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DNA ORIGAMI SITE-SPECIFIC ARRANGEMENT OF GOLD NANOPARTICLES

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Controlling matter at the nanoscale holds a lot of promise in nanotechnology. The DNA origami is promising if used as a template to design and arrange matter at the nanoscale. We have used the DNA origami approach to engineer staple strands at selected sites for attachment of gold nanoparticles. The covalent attachment of thiol-modified DNA oligomers was used to functionalize gold nanoparticles. These oligomers then hybridize with complementary strands extended on selected staple strands on the DNA origami surface with nanometer precision. Gold nanoparticles of 5 nm diameter were arranged across a DNA origami tube to form a C-shape which has potential use in electronics and plasmonics. Agarose gel electrophoresis, AFM, UV-Vis spectroscopy and TEM were used to characterize the structure.

Keywords: DNA origami; thiol-modified; self-assembly; TEM; agarose gel electrophoresis.

1. Introduction

The quest to build miniaturized structures for advanced material design and high performance is the main aim of nanoscience. When Seeman[1] laid down the theoretical framework using DNA as a building material, the pursuit and construction of devices on the nanometer scale with this biological material had begun. To this end, scientists have

tried in various ways to build these small two- or three-dimensional (2D or 3D) structures in a bottom-up approach using DNA[2–4] as the building block since the top-down approach has so many challenges including fabrication of structures with nanoscale precision. The use of DNA to self-assemble is due to the specific binding of its bases. This self-assembly gives a large amount of control over assembly of nanoscale devices. The DNA origami method[4] is particularly significant as it is robust. This method which can produce both 2D and 3D structures folds a long single-stranded DNA (scaffold) with the help of shorter stranded DNAs (staples) into pre-arranged shapes.[4] With the development of these nanoscale synthesis approaches have come the formation of diverse nanoparticle structures. There is a great deal of potential including both plasmonic and electronic applications when DNA is used to structure metals at the nanoscale. The DNA origami approach developed by Rothemund[4] has improved the addressability of using DNA as a template for organizing nanoparticles. The folding process encompasses mixing the approximately 7200 base scaffold and hundreds of staples in a suitable buffer solution. The mixture is then annealed over a certain temperature range.[4–6] The use of DNA origami as a template to assemble nanoparticles is due to the fact that the individual staples can be extended with additional sequences (sticky ends) which protrude on the surface. After acquiring the desired shape, the attachment sites are also designed. Each individual staple involved in binding nanoparticles to the DNA origami template can be modified and extended with sticky ends. Gold nanoparticles (AuNPs) functionalized with complementary sequences can then hybridize with these sticky ends and attach themselves on the surface by making use of the covalent bond between sulfur and gold.[7–17] This approach has been used extensively to organize metal nanostructures into several different shapes.

Recently, Pilo-Pais and coworkers[18] used rectangular DNA origami to arrange gold nanoparticles into several shapes including rings, pairs of parallel bars and H shapes which had outer dimensions that were close to that of the DNA origami. Ding *et al.*[19] also made use of triangular DNA origami templates to rationally arrange a self-similar linear chain of six metal nanospheres. The use of DNA origami templates to arrange chiral plasmonic structures have been demonstrated.[20,21] Shen and his co-authors[21] used a DNA origami template to design gold attachment sites and attached one gold nanoparticle to each of these sites. They then added DNA folding strands which rolled up the rectangular sheets into a hollow DNA tube resulting in the nanoparticles being arranged into a helical geometry. To show the helical arrangement of nanoparticles using DNA origami, Kuzyk and colleagues[20] used the caDNAno honeycomb-pleated approach to design a hollow DNA origami tube as template. They designed Left- and Right-handed nanohelices that were formed by nine gold nanoparticles each of diameter 10 nm. Acuna *et al.*[22] used DNA origami structures as breadboard to position both fluorophore and metal nanoparticles with nanometer precision. They then performed single molecule fluorescence quenching studies of a fluorophore by metallic nanoparticles.

Due to the unique optical properties of metal nanoparticles, it will be of particular interest if they are structured with precise shapes and separation between them. When these precise parameters are achieved, the electrical,[23] plasmonic,[20,21] and CD[20,21] characteristics of these metal nanostructures can be studied. However, arrangement of nanoparticles in the form of a half-circle on a DNA nanotube has not been seen in the literature.

