Supporting Information

DNA Origami/Gold Nanorods Hybrid Nanostructures for the Circumvention of Drug Resistance

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Experimental Methods

1. Materials

All oligonucleotides were purchased from Invitrogen China. The origami staple strands were stored in 1.5 mL Eppendorf tubes and purified by polyacrylamide gel electrophoresis. The 3' thiol-modified DNA strands were also purchased from Invitrogen China and purified by High-Performance Liquid Chromatography. All the strands were normalized the concentration to 100 μM. Doxorubicin was purchased from Beijing Huafeng United Technology (China). RIPA lysis buffer was purchased from Solarbio Life Science. M13mp18 phage DNA (N4040S) was purchased from New England Biolabs (USA). Auric acid ($HAuCl_4$), cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), and tris(carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (USA). The primary anti-P-gp mAb and primary anti-actin mAb was purchased from (Abcam Biotechnology, USA). Horseradish peroxidase conjugated secondary antibody was purchased from Santa Cruz Biotechnology, USA. All the other reagents were purchased from Sigma-Aldrich (USA).

2. Preparation of DNA-gold nanorods(AuNRs) conjugates

AuNRs were synthesized by the seed-mediated growth method published by Nikoobakht and El-Sayed[1].

a. Synthesis of gold seeds:

10 mL of 100 mM cetyltrimethylammonium bromide (CTAB) and 50 μL of 2% (w/v) $HAuCl_4$ were mixed in a 10 mL flask, then 1000 μL of 6 mM $NaBH_4$ was added into the system with stirring quickly. The color of the solution changed to tawney, yielding nanospheres with size smaller than 5 nm as seed nanoparticles. The reaction was incubated at 30 °C for 2h and the solution act as nucleation points of gold nanorods in the next step.

b. The growth of AuNRs:

50 mL of 200 mM CTAB was added into a 100 mL flask. Keeping stirring, 2.5 mL of 4 mM $AgNO_3$, 50 mL of 1 mM $HAuCl_4$, 700 μL of 78 mM L-ascorbic acid, and 120 μL of fresh seeds were added to the flask graduation. The system got brown finally (With the absorption peak 820 nm). After stirring for 1 min, the final solution was kept still at 30°C over 12 h for nanorod growth. After centrifuged at 8000 rpm for 30 min, the supernatant of AuNRs solution was discarded and the pellet was suspended in ddH_2O to remove excess CTAB molecules.

c. Modification of AuNRs with thiolated DNA:

The disulfide bond in the thiolated oligonucleotides (50 μL,100 μM) was reduced to monothiol by TCEP (200 folded excess, 2h) in ddH_2O. Then the oligonucleotides was added to 5 mL modified buffer, which was containing 1×TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA; pH 8.0), 0.01% (w/v) SDS and 500 mM NaCl (pH 4~6).50 μL fresh AuNRs solution was quickly added and with the molecular ratio of DNA to AuNRs at 1000:1. The mixed solution was incubated at 30°C for 12 h. AuNR-DNA conjugates were centrifuged twice at 8000 rpm for 30 minutes to remove redundant ssDNA.

3. Self-assembly of the AuNRs and doxorubicin (DOX) to DNA origami template

a. Synthesis DNA origami:

According to Rothemund's work[2], staple strands, modified target strands, modified capture strands (short oligonucleotides) and 10 nM scaffold (M13 with 7249 nt), were mixed into 1×TAE/Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, 12.5 mM magnesium acetate; pH 8.3), and the ratio of short oligonucleotides to M13 was 10: 1. DNA origami nanostructures were assembled by slowly cooling from 95 °C to room temperature over 12 h.

b. DOX loading to DNA origami:

DOX (100 μM) was mixed with DNA origami (10 nM) and incubated at room temperature for 12 h. DNA origami-DOX was then filtered with 100 kDa MWCO centrifuge filters (Amicon, Millipore, USA) to remove extra DNA staple strands and redundant drugs. The concentration of DOX-loaded DNA origami was measured by Glow phosphorescence photometer (Perkin Elmer Instruments) at 490 nm.

c. Assembling AuNRs to DOX-loaded DNA origami:

Purified DNA origami-DOX was mixed with AuNRs with a ratio of six AuNRs for one DNA origami. The mixture was annealed from 37 °C to 25 °C in 1.5 h for 7 cycles.

d. Purification:

1% EtBr-free agarose gel was used to separate MUC-1 aptamer origami-DOX-AuNRs (MODA) complex from unassembled AuNRs. The target bands were cut off and the purified MODA were extracted from the gel were with Freeze-Squeeze columns (Bio-Rad) at 4°C.

4. Characterization of MODA by TEM

6 μL of the sample was deposited onto negative glow discharged carbon-coated grid for 10 min, and then filter paper was used to blot up the drop. For negative staining of DNA nanostructures, 10 μL of 0.7% uranyl acetate solution was dropped onto the grid and quickly removed; another 10 μL of 0.7% uranyl acetate solution was kept on the

grid for 40 s. TEM imaging was performed by a HT700 （Hitachi Limited）, operated at 80 kV in the dark-field mode.

