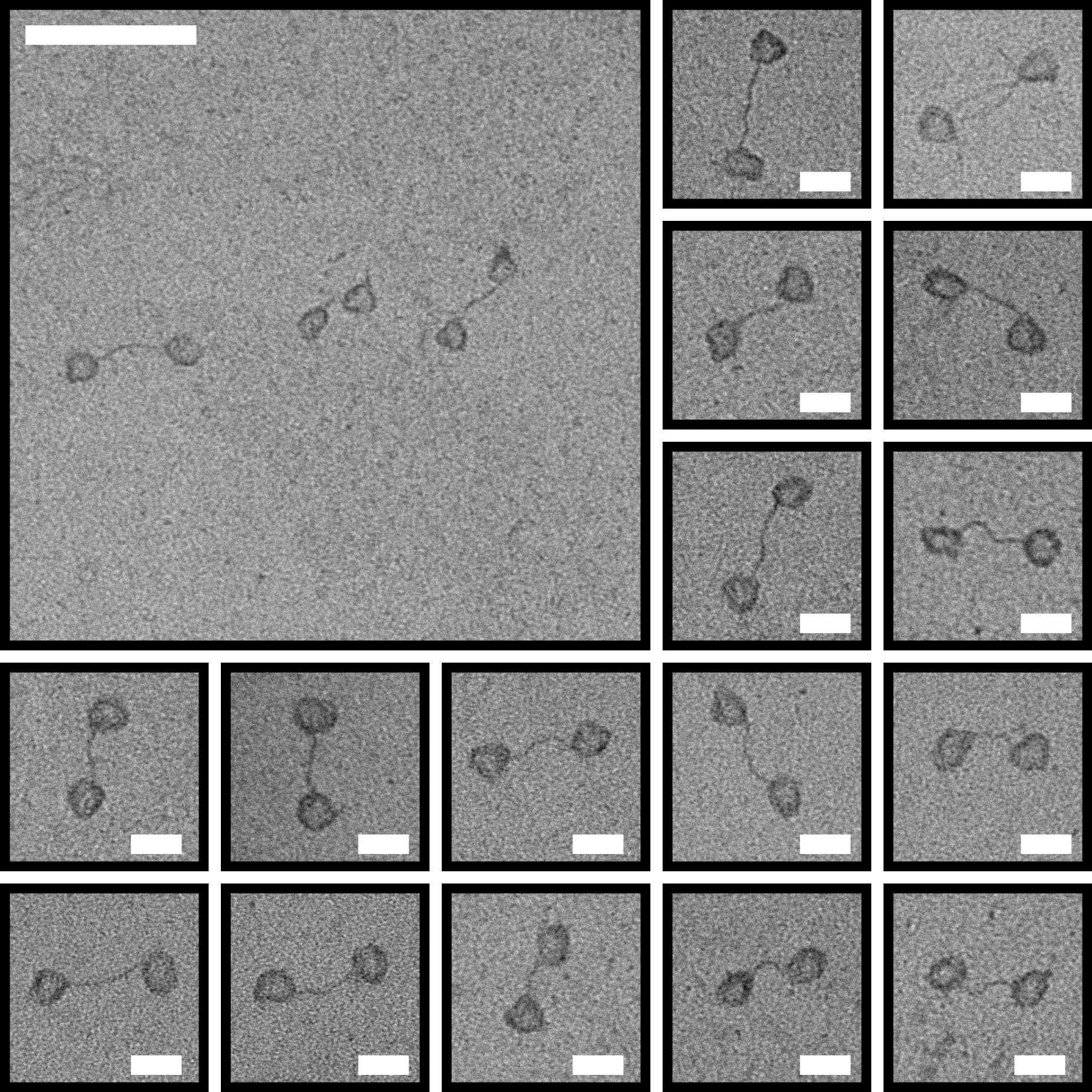
**Supplemental Figures**



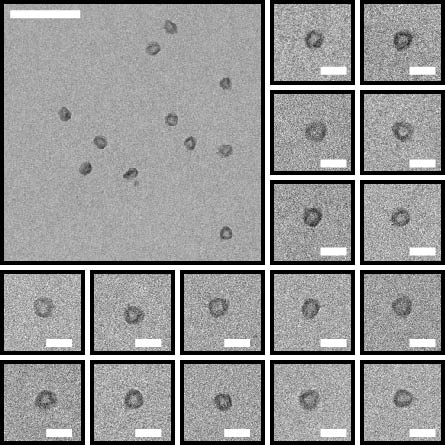
**Figure S1.** Fluorescent image of a 1.5% agarose gel containing crude DNA folding mixtures stained with Ethidium bromide. From left to right, L = 1 kb ladder, m13 = m13 DNA scaffold, 1 = Closed sphere containing all 9 equator crossovers, 2 = Sphere with no equator crossovers, 3 = Sphere with 3 equator crossovers, 4 = Sphere with 3 photo-crossovers. Loop staple strands included for all samples. The bright bands at the bottom of the gel are the excess staple strands. The desired product lies in the fasted migrating band (not including the staple band).