In this work, we demonstrate the rational arrangement of gold nanoparticles into a C-shape by using the DNA origami approach. We used the caDNAno[5,6,24] software to design a 3D DNA origami double tube with the outside tube wrapped around the inside tube. Our structure has been characterized and described.[25] We determined the specific positions on the outside tube where gold nanoparticles were to be attached from the caDNAno software. We then designed these attachment sites by extending specific staples with sticky ends that will hybridize with complementary sticky ends conjugated to the gold nanoparticles by thiol chemistry.

2. Materials and Methods

2.1. *Materials*

All unmodified staple strands were purchased from Sangon Biotech (Shanghai) Co. Ltd. They were suspended in ultrapure water and used without further purification. All 3'- and 5'- thiol-modified HPLC purified DNA strands were also purchased from Sangon Biotech (Shanghai, China) Co. Ltd.

Tris (carboxyethyl) phosphine hydrochloride (TCEP) and Bis (*p*-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BSPP) were purchased from Sigma-Aldrich, USA. 5 nm AuNP colloid was purchased from Ted Pella Inc. Other chemical reagents were bought from Sinopharm Chemical Reagent Co. Ltd. Carbon copper grids and mica were bought from Beijing Zhongjingkeyi Technology Co. Ltd.

2.2. *Experimental methods*

The following experimental procedures were adapted.[6,26,27]

2.2.1. *Phosphination and concentration of AuNPs*

We stabilized 5 nm AuNPs with absorption of BSPP. BSPP (15 mg) was added to the colloidal nanoparticle solution (50 mL) and the mixture shaken overnight with a constant temperature incubator at room temperature. Sodium chloride (solid) was slowly added to the mixture and stirred until the color changed from deep burgundy to light purple. The resulting mixture was centrifuged at 3000 rpm for 30 min and the supernatant was carefully removed with a pipette. AuNPs were resuspended in 1 mL solution of BSPP (0.5 mM). Approximately 500 μL methanol was then added to precipitate the particles and the mixture was centrifuged at 3000 rpm for 30 min again. The supernatant was carefully removed and AuNPs were resuspended in 1 mL of 0.5 mM BSPP. The concentration of the AuNPs was estimated by the optical absorbance at 520 nm. Phosphine coating increases the negative charge on the particle surface and therefore stabilizes the AuNPs in high electrolyte concentrations at high particle density.

2.2.2. *Preparation of AuNP-DNA conjugates*

The disulfide bond in the thiol-modified oligonucleotides was reduced to a monothiol using TCEP (20 mM, 1 h) in water. The oligonucleotides were purified using size exclusion columns (G-25, GE Healthcare) to remove smaller molecules. Monothiol-modified oligonucleotides and phosphinated AuNPs were then combined (DNA to AuNP molar ratio of more than 200:1) in 0.5X TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) containing 50 mM NaCl for 40 h at room temperature to ensure the AuNPs were fully covered by thiolated DNA. AuNP−DNA conjugates were washed with 0.5X TBE buffer in Microcon (100 kDa, Millipore, Billerica, MA) columns to remove the excess oligonucleotides. The concentration of these AuNP−DNA conjugates was estimated from the optical absorbance at 520 nm. Freshly prepared AuNPs, fully covered with DNA strands did not precipitate in buffer (5 mM Tris, 1 mM EDTA, 12 mM $MgCl_2$), which is preferred for the folding in DNA origami. This high salt resistant property of fully covered AuNPs makes it possible to assemble them on the DNA origami template. The DNA sequences used in the conjugation are given as S1 and S2 in Table S1 (Supplementary Information).

2.2.3. *Self-assembly of DNA origami template*

The preparation and characterization of the DNA origami roller structure has been described.[25] The structure was designed by caDNAno[5] software using single stranded M13mp18 DNA (7249 nt, New England Biolab.) as the scaffold and the 201 generated staples. A molar ratio of 1:10 between the long, viral ssDNA and the staples (used as purchased without further purification) was used. DNA origami was assembled in 5 mM Tris, 1 mM EDTA, 12 mM $MgCl_2$ (pH 7.9, 20°C) buffer, and annealed slowly from 65°C to 60°C over 50 min, then 60°C to 24°C over 72 h on the thermal ramp. The DNA origami was filtered by performing three buffer exchanges in 1x TAE/12 mM Mg^{2+} buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.2) with a single ultrafiltration centrifuge unit (100 kDa MWCO, Millipore) to remove the extra staple strands. The single strand staple DNA sequences used in folding are given in Table S2 (Supplementary Information).