5. Absorption spectra by Ultraviolet-visible

The concentration of the purified samples was measured by UV-vis spectrophotometer (Shimadu, Japan). DNA concentration was determined by the absorption peak at 260 nm and AuNRs concentration was determined by the absorption peak at 820 nm.

6. Doxorubicin fluorescence by Glow phosphorescence photometer

DOX concentration was measured by Glow phosphorescence photometer (Perkin Elmer Instruments), which was excited at 490 nm and determined at the emission peak 590 nm. To the DOX-loaded DNA origami, it was incubated at 95 °C for 10 min to separate origami-DOX assembly. Afterward, this uploaded DOX concentration was also measured by the above means.

7. Cell culture

MCF-7 cell is a human breast adenocarcinoma cancer cell line. The doxorubicin-resistant MCF-7 cell (MCF-7/ADR) subline was obtained by exposing regular MCF-7 cells to increasing amounts of doxorubicin, which was kindly provided by Prof. Xingjie Liang (NCNST). MCF-7/ADR cell was cultured in RPIM 1640 complete medium (Hyclone, Thermo Scientific) supplemented with 10% fetal bovine serum (Hyclone, Thermo Scientific) and 1% penicillin, and streptomycin (GIBICO, Invitrogen). The cells were cultured at 37 °C under an atmosphere of 5% CO_2.

8. Confocal imaging

MCF-7/ADR cells were seeded in 35 mm confocal dishes and were cultured overnight. The cells were then incubated with purified MODA (1 nM) or AuNRs (2 nM), or DOX (2 μM) for 12 hours. After washing with PBS, the living cells were visualized by laser confocal fluorescent microscopy (Olympus).

9. Photothermal ablation of tumour cells

MCF-7/ADR cells were seeded in 96 well plate and were cultured overnight. DOX, AuNRs, origami-AuNRs (OA), MUC-1 aptamer origami-AuNRs (MOA), MODA

were diluted to the equal concentration (DOX: 2 μM, AuNRs: 2 nM, MUC-1 origami: 1 nM) by fresh RPIM 1640 complete culture medium. After 12 h incubation, the above medium in each well was replaced and the cells were irradiated with NIR laser (808 nm, 1.5 W) for 5 min. After incubation for another 24 h, 100 μL of fresh serum-free medium containing 0.5 mg/ml 3-（4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was added to each well, and the cells were incubated for 4 h at 37 °C for the cytotoxicity assay. Then MTT solution was replaced by 150 μL DMSO and the absorption at 570 nm was measured using a microplate reader.

10. ICP-MS

MCF-7/ADR cells were seeded in 6 well plate and were cultured overnight. Then AuNRs and MODA were set at corresponding concentration (AuNRs: 2 nM, MUC-1 origami: 1 nM) with fresh RPIM 1640 complete culture medium and added each well 1 mL. The cells were soaked in aqua regia and completely digested in acid (3:1 mixture of HNO_3 and H_2O_2) on a hot plate prior to ICP analysis. The detection of gold contents in samples was performed by mass spectrometry (PerkinElmer).

11. Western blotting analysis

After incubated with different assembled complex overnight, MCF-7/ADR cells were exposed to 808 nm NIR laser with 1.5 W for 5 min. All the cells were washed with 1×PBS buffer for three times and collected for the whole cell protein extraction by RIPA lysis buffer. P-glycoprotein (P-gp) expression levels in MCF-7/ADR cells treated with different groups were detected by carrying out denaturing SDS-PAGE. Separated on 10% SDS-PAGE mini-gels with vertical cell (DYCZ-24DN, Liuyi, Beijing), proteins were electroblotted onto polyvinylidene fluoride (PVDF) membranes (Amicon, Millipore, USA) using a wet transfer cell (ZY5, Junyi, Beijing). The membrane was blocked by 1% BSA (1×TBST buffer: 1.5 M NaCl, 20 mM Tris-HCl, 0.05% Tween-20) for 3 h. Washed three times with 1×TBST buffer, membranes were incubated with primary anti-P-gp mAb (Abcam Biotechnology, USA) (diluted 1: 1000 in 1×TBST buffer) or primary anti-actin mAb (Abcam

Biotechnology, USA) for 12 hours at 4 ℃. Horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, USA), diluted 1: 1000 in 1×TBST buffer, was added and blots were incubated for 2 hours at room temperature, successively. Finally, the protein bands were detected with a SuperSignal West Pico Trial Kit (Thermo Scientific, Waltham, MA, USA) and the enhanced chemiluminescent imaging was performed by gel-imager (Tanon, Shanghai).

Additional Figures

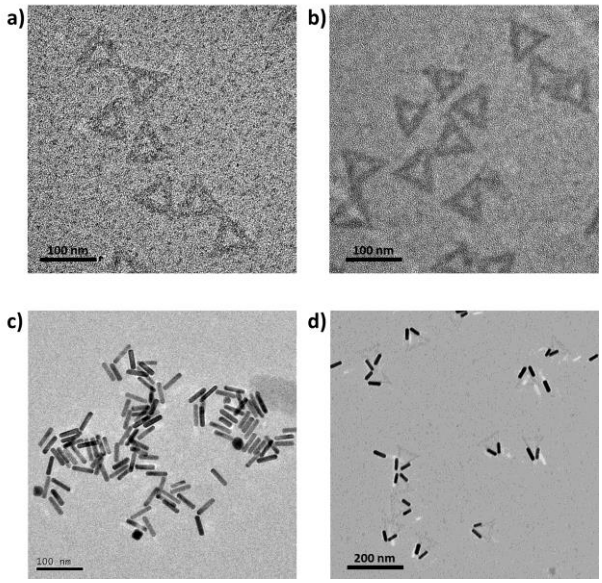


Figure S1. Additional TEM images. (a) MUC-1 Origami, (b) MUC-1 Origami-DOX, (c) AuNRs, and (d) MUC-1 Origami-DOX-AuNRs. The triangle shape of DNA template is visible as grey color after negative staining.