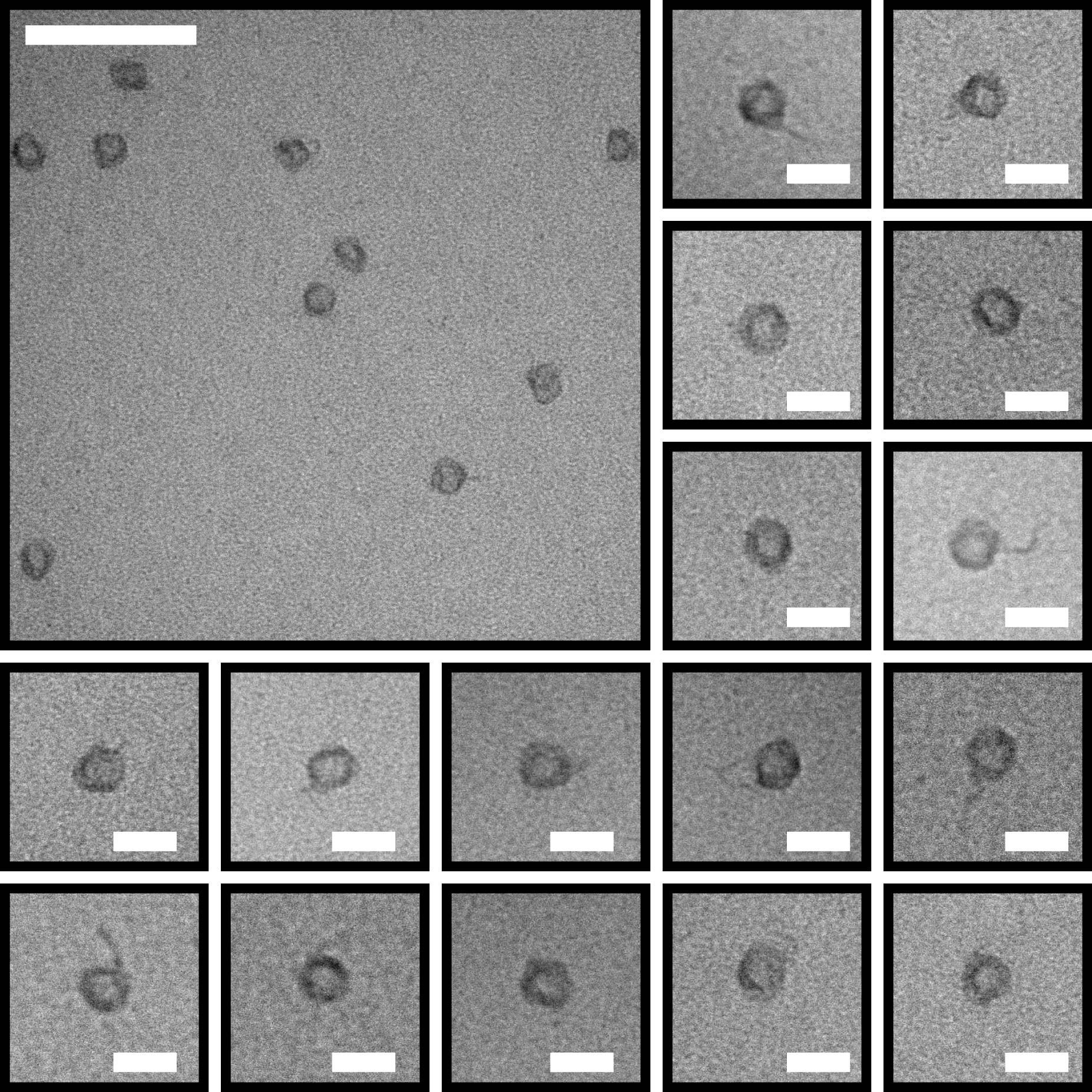
**Figure S2.** TEM images of closed spheres. Top left scale bar = 200 nm. All other scale bars = 50 nm.