2.2.4. *Preparation and purification of origami-AuNPs complexes*

The purified DNA origami was then mixed with AuNP−DNA conjugates in a ratio of 1:10 and annealed from 40°C to room temperature. The annealed product of the DNA origami and AuNPs was loaded in a 1.0% Gel-red (Biotum) stained agarose gel (running buffer 0.5X TBE containing 11 mM $MgCl_2$, loading buffer 50% glycerol, 70 V

constant Voltage). Selected bands were cut out and the DNA Origami−AuNPs complexes were extracted from the gel with Freeze'N'Squeeze columns (Bio-Rad Laboratories Inc.) at 4°C.

2.2.5. *UV-visible measurement*

Approximately 10 μL of the origami and AuNP–DNA conjugate mixture was diluted in a cuvette and the absorbance measured before and after annealing with a UV-visible spectrophotometer (METASH UV-6100).

2.2.6. *AFM imaging*

A sample of the DNA origami (2 μL) was first deposited on freshly cleaved mica and left to adsorb onto the surface for 2 min. The sample was rinsed with a few drops of ddH_2O to get rid of salts, and then blown dry with canned nitrogen. Imaging was performed with an Asylum Research MFP-3D^{TM} AFM using NP-S tips (Veeco Instruments Inc.) in the tapping mode.

2.2.7. *TEM imaging*

Transmission electron micrographs were obtained with a JEM-2100 (HR) TEM. One sample was negatively stained, and another was not. A 3 μl sample solution was deposited onto the carbon-coated side of the TEM grid and allowed to adsorb for about 5 min. The grid sample-side was first immersed into a 2% uranyl acetate (Structure Probe, Inc) stain-solution droplet and incubated for 40 s. Excess liquid was dabbed off with the edge of filter paper, and the grid allowed to dry completely. Images were taken at different accelerating voltages.

3. Results and Discussion

Figure 1 depicts the procedure used to produce purified C-shaped Au nanoparticles on the DNA origami roller template. The schematic drawing of the design process for the site-specific metallization of the DNA origami roller template to form the C-structure is illustrated in Fig. 1(a). Our previously reported ~74 nm DNA origami roller structure was