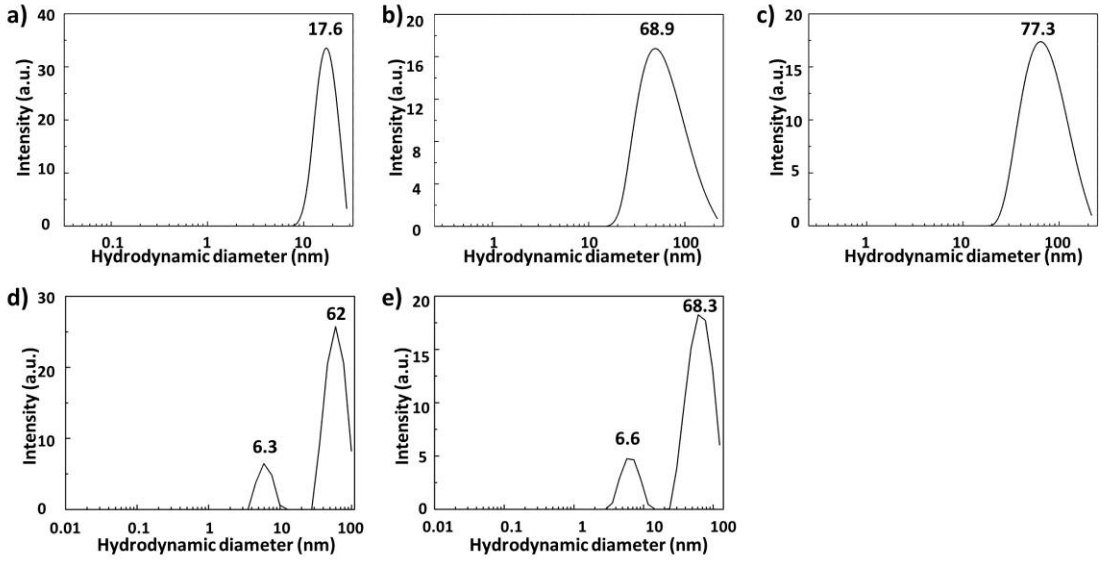


Figure S2. Dynamic Light Scattering results of different product：(a) AuNRs, (b) MUC-1 aptamer DNA origami, (c) MUC-1 aptamer DOX-origami, (d) MUC-1 aptamer DNA origami-AuNRs, (e) MUC-1 aptamer DNA origami-DOX-AuNRs

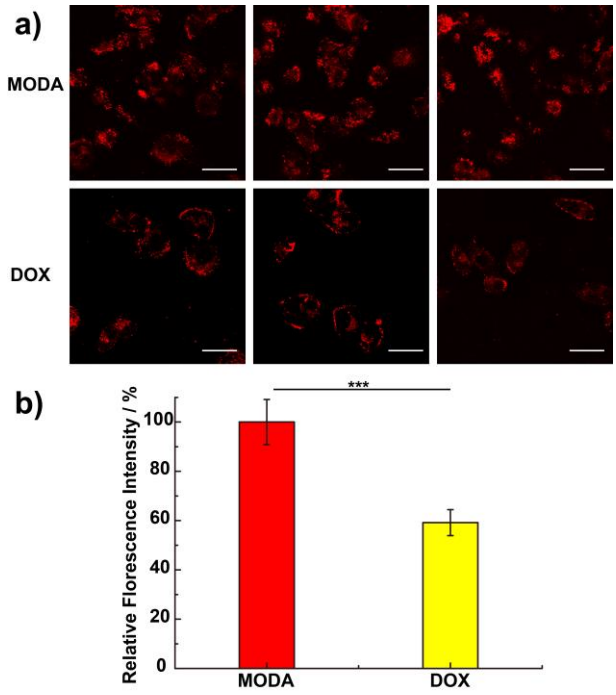


Figure S3. Single-photon confocal fluorescence of DOX (a), and quantification by image J (b) (* $p < 0.001$), Scale bars = 40 μm. DOX internalization in MCF-7/ADR cells was detected by single-photon laser scanning confocal microscope. With the help of origami carrier containing targeting groups, the DOX signal detected in the MODA group was 2 times higher than free DOX group.**