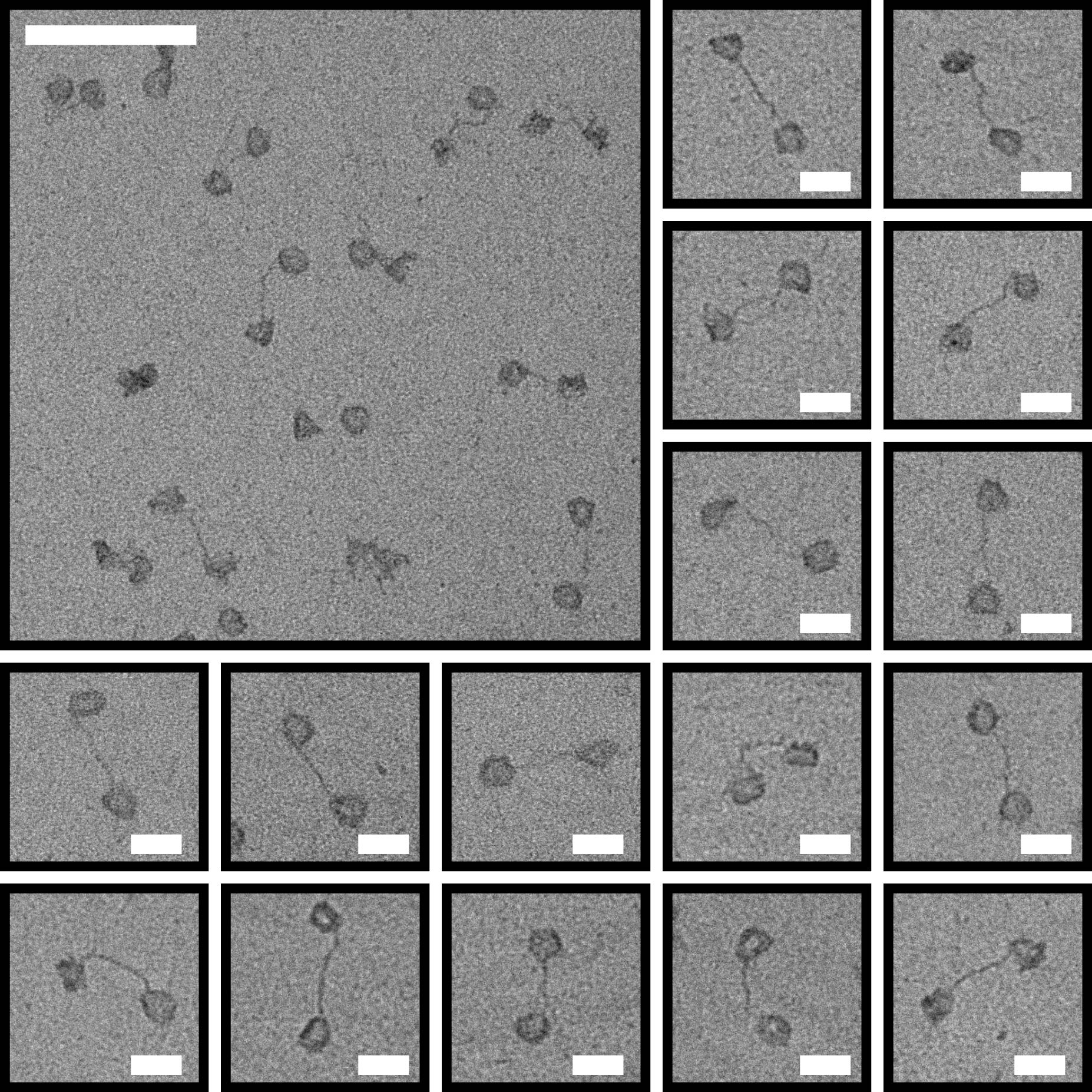
**Figure S3.** TEM images of structures created without the equator crossovers. Top left scale bar = 200 nm. All other scale bars = 50 nm.