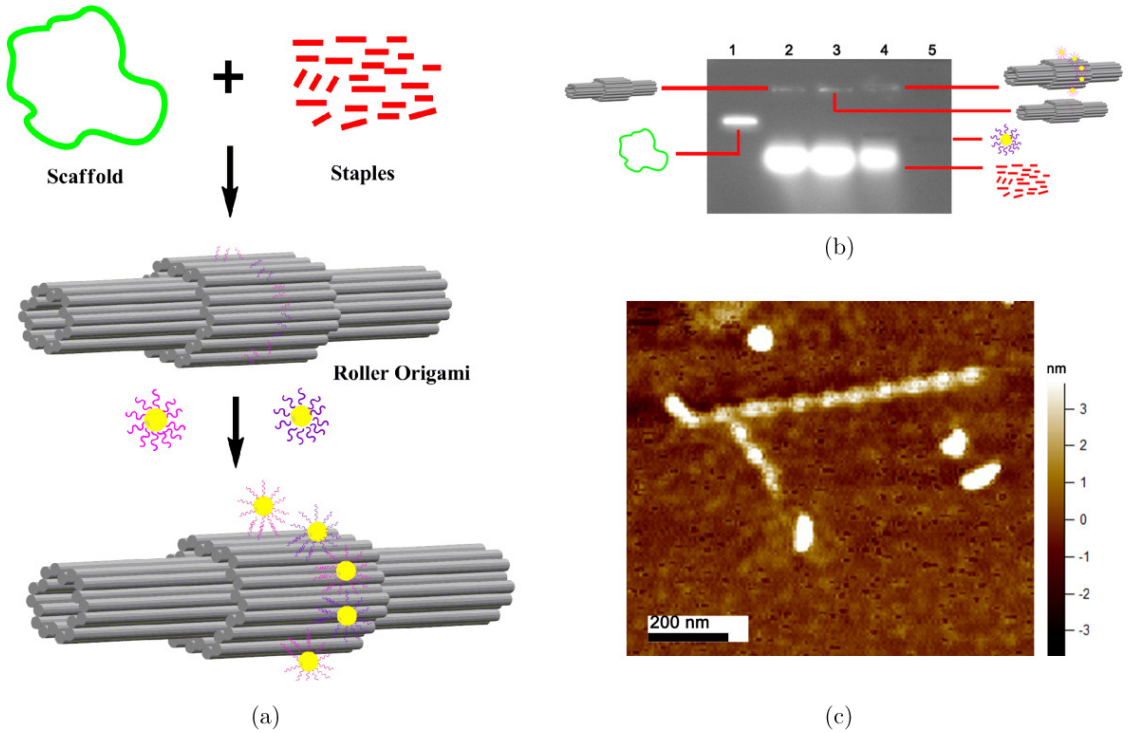


Fig. 1. Assembly steps of DNA origami metalized C-structure. (a) A schematic drawing depicting the assembling process of the 5 nm Au nanoparticles to form the C-shape on the DNA origami. The caDNAno[5,6] generated staple strands (red) hybridize with the input long circular single stranded scaffold (green) to generate the roller DNA origami structure with the five attachment sites indicated. The 5 nm Au nanoparticles which are covered separately with the corresponding DNA strands then hybridize with and bind to their complements on the designed sites; (b) Gel-red stained agarose gel electrophoresis of assembled origami and origami/AuNP products and (c) AFM image of assembled roller DNA origami structure (color online).

used as the DNA origami template for the C-shaped metallic structure.[25] We used the 48-helix bundle multilayer in the middle of the roller structure to assemble the gold nanoparticles after making the following modifications to the staple strands at our designed binding sites: We designed different DNA sticky ends at specific locations (see Fig. 2) on the surface of the multilayer by extending the sequence from selected staple strands of the roller template. The attachment sites (two attachment sites are indicated by the 3' (red) ends and 5' ends (green) in Fig. 2) were designed to consist of three adjacent helices where each of the helix contained one end of the staple strand to be extended. The sticky end extensions consisted of two different kinds of 15 nucleotide (nt) long single strands. To avoid nonspecific binding, the three attachment DNA staple strands at a particular site were extended with one kind of the sticky end strands [indicated by pink and violet in Fig. 1(a)] on the 3' end and the neighboring site with the other kind of sticky end strands on the 5' end. Thus, to form the C-structure we needed 15 helices and 15 sticky ends.[20] The attachment sites were positioned such that there were 3 double helices between adjacent sites on the honeycomb lattice. This was approximately 6 nm apart across but not along the roller as shown in Fig. 1(a). After hybridization of our Origami structure all the sticky ends were expected to be displayed on the surface. The DNA origami template was purified from excess staple strands by agarose gel electrophoresis.

We functionalized the Au nanoparticles with multiple strands to allow the attachment of each nanoparticle through more than one strand. After conjugating the 5 nm gold nanoparticles (AuNPs) with the corresponding thiolated DNA strands, we used column filtration to remove the unbound DNA strands before adding the mixture to the origami structure in solution and the new mixture annealed for 4 h.

After the annealing process, the product was analyzed by agarose gel electrophoresis shown in Fig. 1(b). In lane 1, was the single stranded M13mp18. Lane 2 contained the assembled DNA origami structure as indicated. Contained in lane 3 was the origami structure with the extended sticky ends which is shown as the clear band indicated. Lane 4 contained the DNA origami roller conjugated with AuNPs with the band indicated as such. The filtered staple strands in lane 5 are less because of the initial column filtration.

Lane 5 contained only 5 nm AuNPs. Figure 1(c) shows our DNA origami structures that are stacked together since we did not remove the end sequences. The desired band (in lane 4) was analyzed by transmission electron microscopy (TEM). The TEM images are shown in Fig. 3. Figure 3(a) shows a closer look of the gold C-structure. We see two clearly formed C-structures in the first two images on the left but with one missing AuNP. The last two images in Fig. 3(a) show two clearly formed C-shapes with five AuNPs. Figure 3(b) shows uranyl