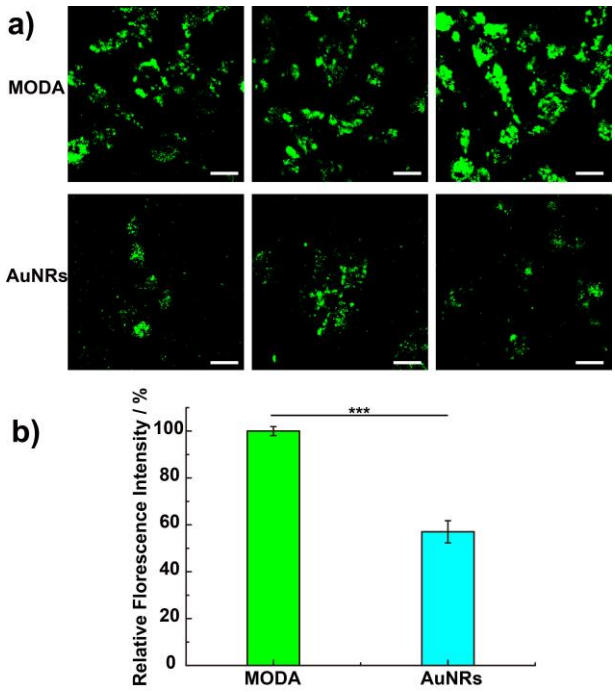


Figure S4. Two-photon confocal fluorescence of AuNRs (a), and quantification by image J (b) (* $p < 0.001$), Scale bars = 40 μm. AuNRs internalization in MCF-7/ADR cells was detected by two-photon laser scanning confocal microscope. With the help of origami carrier containing targeting groups, the AuNRs signal detected in the MODA group was about 1.7 times higher than free AuNRs group.**

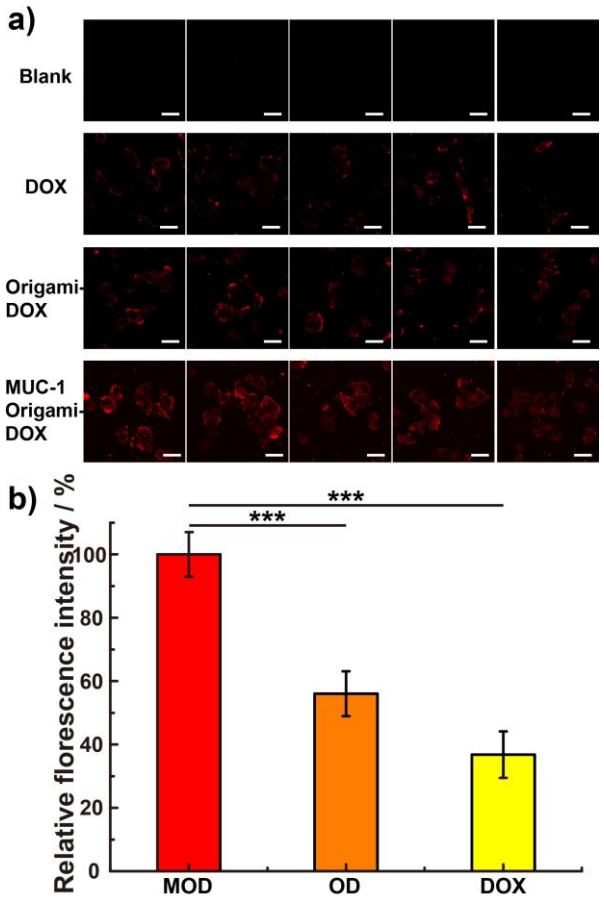


Figure S5. Additional cell images for quantifying the average intracellular fluorescence intensity (MOD: MUC-1 origami-DOX, OD: origami-DOX, and DOX). (a) Single-photon confocal fluorescence of DOX, Scale bars = 40 μm, and (b) quantification by image J (* $p < 0.001$). Compared with free DOX, the relative fluorescence intensity of DOX increased by 20% delivered by origami, and increased by 60% delivered by MUC-1 aptamer origami. MUC-1 origami increased the internalization of DOX.**

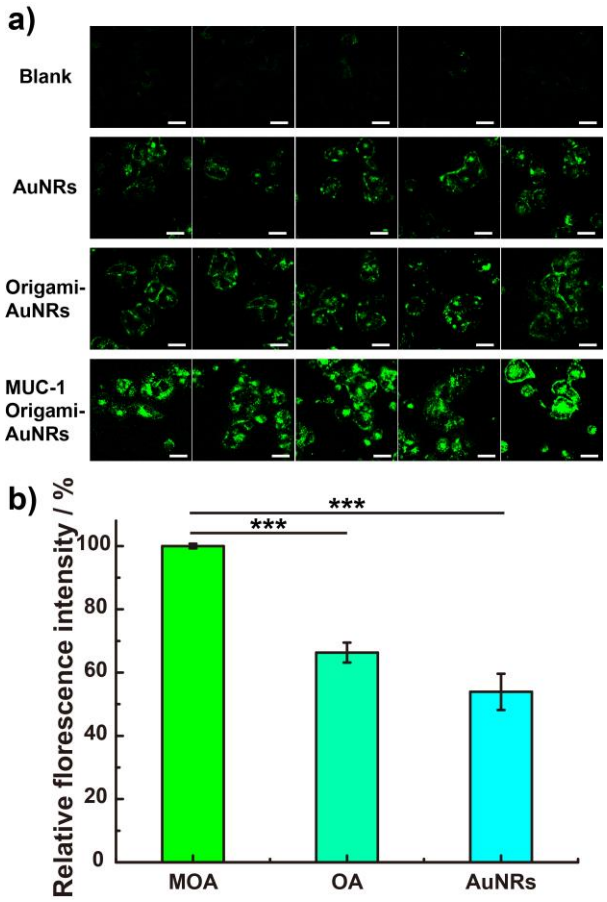
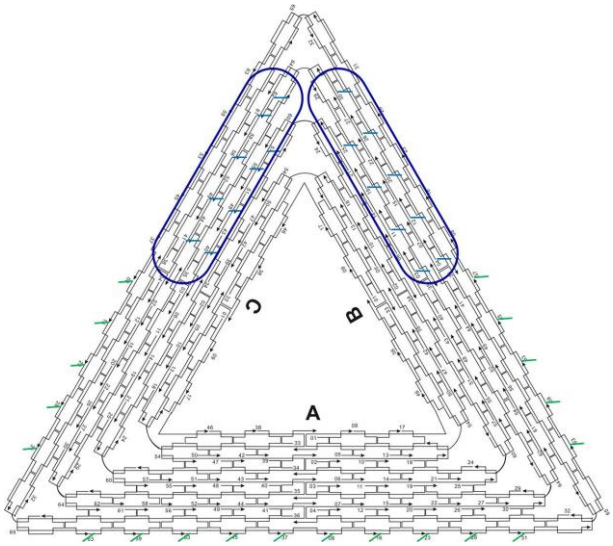