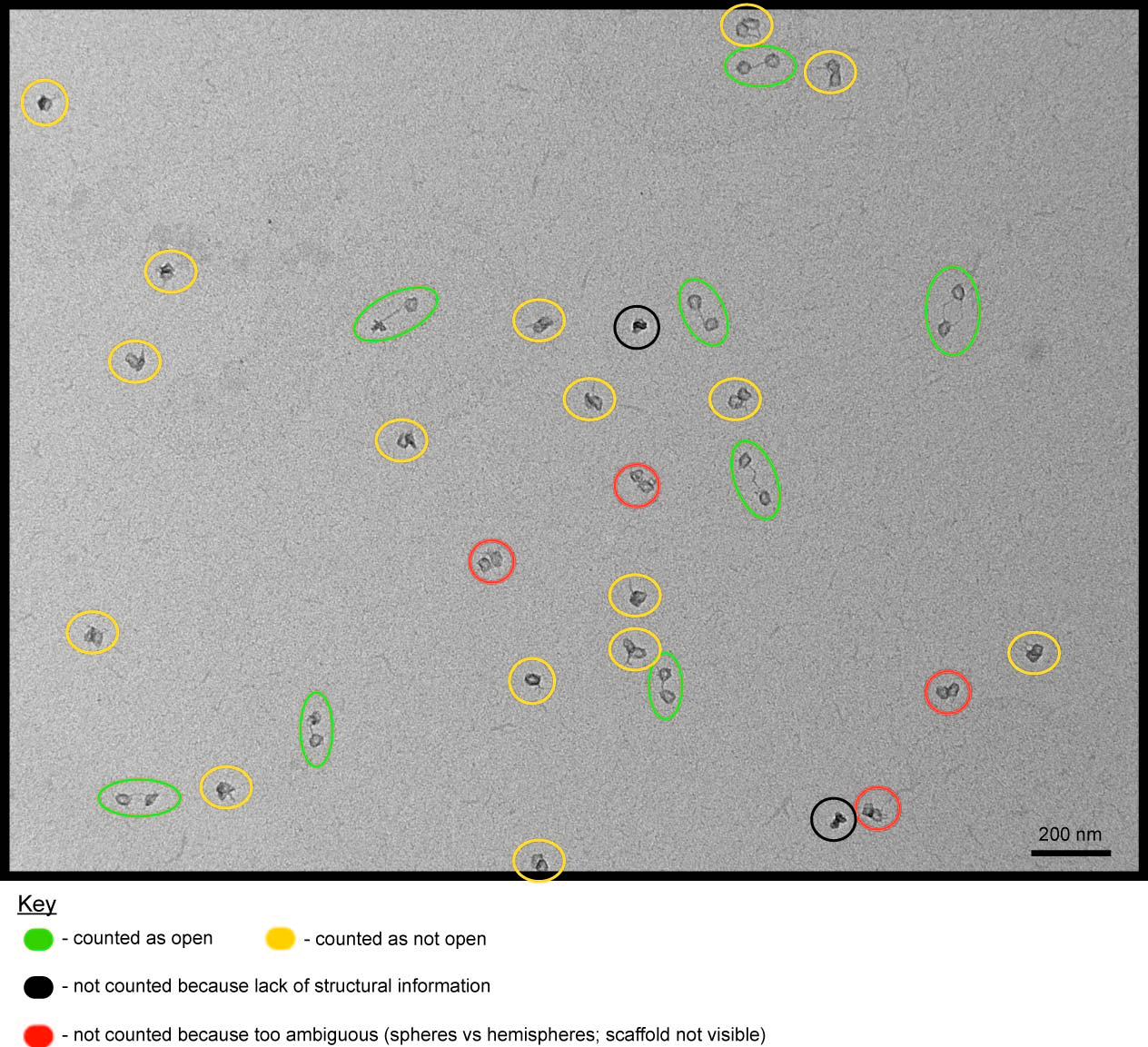
**Figure S4.** TEM images of closed spheres created with 3 equator crossovers. Top left scale bar = 200 nm. All other scale bars = 50 nm.



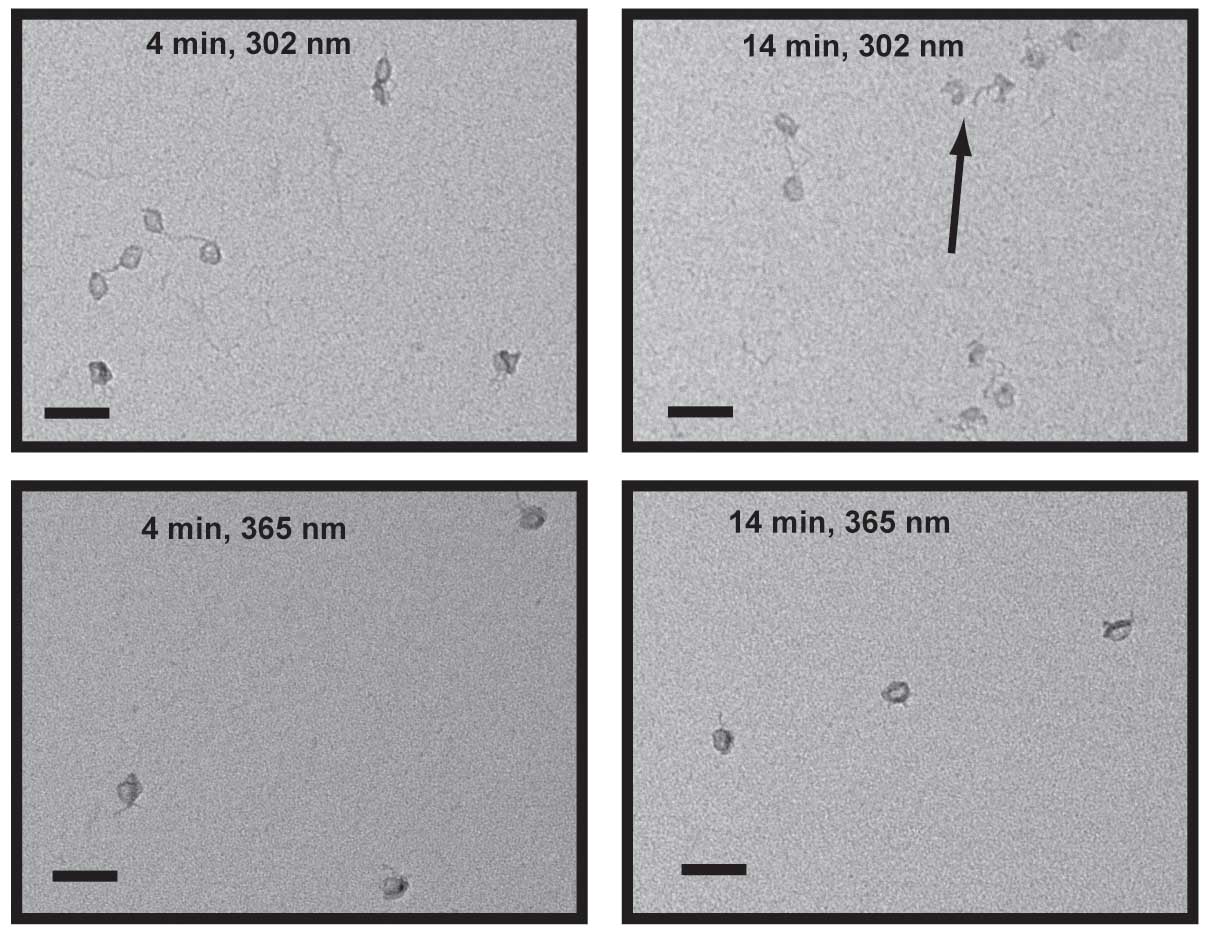
**Figure S5.** TEM images of closed spheres created with *o-NB* photo-crossovers before light illumination. Top left scale bar = 200 nm. All other scale bars = 50 nm.