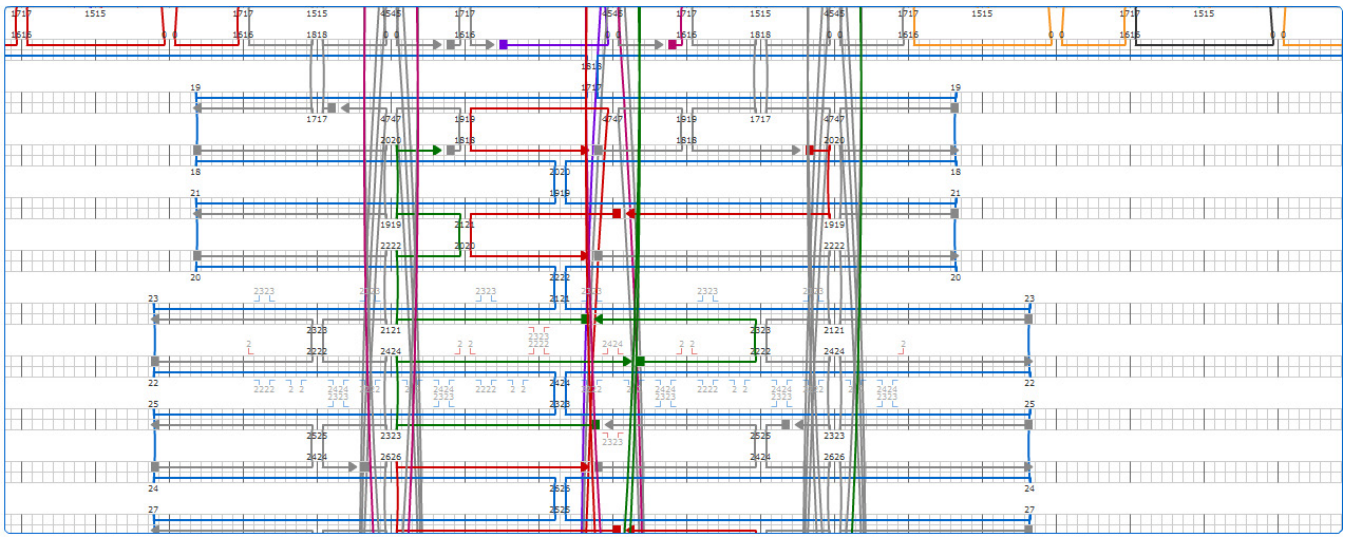
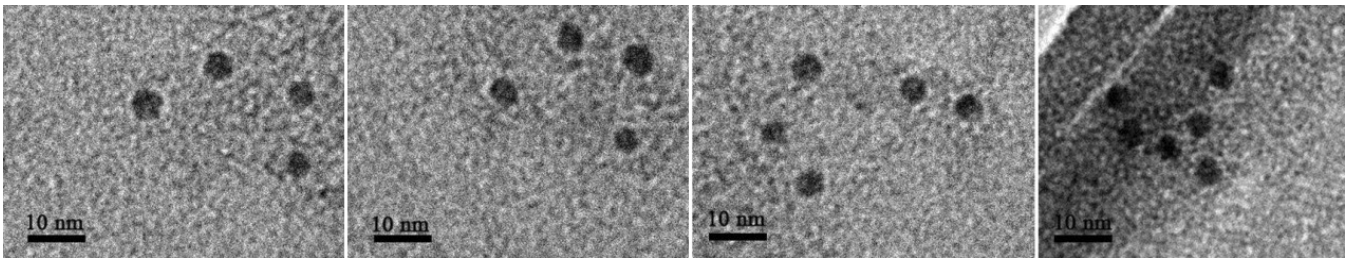
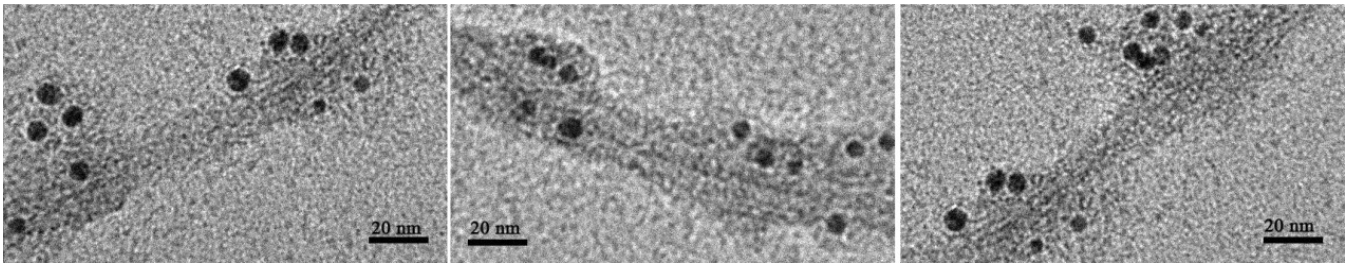


Fig. 2. Two designed attachment sites as seen on the caDNAno interface. The sites are defined by the three different sequences (shown with red and green). We see that there are three double helices between the centers of the designed sites (color online).