Figure S6. Additional cell images for quantifying the average intracellular fluorescence intensity (MOA: MUC-1 origami-AuNRs, OA: origami-AuNRs, and AuNRs). (a) Two-photon confocal fluorescence of AuNRs, Scale bars = 40 μm, and (b) quantification by image J (* $p < 0.001$). Compared with free AuNRs, the relative fluorescence intensity of AuNRs increased by 10% delivered by origami, and increased by 50% delivered by MUC-1 aptamer origami. MUC-1 origami increased the internalization of AuNRs.**

DNA strands used in the experiment



Scheme S1. The design of the MUC-1 origami-AuNR.

1. thiolated DNA used in the AuNRs modification.

S 15: TTTTTTTTTTTTTTTAGCG-SH

S 10: TATTATTATTATTATTTTT-SH

2. Capture strands for triangle origami

B03-cap,

AAAAAAAAAAAAAAA

GGCATCAAATTTGGGGCGCGAGCTAGTTAAAG

B04-cap,

AAAAAAAAAAAAAAA

TTCGAGCTAAGACTTCAAATATCGGGAACGAG

B11-cap,

AAAAAAAAAAAAAAA

CATCCAATAAATGGTCAATAACCTCGGAAGCA

B12-cap,

AAAAAAAAAAAAAAA

AACTCCAAGATTGCATCAAAAAGATAATGCAGATACATAA

B19-cap,

AAAAAAAAAAAAAAA

TTAGCAAATAGATTTAGTTTGACCAGTACCTT

B20-cap,

AAAAAAAAAAAAAAA

TAATTGCTTTACCCTGACTATTATGAGGCATAGTAAGAGC

B25-cap,

AAAAAAAAAAAAAAA

TAAAGCTATATAACAGTTGATTCCCATTTTTG

B26-cap,

AAAAAAAAAAAAAAA

CGGATGGCACGAGAATGACCATAATCGTTTACCAGACGAC

B30-cap,

AAAAAAAAAAAAAAA

TGCTGTAGATCCCCCTCAAATGCTGCGAGAGGCTTTTGCA

C40-cap,

ATAATAATAATAATA

GCCAGTGCGATCCCCGGGTACCGAGTTTTTCT,

C41-cap,

ATAATAATAATAATA

TTTCACCAGCCTGGCCCTGAGAGAAAGCCGGCGAACGTGG,

C48-cap,

ATAATAATAATAATA

GGTTTTCCATGGTCATAGCTGTTTGAGAGGCG,

C49-cap,

ATAATAATAATAATA

GTTTGCGTCACGCTGGTTTGCCCCAAGGGAGCCCCCGATT,

C55-cap,

ATAATAATAATAATA

TGCTGCAAATCCGCTCACAATTCCCAGCTGCA,

C56-cap,

ATAATAATAATAATA

TTAATGAAGTTTGATGGTGGTTCCGAGGTGCCGTAAAGCA,

C57-cap,

ATAATAATAATAATA

TGGCGAAATGTTGGGAAGGGCGAT,

C61-cap,

ATAATAATAATAATA

TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAAT,

C62-cap,

ATAATAATAATAATA

GCGCTCACAAGCCTGGGGTGCCTA,

3. MUC-1 target strands for triangle origami

A08 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

GACGGGAGAATTAACTCGGAATAAGTT

A16 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

GTCAGAGGGTAATTGATGGCAACATAT

A23 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

GATAACCCACAAGAATGTTAGCAAACG

A28 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

ATAAGAGCAAGAAACATGGCATGATTA

A31 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

TATCTTACCGAAGCCCAAACGCAATAA

A37 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

AGAGAATAACATAAAAACAGGGAAGCGCATTA

A45 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

AACGTCAAAAATGAAAAGCAAGCCGTT

A53 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

TCCCAATCCAAATAAGATTACCGCGCC

A59 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

GCCAGTTACAAAATAATAGAAGGCTTA

A63 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