**Figure S6.** TEM images of well separated hemispheres created by light irradiation (10 minutes, 302 nm) of closed spheres containing *0-NB* photo-crossovers. Top left scale bar = 200 nm. All other scale bars = 50 nm.

****

**Figure S7.** Illustration of the open sphere quantification process. Image of light sensitized spheres irradiated for 4 minutes with 302 nm light. Percent of opened structures is determined by number of open structures (green) compared to the total number of identified structures (green + orange). Particles are not counted if they lack convincing structural information (black) or if their orientation relative to other structures makes identification ambiguous (red).

**Figure S8.** Photo damage accumulation over time. TEM images of light-sensitized spheres irradiated at 4 or 14 minutes with 302 or 365 nm light. 302 nm light irradiation effectively opened spheres over time (top left, top right) however photo damage became apparent at long irradiation durations (top right, arrows). Photo damage does not appear when 365 nm light is used however this wavelength is ineffective at opening spheres (bottom left, bottom right). Scale bar =100 nm.

**Staple oligonucleotide list**

Designs were obtained using caDNAno (high resolution images and .json files separately attached). Staple oligonucleotide lists were obtained directly from caDNAno software.

Northern Hemisphere (70 staples):

GTGAATTACCTTAATGTTTAGA

ACCGCCACCCTCCTATTATTCTGAAA

CGTACTCAGGAGGGATTAGCGGGGTTT

CTAAAACACTCATCCGCTTTTGCGGGA

AGCCAGAATGGAACGGATAAGTGCCGT

AACGAAAGAGGCACCATGTTACTTAGCC

AGATGAACGGTGTACAGAATGCCCCCTGC

CGAGAGGGTAACAAAGTACAACGGAGATT

TTAATTTCAACACAAATAAATCCTCATTAA

ATCAAGAGTAATCTTGTGAGATTTAGGAATA

CAAGCCCAATTTTTCTGTATGTGAATTTCTT

TCAGTGAATAAGGCTTGGAAGAAAAATCTAC

ATTGCGTGCTTTCGAGGGGATTTTGCTAAGGA

GTCAGTGCCTTGAGTAGAACCGCCTCCCTCAG

GTTTCCATTAAACGGGCAACTTTGAAAGAGGAC

TTCCAGTAAGCGTCATCAGAACCACCACCAGAGC

CTAAAGTAAACTACAACGCCTGATAGGTGTATCAC

ACAACAACCAGCGAAAGACAGCAGCACCAACCTAA

AAATGAAAGGAACCCATGTACCCGCCACCCTCAGA

AAACAGTTACCAGGCGCATCATAATCAAAATCACCG

TGCTCAGTACCAGGAGCGCAGAGGTTGAGGCAGGTC

TCGTCACCCTCAGCATCGCCCACGCATATCCAAAAG

CATGAAAGTATTAAACTGGTGCCACCCTCAGAGCCA

GGAACGAGGCGCAGTCAACGGAACAACATTATTACAG

CCACATTCAATGCCATCTTTTAGGCTGGCTGACCTTC

TGTATCATCGCCTGAGTAAATTTAAGAACTGGCTCATT

TACATAACGAGGAAGCCCGAAAGACTTCACATGATTAAG

ACTCCTTATTTAGCCCCCTTATTAGCGTTCTAATGCAGA

CATTAAAGGTTCCAATACTGCGGAATCGTATGTTTTAAA

TATGCAACTAAGAATTAACTGAACACCCTGGGAAATTATT

AAAGAACTGGAATATCGCGTTTTAATTCGACTACTAATAG

AAACAGCTTGGAGGCTTTGAGGACTAAAGATCAGGGATAG

TAGTAGCATTAGCCTTAAATCAAGATTAGTGGAATACCCA

TACATAAAGTCAGACTGTAGCGCCACCGACAGTGCCCGTAT

ATATGGTGCCGGAAACGTCACCGCCACACATGGCTTTTGATGA

GAGCCTTTTTTTCACGTTGAAAATATAGAAGCGGAGTAACGAT

CAGACAGCCCGGCTTGCAGGGAGTTAAAGGCTTTGACCCCCAG

AGATTAAGCCAAAAGGAATTACATCAGTACAAGAACCGGATATT

CAACCGATCCAGCAAAATCACCTGACAGGTCTCTGAATTTACCG

TGACTATTTCATAACCCTCGTTCTAACGTAACAAAGCTGCTCAT

AACGAGAAAATAGCGAGAGGCTACGTTGGCCCTGACGAGAAACA

CACGGAATCGTAATCAGTAGCGAGAACCAATAAGTTTTAACGGG

ATTCATTGGGGGTAATAGTAAATGCGATTTGGGCTTGAGATGGT

CTATTTCGGAACAGAGCCACCACCCTCATTTCTTTTTCATGAGGAA

AGTTGCGCTATCGGTTTATCAGCTAATAATAATTAATTGCGACAATG

GCCACCCTCAGAACGTAACACTGAGTTTCGTCACCAGTACTTTGTCGT

GTAGAAAGATTCGAGGCATAGTAAGAGCAACACTAATAGTCAGAAGCA

CGATTATACCAAGCGCGATGATATAAGTATAGCCCGGATAGCATTCCA

GAACCAGAGCCACGTTTTCATCGGCATTTTCGGTCAACGCAGTATGTT

CTTTCCAGACTTTCAACAGTTTCAAGGAACAACTAAAACAACGTTAGT

CGCCGCCAGCATAGTAGCACCATTACCATTAGCAAGTTACCAGCGCCA

AATGCCACTACGAAGTCGGAACGAGGGTAGCAACGGCTACAATACCGAT

CCACCCTCAGAGCCAATGAAACCATCGATAGCAGCACAAGTTTATTTTG

GTTAATAAAACGAATACCAGACGACGATAAAAACCAATGACCATAAATC

AAGACAAAAGGGAACCCACAAGAATTGAGTTAAGCCCGAAACGATTTTTT

AGCCGCCACCCTCACAGAATCAAGTTTGCCTTTAGCGGTGGCAACATATA

AAGCGGATTGCAGACCGGAAGCAAACTCCAACAGGTCTATTTTCATTTGG

AAAGAAACGCATTAAGAAAAGTAAGCAGATAGCCGAACAACGCTAACGAG

TACAGGAGTGTGAGGCTGAGACTCCTCAAGAGAAGGATTAGTTTAGTACC

AGCAAACGTAGAGAAACCGAGGAAACGCAATAATAACTGCTATTTTGCAC

TAAATATTGACAACAAAGTCAGAGGGTAATTGAGCGCCTTTACAGAGAGA

GCTTTAAACAGGGCTTAGAGCTTAATTGCTGAATATAGTTGATTCCCAAT

ATACCAGTCAGGTTTGCAAAAGAAGTTTTGCCAGAGAATCCCCCTCAAAT

CATTACCCAAAACGGTCAATCATAAGGGAACCGAACTGACTAAAATACGT

TCACAATCAATAAAACAATGAAATAGCAATAGCTATCACAAAATAAACAG

CCAGAACGAGTATAAATTGTGTCGAAATCCGCGACCTGCTAAAGAATACA

AGACGATTGGCCTCTTGAGCCATTTGGGAATTAGAGTGAGGGAGGGAAGG

AAAAATCAGGTCCTTTAATTGCTCCTTTTGATAAGAGATTAGATACATTT

CTGGATAGCGGAATTATCACCGTCACCGATGATATTCACAATTTAATCATT

CGGTCGCTGATCATAGTTAGCGTGAGACTCCAAAAAAAAGGCACCGATATATT

Southern Hemisphere (70 staples):