(a)



(b)

Fig. 3. TEM images of the C-shaped AuNP assemblies. (a) The nonstained TEM image and (b) uranyl acetate stained image of the structure formed on DNA origami roller structure.

acetate stained TEM images of the structure in which is seen also the origami template. All the images show some distortions in the shapes formed. We think the distortions might be due to the processing on the TEM grid for imaging. The average distance between adjacent AuNPs [last image in Fig. 3(a)] is approximately 6 nm. We determined the inter-attachment site distances from the flat 2D Path panel (see Fig. 2) using caDNAno to be 6 nm. But since this flat surface will bend during folding, the inter-attachment distances might have stretched on the origami surface during folding. It has also been reported that functionalized particles avoid attachment if the adjacent binding sites are closer than approximately 20−30 nm.[18] Our C-structure formed possibly because of the bending during folding as it was not arranged linearly and thus accommodating our nanoparticles.

We also observed that some of the C-structures were not well formed. There were some missing AuNPs [see Fig. 3(a)] in binding sites which could be due to stearic hindrance and electrostatic repulsion effects associated with the metals themselves or the negatively charged DNA used to functionalize the metals.[21]

We have also measured the UV-Vis spectra for both AuNP/DNA origami mixtures before and after annealing. These are shown in Fig. 4. From the figure we see a plasmon band shift from 521 nm to 525 nm after annealing. It is well known that the size, shape and the orientation of nanoparticles and their assemblies affect their optical properties. It is known that the interaction between light and free electrons in gold nanoparticles could give rise to collective oscillations called surface plasmons.

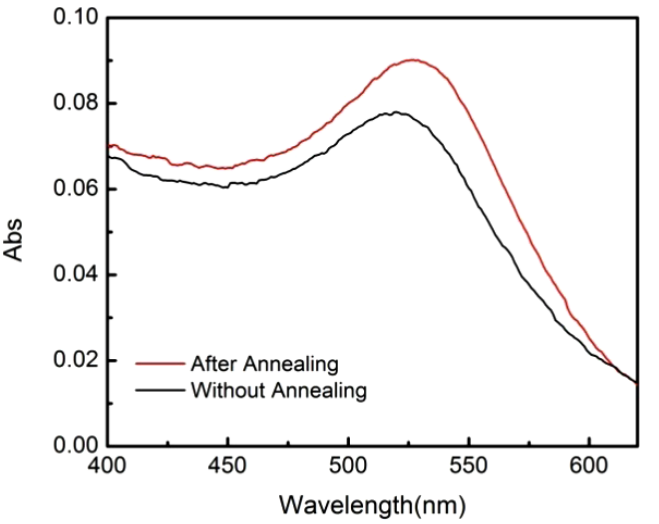


Fig. 4. UV-Vis absorbance spectra of DNA origami/AuNPs mixtures both before (black) and after (red) annealing. Clearly seen is the plasmon band peak shift from 521 nm to 525 nm due to the annealing process (color online).

Surface plasmons have the potential to confine light to the interface between metal and dielectric and in turn generate intense electromagnetic fields locally. It is clear from Fig. 4 that there is plasmonic interaction involving the AuNPs in the annealed structure. Halas and coworkers have also reported that if the plasmonic nanocrystals are strongly interacting, then the absorption peak will be red shifted.[28] Our C-shape AuNPs structure could have the potential to generate such intense localized electromagnetic fields and as such the possibility to be used as components for nanoelectronic, plasmonic or metamaterial applications.

4. Conclusion

We have successfully arranged AuNPs on a double tube DNA origami structure to form a C-shape which has the possibility of being used as a component for nanoelectronic, plasmonic or metamaterial applications. We have also shown that if the nanoparticles are arranged across the DNA nanotube length instead of along it, then metallic nanostructures could be formed even if the separation between nanoparticles is as small as 6 nm.