ACGCTAACGAGCGTCTGGCGTTTTAGC

B37 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

ACAGGTAGAAAGATTCATCAGTTGAGATTTAG

B45 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

TTAATAAAACGAACTAACCGAACTGAC

B53 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

ACCAGTCAGGACGTTGGAACGGTGTAC

B59 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

ACCTTATGCGATTTTATGACCTTCATC

B63 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

TGGTTTAATTTCAACTCGGATATTCAT

C08 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

TTGACGAGCACGTATACTGAAATGGAT

C16 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

AGAATCAGAGCGGGAGATGGAAATACC

C23 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

TTAAAGGGATTTTAGATACCGCCAGCC

C28 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

GAATCCTGAGAAGTGTATCGGCCTTGC

C31 MUC-1,

GCAGTTGATCCTTTGGATACCCTGG

GCCACCGAGTAAAAGAACATCACTTGC

4. Sequences of unmodified staple strands

A00, TAACGAAAATCACCAG

A01, CGGGGTTTCCTCAAGAGAAGGATTTTGAATTA

A02, AGCGTCATGTCTCTGAATTTACCGACTACCTT

A03, TTCATAATCCCCTTATTAGCGTTTTTCTTACC

A04, ATGGTTTATGTCACAATCAATAGATATTAAAC

A05, TTTGATGATTAAGAGGCTGAGACTTGCTCAGTACCAGGCG

A06, CCGGAACCCAGAATGGAAAGCGCAACATGGCT

A07, TATTTCCAGCGCCAAAGACAACATTTTCGGTCATAGCCAAAATCA

A09, GATAAGTGCCGTCGAGCTGAAACATGAAAGTATACAGGAG

A10, TGTACTGGAAATCCTCATTAAAGCAGAGCCAC

A11, CACCGGAAAGCGCGTTTTCATCGGAAGGGCGA

A12, CATTCAACAAACGCAAAGACACCAGAACACCCTGAACAAA

A13, TTTAACGGTTCGGAACCTATTATTAGGGTTGATATAAGTA

A14, CTCAGAGCATATTCACAAACAAATTAATAAGT

A15, AAAAGCGATTGAGGGAGGGAATTTAGCGTCAGACTGTCCGCCTCC

A17, TAGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG,

A18, CCTTGAGTCAGACGATTGGCCTTGCGCCACCC,

A19, TCAGAACCCAGAATCAAGTTTGCCGGTAAATA,

A20, TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGA,

A21, CAGAGCCAGGAGGTTGAGGCAGGTAACAGTGCCCG,

A22, TAGAAAATTATTCATTAAAGGCCGTAATCAGTAGCGAGCCACCCT

A24, GCCGCCAGCATTGACACCACCCTC

A25, AGAGCCGCACCATCGATAGCAGCATGAATTAT

A26, CACCGTCACCTTATTACGCAGTATTGAGTTAAGCCCAATA

A27, GACTCCGACTTGAGCCATTTAAACGTCACCAATGAACACCAGAACCA

A29, CCATTAGCAAGGCCGGGGGAATTA

A30, GAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGC

A32, CAGAAGGAAACCGAGGTTTTTAAGAAAAGTAAGCAGATAGCCG

A33, CCTTTTTTCATTTAACAATTTCATAGGATTAG

A34, TTTAACCTATCATAGGTCTGAGAGTTCCAGTA

A35, AGTATAAAATATGCGTTATACAAAGCCATCTT

A36, CAAGTACCTCATTCCAAGAACGGGAAATTCAT

A38, AAAACAAAATTAATTAAATGGAAACAGTACATTAGTGAAT

A39, TTATCAAACCGGCTTAGGTTGGGTAAGCCTGT

A40, TTAGTATCGCCAACGCTCAACAGTCGGCTGTC

A41, TTTCCTTAGCACTCATCGAGAACAATAGCAGCCTTTACAG

A42, AGAGTCAAAAATCAATATATGTGATGAAACAAACATCAAG

A43, ACTAGAAATATATAACTATATGTACGCTGAGA

A44, TTTATGAAACCAATCAATAATAGGGCTTAATTGAGAATCATAATT

A46, GAGCAAAAGAAGATGAGTGAATAACCTTGCTTATAGCTTA

A47, GATTAAGAAATGCTGATGCAAATCAGAATAAA

A48, CACCGGAATCGCCATATTTAACAAAATTTACG