TATCAAAATTATTGTGAATAAC

ATGGTTGCTTTGTAAAGCACTAAATC

GAATAGCCCGAGAAGAAAGCGAAAGGA

CTGAACCTCAAATATTGAATGGCTATT

GTAACCACCACAGCCGGCGAACGTGGC

GAAAAATCTAAAGCTAATAGATTAGAGC

GCGGGCGCTGCAAATCAACAGTTGAAAGG

CTCGTATTAAATCCTTTAAGTTTTTTGGG

TGGAAGGGTTAAATCCCTTATAAATCAAAA

AGAATCAGAGCAATACTTCTATTGGCAGATT

TGAGTAACATTATCATTGCTTTGAATACCAA

CTGATTATCAGATGATTTTAACGTCAGATGA

CCAGAAGATTATTTACTTGATTAGTAAAATAT

AGGGCGATGGCCCACTCCAGGGTGGTTTTTCT

GGTCAGTATTAACACCTACAAACAATTCGACAA

TTGGAACAAGAGTCCAGAGTTGCAGCAAGCGGTC

TAAAAGAGAACGGTACGCCAGAGGTCACGCTGCGC

CTGGCCAACGAACTGATAGCCCTCCAGCAGCAAAT

GTTGTAGCGGGAGCTAAACAGGCAGGGCGCGTACT

AGTCTTTAATGCGCAGAGATAGAACCCTGAAATACC

GGAACCCTAAAGGGAAAGGGAACAGCTGATTGCCCT

GAGAAAGGAAGGGATAGGGTCAGGCGAAAATCCTGT

CCCAAATCGCCCGAACGTTCAACGCGCGGGGAGAGG

CGTCAATAGATAATGAGCGGTTACATCGGGAGAAACA

GTTACAAAATAATGAATCGGCATTAATTTTAAAAGTT

AATTGAGGAAGGTTTTTGGATAAAACAGAAATAAAGAA

CGAATTATTAACTATATGTAAATGCTGATGGCACCGCTT

GGTCGACTCTTAAATCGTCGCTATTAATTAAGCCTGTTT

CTGGTGCCGGCTGTCGTGCCAGCTGCATTCGCGCAGAGG

CACCAGTCACCGAACCACCAGCAGAAGATATTTCCTCGTT

AGTATCATATCGCCATCAAAAATAATTCGCCATGCCTGCA

CCAGCTTTCCGCAAATCCAATCGCAAGACAGTCCAGACGA

CGACAATAAAATCTACAAAGGCTATCAGGTCGCACTCCAG

CTGCGCAATTGCGTTGCGCTCATGGGCGACGTGAACCATCA

GCGATTATTCCACACAACATACTGAGACTATTAAAGAACGTGG

TACATTTTGCCAGCCATTGCAACACTATCGAAGAACCACCGAG

GTGTTTTTATTACGTGGCACAGACAATATTTTCAAACCCTCAA

ACGCTGAGTCATTTGAATTACCTTCAGGGGCAATTCATCAATAT

GGTTATATCATTTCAATTACCTCCTGATTTTGCGGAACAAAGAA

CGCTATTAGCCTGGGGTGCCTAACGGGCCGAAAAACCGTCTATC

GACGTTGTATAGCTGTTTCCTGCCCCAGTGAGTGTTGTTCCAGT

GGTCTGAGAACATCAAGAAAACTACCTTAATTATCATCATATTC

CCTTAGAAATCAATATATGTGATGCACGTTATACTTCTGAATAA

GTCGAGGTGCCGACGAGCACGTATAACGTGCAAACAGAGGTGAGGC

TAATAAAATCAATCGTCTGAAATGCAATATTACCGACGCGGGACATT

TCAATATCTGGTCAGTTGAGGGCGCTGGCAAGTGTAGCATCCTGAGAA

TTAATGCGCCGCTAAGGCCGATTAAAGGGATTTTAGACAGGTCTGTCC

CACGCTGGTTTGTGTGAAATTGTTATCCGCTCACAAAGTTGGGTAACG

CGGTTTGCGTATCTGCCCGCTTTCCAGTCGGGAAACAAACCAGGCAAA

ATAACGGATTCGGAGCAAAAGAAGATGATGAAACAAGACTACCTTTTT

ATCACGCATCACTTGCCTGAGTAGGCCTTGCTGGTTAACAAATTAACC

TCACCGCCTGGCCCGAGCCGGAAGCATAAAGTGTAAACGCCAGCTGGCG

ATATACAGTAACAGAAAATTAATTACATTTAACAATTAAGAGTCAATAG

GTGCCACGCTGAGAGAAAACATCGCCATTAAAAATACCGAAACGACCAG

GATCGGTGCGGTGGGCGCATCGTAACCGTGCATCTGCGGTAATCGTAAAA

AATCCTGATTGATCTAAAATATCTTTAGGAGCACTAACAACATCACCTTG

AAAGGGGGATGTGGCGGATTGACCGTAATGGGATAGGTGATAATCAGAAA

ACTCCAACGTCAGCCCCCGATTTAGAGCTTGACGGGGAAACCCGCCGCGC

TGAATTTATCAAAATTTAATGGTTTGAAATACCGACCCGCCAACATGTAA

ACCACCAGAAGACATTTGAGGATTTAGAAGTATTAGACTTGCCTGCAACA