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Supplementary Material

Table S1. DNA staple sequences that were extended with sticky end sequences for attachment of AuNPs at the binding sites. I8′, I9′, etc were extended at the 3′ end and H10′, H11′, etc were extended at the 5′ end. S1 and S2 that have thiol extensions are complementary to the sticky end sequences.

I8′	TAGGCAAAAGAAGTTTTCCAGACGACAAAAAAAAAAAAAAA	41
I9′	TAGAAAAAAGTTTATTAACCCTCGTTTAGCCAGAGGGGGTCGAAAAAAAAAAAAAAA	57
I10′	ATAGACGGAATTACATACGCAGTAAAAAAAAAAAAAAA	38
I11′	TTAACGTTGGATTACCTTAAAAAAAAAAAAAAAA	35
I12′	ACTGCCCGTAATCAGCAGAACGAGTAGTATGCGATTTTAAGAAAAAAAAAAAAAAAA	57
I13′	GCGAATCAAGGCAGCACAGCAAAATCACCAGCATTTGGAAAAAAAAAAAAAAA	53
I15′	TTACAACTGCTCCATGTTACTTAGCAACCGAACTGACCAACTAAAAAAAAAAAAAAA	57
I16′	CACAAACCCTCAGAGCCGCCACACCCTCAAAAAAAAAAAAAAA	43
I17′	GTGTACTAAATCCTCATTAAATGATATTAAAAAAAAAAAAAAA	43
H10′	AATAATAATAATAATACACTATCATTTGGTCAGTTGAGATTTAGGAATGTAAGAGCA	57
H11′	AATAATAATAATAATGATTGAGGGAGTCACAATCA	35
H12′	AATAATAATAATAATTTCAGAAGGCTCATTATACCAGTCAGGTTACAGGTAGAAAGA	57
H13′	AATAATAATAATAATACACTATAAGGCTGCGCAACCCGAGCT	42
H14′	AATAATAATAATAATGATATTCATTACCCAAATCAACCTTCATCGGAA	48
H15′	AATAATAATAATAATTCAAAATCACCGGACCTCCGGCTGA	40
S1	HS-TTTTTTTTTTTTTTTTTT	19
S2	ATTATTATTATTATTTTTT-SH	19

Table S2. DNA sequences generated with caDNAno. The DNA sequences used in the construction of the DNA origami structure. The sequences are from the 5′ end to 3′ end.

A1	AATAATAAGACCACAAGAATTGAGTT	26
A2	AACCCTCTTGAAAGGAATTGAAGTAACAATTCCTGCAGT	39
A3	CTCAAATAGCACGCTAATATC	21
A4	CTATCAACACTAACAACTAATTATT	25
A5	GTTGGCAAAAACAAAGAAACC	21
A6	ATCTGTTATTAATTTTAAAAGTTTGGGAAGGTAAC	35
A7	GAACTTAGGAGATCTAAAGAAGCCCCAAAGTCAGAG	36
A8	AAAGATTGTTAGAAATTGCGTAGATTTTCAG	31
A9	CAACTCGAGATTAGGGGCGAAAAGCAGAGAATTAACTGA	39
A10	ACCAGAAGGAGCCGGGAGAAACAA	24
A11	CATGGAATTATCATCATTTATCATTTTGCGGTCAACAGAATC	42
A12	TAACGGATTCGCCTGATTGTTAATTAATTTTCCCTTAG	38
A13	AATCCTTGAAAACATAGCGTTAATGGTTTGA	31
A14	TTGTCGCTACTTTGAAAGTACCTTTTA	27
A15	ATTTCATCTTCTGACCTAAATATAGC	26
A16	TTAGTTAAGAAGAGATAA	18
A17	CAAGAATCATAATTACTAGAAAAAAACGCG	30
A18	AGAAAACTATCAAAATAAATCA	22
A19	AATACCGACCGTTATAAAGTACCG	24
A20	GTTAAATTTCGAGCACGACGACAAT	25
A21	ACAAAAGGTAAAGTAATTCACCGCACTCATCGAGAACA	38
A22	TCAACAATAGAAACCAATCAAAGGCTTA	28
A23	CGGAACGCGCTAACAACGCCAACA	24
A24	AGCAAGCCGTTTTTATTTTAACGCTATACAAAAAGATA	38
A25	AGCCTAATTTGCCAGTACGAGCGTCTTTCCAG	32
A26	ACGCAAGAAACAATGAAATATCA	23
A27	AGAGTAAACAGCCATATTTCCTGAATCTTACCCATCGTAACCA	43
A28	GGTCCAATCCAAATAAGTTGCACCCAGCTACGCCCAATCATTC	43
A29	TGAATTTTTAAGAAAAGTAAACCGT	25