A49, AGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTTT

A50, ACATAGCGCTGTAAATCGTCGCTATTCATTTCAATTACCT

A51, GTTAAATACAATCGCAAGACAAAGCCTTGAAA

A52, CAATAAATAATATCCCATCCTCGCCAACATGTAATTTAATAAGGC

A54, TCCCTTAGAATAACGCGAGAAAACTTTTACCGACC

A55, GTGTGATAAGGCAGAGGCATTTTCAGTCCTGA

A56, ACAAGAAAGCAAGCAAATCAGATAACAGCCATATTATTTA

A57, GTTTGAAATTCAAATATATTTTAG

A58, TCCGGTTATCAACAATAGATAGAGCCAGTAATAAGAGATTTAATG

A60, TTCTGACCTAAAATATAAAGTACCGACTGCAGAAC

A61, GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT

A62, GAACCCAACATGTTCAGCTAAAAAAGGTAAAGTAATT

A64, ACGACAATAAATCCCGACTTGCGGGAGATCCTGAATCTTACCA

A65, TGCTATTTTGCACCCAGCTACAATTTTGTTTTGAAGCCTTAAA

B01, TCATATGTGTAATCGTAAAACTAGTCATTTTC

B02, GTGAGAAAATGTGTAGGTAAAGATACAACTTT

B05, ACAGTCAAAGAGAATCGATGAACGACCCCGGTTGATAATC

B06, ATAGTAGTATGCAATGCCTGAGTAGGCCGGAG

B07, AACCAGACGTTTAGCTATATTTTCTTCTACTA

B08, GAATACCACATTCAACTTAAGAGGAAGCCCGATCAAAGCG

B09, AGAAAAGCCCCAAAAAGAGTCTGGAGCAAACAATCACCAT

B10, CAATATGACCCTCATATATTTTAAAGCATTAA

B13, CGTTCTAGTCAGGTCATTGCCTGACAGGAAGATTGTATAA

B14, CAGGCAAGATAAAAATTTTTAGAATATTCAAC

B15, GATTAGAGATTAGATACATTTCGCAAATCATA

B16, CGCCAAAAGGAATTACAGTCAGAAGCAAAGCGCAGGTCAG

B17, GCAAATATTTAAATTGAGATCTACAAAGGCTACTGATAAA

B18, TTAATGCCTTATTTCAACGCAAGGGCAAAGAA

B21, ATAAAGCCTTTGCGGGAGAAGCCTGGAGAGGGTAG

B22, TAAGAGGTCAATTCTGCGAACGAGATTAAGCA

B23, AACACTATCATAACCCATCAAAAATCAGGTCTCCTTTTGA

B24, ATGACCCTGTAATACTTCAGAGCA

B27, TAATTGCTTGGAAGTTTCATTCCAAATCGGTTGTA

B28, GATAAAAACCAAAATATTAAACAGTTCAGAAATTAGAGCT

B29, ACTAAAGTACGGTGTCGAATATAA

B31, AAAGAAGTTTTGCCAGCATAAATATTCATTGACTCAACATGTT

B32, AATACTGCGGAATCGTAGGGGGTAATAGTAAAATGTTTAGACT

B33, AGGGATAGCTCAGAGCCACCACCCCATGTCAA

B34, CAACAGTTTATGGGATTTTGCTAATCAAAAGG

B35, GCCGCTTTGCTGAGGCTTGCAGGGGAAAAGGT

B36, GCGCAGACTCCATGTTACTTAGCCCGTTTTAA

B38, CCTCAGAACCGCCACCCAAGCCCAATAGGAACGTAAATGA

B39, ATTTTCTGTCAGCGGAGTGAGAATACCGATAT

B40, ATTCGGTCTGCGGGATCGTCACCCGAAATCCG

B41, CGACCTGCGGTCAATCATAAGGGAACGGAACAACATTATT

B42, AGACGTTACCATGTACCGTAACACCCCTCAGAACCGCCAC

B43, CACGCATAAGAAAGGAACAACTAAGTCTTTCC

B44, CAACTCCTGATAAATTGTGTCTCAGCAGCGAAAGACACCATCGCC

B46, AGGTTTAGTACCGCCATGAGTTTCGTCACCAGGATCTAAA

B47, GTTTTGTCAGGAATTGCGAATAATCCGACAAT

B48, GACAACAAGCATCGGAACGAGGGTGAGATTTG

B49, TATCATCGTTGAAAGAGGACAGATGGAAGAAAAATCTACG

B50, AGCGTAACTACAAACTACAACGCCTATCACCGTACTCAGG

B51, TAGTTGCGAATTTTTTCACGTTGATCATAGTT

B52, AGACCGAAACAAAGTACAACGAGCAACGGCTACAGAGGATACCGA

B54, ACAGACAGCCCAAATCTCCAAAAAAAAATTTCTTA

B55, AACAGCTTGCTTTGAGGACTAAAGCGATTATA

B56, CCAAGCGCAGGCGCATAGGCTGGCAGAACTGGCTCATTAT

B57, CGAGGTGAGGCTCCAAAAGGAGCC

B58, AAGAGCATCTTTGACCCCCAGACTTTTTCATGAGGAACTTGCTTT

B60, CGGTTTATCAGGTTTCCATTAAACGGGAATACACT

B61, AAAACACTTAATCTTGACAAGAACTTAATCATTGTGAATT

B62, TACCCACGAAAGAGGCAAAAGTAAAATACGTAATGCC

B64, ACCAACCTAAAAAATCAACGTAACAAATAAATTGGGCTTGAGA