TTTCACCAGTGAGATGAGTGAGCTAACTCACATTAACTGTTGGGAAGGGC

ATTGCGTAGATTTTTTTTAATGGAAACAGTACATAATCCTTGAAAACATA

TGCCAAGCTTGGTCTGGCCTTCCTGTAGCCAGCTTTCCGCATTAAATTTT

TTGATGGTGGTTCAGCTCGAATTCGTAATCATGGTCAAAACGACGGCCAG

GCGATAGCTTACGTTAAATAAGAATAAACACCGGAATCTCAACAGTAGGG

GCGCCATTCGCCACGACAGTATCGGCCTCAGGAAGATCATTGCCTGAGAG

AACCTCCGGCTCTTTTTCAAATATATTTTAGTTAATTATAAGAGAATATA

CCAGGGTTTTCCGAGCGAGTAACAACCCGTCGGATTCGCAAATATTTAAA

CTTGCTTCTGAGAGGATCCCCGGGTACCGCGAAATCGGCAAGAACCTACCA

AGCGTAAGAAAATCAGTGAGGCTCAAAGGAAAAACGCTCATGTCTGACCTGAA

Equator Crossovers (18 staples):

ATAACATAAAAATCATTTTTTAACCAATAGGAAGCGTTATA

TGTTAAATCAGCCAGGGAAGCGCATTAGACGGGAAGTACGG

TGTCTGGAAGTTTATTTTGTTAAAATTATCAACATTAAATGTCAGTCAC

AAGTACCGACAATATCCTGAATCTTACCAAAGTTACCAGAAGAAATACA

CAAATTCTTACCAATGAAAATAGCAGCTAATATCAGAGAGATCGACATT

CGTCTTTCCAGAATTTTCGAGCCAGTATCATCTTCTGACCTAAATCATA

GGCGCGAGCTGAAGAGAATCGATGAACCAGTTTGAGGGGACGATTCAGG

CTAGCATGTCAATAACCTGTTTAGCTAAGGATTAGAGAGTACTTTACCC

CTTAATTGAGAATCCCAATCCAAATAAAATAATAAGAGCAAGGAAAATTC

TCTGCGAACGAGAGGAAGATTGTATAATCCGTGGGAACAAACGCTGCAAG

TTGTAAACGTTAATCATTCCATATAACAATGCTGTAGCTCAACCATAAAT

GTTTAACGTCAAAAGTATAAAGCCAACGCATAATTACTAGAAAAATTTTC

CCATATTATTTATCGCCATATTTAACAAGTGTGATAAATAAGGGATTAAG

AGCCCCAAAAACTAGATTTAGTTTGACCGTCATTTTTGCGGATTTCAGAA

TTTAGGCAGAGGCGCCTAATTTGCCAGTTTTACCGAAGCCCTTTAAGACAC

CGCAAATGGTCAATCATATGTACCCCGGTTCACGTTGGTGTAGAGCCTCTT

TCTGGAGCAAACAAAAGGTGGCATCAATTGCTTCAAAGCGAACCATCAAAA

CCAGCTACAATTTAAGGTAAAGTAATTCTAAGAACGCGAGAAAATAGGTTG

Photo-Crossovers (6 staples)

*o*-nb = *ortho*-nitrobenzyl

ATAACATAAAAA *o*-nb TCATTTTTTAACCAATAGGAAGCGTTATA

TGTTAAATCAGC *o*-nb CAGGGAAGCGCATTAGACGGGAAGTACGG

CGTCTTTCCAGA *o*-nb ATTTTCGAGCCAGTATCATCTTCTGACCTAAATCATA

CTAGCATGTCAA *o-*nb TAACCTGTTTAGCTAAGGATTAGAGAGTACTTTACCC

TTTAGGCAGAGGC *o*-nb GCCTAATTTGCCAGTTTTACCGAAGCCCTTTAAGA CAC

CGCAAATGGTCAA *o*-nb TCATATGTACCCCGGTTCACGTTGGTGTAGAGCCT CTT

Loop (12 staples):

CCAATAAATCATACAGGCAAGGCAAAGAATTAGCAAAATTAAGCAATAAA

GCCTCAGAGCATAAAGCTAAATCGGTTGTACCAAAAACATTATGACCCTG

TAATACTTTTGCGGGAGAAGCCTTTATTTCAACGCAAGGATAAAAATTTT

TAGAACCCTCATATATTTTAAATGCAATGCCTGAGTAATGTGTAGGTAAA

GATTCAAAAGGGTGAGAAAGGCCGGAGACAGTCAAATCACCATCAATATGA

TATTCAACCGTTCTAGCTGATAAATTAATGCCGGAGAGGGTAGCTATTTTT

TGTTCAGCTAATGCAGAACGCGCCTGTTTATCAACAATAGATAAGTCCTG

AACAAGAAAAATAATATCCCATCCTAATTTACGAGCATGTAGAAACCAAT

CAATAATCGGCTGTCTTTCCTTATCATTCCAAGAACGGGTATTAAACCAA

GTACCGCACTCATCGAGAACAAGCAAGCCGTTTTTATTTTCATCGTAGGA

ATCATTACCGCGCCCAATAGCAAGCAAATCAGATATAGAAGGCTTATCCG

GTATTCTAAGAACGCGAGGCGTTTTAGCGAACCTCCCGACTTGCGGGAGGT