B65, CCTGACGAGAAACACCAGAACGAGTAGGCTGCTCATTCAGTGA

C00, CTGAGCGCCATTAAAA

C01, TCGGGAGATATACAGTAACAGTACAAATAATT

C02, CCTGATTAAAGGAGCGGAATTATCTCGGCCTC

C03, GCAAATCACCTCAATCAATATCTGCAGGTCGA

C04, CGACCAGTACATTGGCAGATTCACCTGATTGC

C05, TGGCAATTTTTAACGTCAGATGAAAACAATAACGGATTCG

C06, AAGGAATTACAAAGAAACCACCAGTCAGATGA

C07, TATTTAATAAAAGGGACATTCACCTCAAATATCAAACACAGTTGA

C09, CCTGATTGCTTTGAATTGCGTAGATTTTCAGGCATCAATA

C10, TAATCCTGATTATCATTTTGCGGAGAGGAAGG

C11, TTATCTAAAGCATCACCTTGCTGATGGCCAAC

C12, AGAGATAGTTTGACGCTCAATCGTACGTGCTTTCCTCGTT

C13, GATTATACACAGAAATAAAGAAATACCAAGTTACAAAATC

C14, TAGGAGCATAAAAGTTTGAGTAACATTGTTTG

C15, TACATAACCCTTCTGACCTGACAAATGAAAAATCTAAAATATCTT

C17, GCGCAGAGGCGAATTAATTATTTGCACGTAAATTCTGAAT

C18, AATGGAAGCGAACGTTATTAATTTCTAACAAC

C19, TAATAGATCGCTGAGAGCCAGCAGAAGCGTAA

C20, GAATACGTAACAGGAAAAACGCTCCTAAACAGGAGGCCGA

C21, TCAATAGATATTAAATCCTTTGCCGGTTAGAACCT

C22, ATTGCGGCACAGACAATATTTGCCTGCAACAGTGCCATAGAGCCG

C24, ACAATTCGACAACTCGTAATACAT

C25, TTGAGGATGGTCAGTATTAACACCTTGAATGG

C26, CTATTAGTATATCCAGAACAATATCAGGAACGGTACGCCA

C27, GGTACTTTAATGCGCGAACTAAAACAGAGGTGAGGCTTAGAAGTATT

C29, ACCACCAGCAGAAGATGATAGCCC

C30, TAAAACATTAGAAGAACTCAAACTTTTTATAATCAGTGAG

C32, TCTTTGATTAGTAATAGTCTGTCCATCACGCAAATTAACCGTT

C33, CGCGTCTGATAGGAACGCCATCAACTTTTACA

C34, AGGAAGATGGGGACGACGACAGTAATCATATT

C35, CTCTAGAGCAAGCTTGCATGCCTGGTCAGTTG

C36, CCTTCACCGTGAGACGGGCAACAGCAGTCACA

C37, CGAGAAAGGAAGGGAAGCGTACTATGGTTGCT

C38, GCTCATTTTTTAACCAGCCTTCCTGTAGCCAGGCATCTGC

C39, CAGTTTGACGCACTCCAGCCAGCTAAACGACG

C42, GTAACCGTCTTTCATCAACATTAAAATTTTTGTTAAATCA

C43, ACGTTGTATTCCGGCACCGCTTCTGGCGCATC

C44, CCAGGGTGGCTCGAATTCGTAATCCAGTCACG

C45, TAGAGCTTGACGGGGAGTTGCAGCAAGCGGTCATTGGGCG

C46, GTTAAAATTCGCATTAATGTGAGCGAGTAACACACGTTGG

C47, TGTAGATGGGTGCCGGAAACCAGGAACGCCAG

C50, GGATAGGTACCCGTCGGATTCTCCTAAACGTTAATATTTT

C51, AGTTGGGTCAAAGCGCCATTCGCCCCGTAATG

C52, CGCGCGGGCCTGTGTGAAATTGTTGGCGATTA

C53, CTAAATCGGAACCCTAAGCAGGCGAAAATCCTTCGGCCAA

C54, CGGCGGATTGAATTCAGGCTGCGCAACGGGGGATG

C58, TGTCGTGCACACAACATACGAGCCACGCCAGC

C59, CAAGTTTTTTGGGGTCGAAATCGGCAAAATCCGGGAAACC

C60, TCTTCGCTATTGGAAGCATAAAGTGTATGCCCGCT

C63, CGATGGCCCACTACGTATAGCCCGAGATAGGGATTGCGTT

C64, AACTCACATTATTGAGTGTTGTTCCAGAAACCGTCTATCAGGG

C65, ACGTGGACTCCAACGTCAAAGGGCGAATTTGGAACAAGAGTCC

Link-A1C, TTAATTAATTTTTTACCATATCAAA

Link-A2C, TTAATTTCATCTTAGACTTTACAA

Link-A3C, CTGTCCAGACGTATACCGAACGA

Link-A4C, TCAAGATTAGTGTAGCAATACT

Link-B1A, TGTAGCATTCCTTTTATAAACAGTT

Link-B2A, TTTAATTGTATTTCCACCAGAGCC

Link-B3A, ACTACGAAGGCTTAGCACCATTA

Link-B4A, ATAAGGCTTGCAACAAAGTTAC

Link-C1B, GTGGGAACAAATTTCTATTTTTGAG

Link-C2B, CGGTGCGGGCCTTCCAAAAACATT

Link-C3B, ATGAGTGAGCTTTTAAATATGCA

Link-C4B, ACTATTAAAGAGGATAGCGTCC

Loop, GCGCTTAATGCGCCGCTACAGGGC

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