Table S2. (*Continued*)

A30	ACATTTTTGTTTAACGTAAATCAAGATTAGTATATAGATAAT	42
A31	AAGCCCAATATCTGGTCA	18
B1	CAATTCATCAATATAATCCTTCCTTTGCCC	30
B2	AACTACCAAGTTACAAATGCTTCTGTAAATCAGATTAAGACGCTG	45
B3	CCTATCGCGCGTCAGATGAATATAATTATCAGATGATGG	39
B4	AGTTGTCCAGCAGTAATAAGAGAAGTGATAAATAAGGC	38
B5	CACAACATGTAGGCAGAGGCATTAAGAATAAACACCGATATATT	44
B6	GAGATAGCTATCTTACCGCATCACCTTGCTGTATCTAAAAT	41
C1	GTTTAACAGAGGCGAATTATTATATATGTGA	31
C2	CATTTCAATTACCTGAGCAAAATGGAAACAGTACATCATAGG	42
C3	GTGATCAATAGTGAATTTTTTT	22
C4	TGTAATTTTCAGCTAATGCAGCTGTCTTTC	30
C5	AAAAGAACGGGTATTAAGGAATCATTACCGCAATTTTAATTTATCAATT	49
C6	CTTATAGCAAGCAAATCAGTGCTATTAAACGATCCC	36
D1	GATAGGGGAGACGGGCAACAGGCTGCATCACAATTCAGG	39
D2	AATAATCAGGGGAAGCAAACTCCAAATTGCTGAATA	36
D3	CCGAAATCGAGAGTTGCAGCAAGCGCTCAAGCCTGGCGAC	40
D4	TGGATTGCACAAATATCGC	19
D5	GCAGGCGAAAATCCTGACGCTGGTTTGCCCCA	32
D6	CCTGGTCGGGAAACCTGTCGTGCCACTGATTGAAAG	36
D7	CCAGCCCTGAGGCAAAAATTATAGAGCTTCAAAG	34
D8	AACTCACATTAATTGCGTTGCGGTCCTTTGA	31
D9	AGCCGGAAGTGCCACAGCTGGCGA	24
D10	TCGCGGGCCTCTTCGCTGCACCGCTTCTGGTATTG	35
D11	GCCAGGGTTTTCCCAGTCAGGTGCCTAATGAGTGAGCT	38
D12	AGGGGACGACGAAAGTTGGGTAAC	24
D13	ACCGTAATGGCCTTCCTCAGGAAGGCAA	28
D14	GCTTTCAACGGCGGGCCGGAAATCGGTGACTCTAG	35
D15	TCGCGTCTGGGATAGGTCTTTCCGATT	27
D16	AAATATTTAAGTCATTGACCA	21
D17	AACTATTTTGGAGATCTCGTTCTAGCTGATAGGTTGTACCA	41
D18	TTTGTTAAATCAGCTCATTTCTGCCAGTTTG	31
D19	CGATGAAAAAAGCCCCAAAAAGTAGCCA	28
D20	AGAGGGTAGCTATTTTTGATTAAAATTCGCATTAAATT	38
D21	AAAAAAATTAAGCAATAAACAGTTGGTGT	29
D22	TAAAGCTAAATCAATTAATGCCGG	24
D23	TAATAGTTGGTCAAAGAGCTTACAGGTCGACCATAAATCAAAAGCCCGA	49
D24	AGAGAACGAGTAAATATTAAT	21
D25	TTCCATATAAGCCTCAGAGCA	21
D26	TAATACATTTCGCAAAAGTAGCACAACGCAGGTGAGAAGAGAAT	44
D27	CTGAAGACTTTCAAAAAGATTAAGAG	26
D28	CGAAAGCTCAACATGTTTTAGATTTAGTTTGAATCATACTTGCG	44
D29	TCGTCAGAAGCAAAGCGGTGGTT	23
D30	GTTTGCAACTAAAGTACGATTCCCAATTCTGCATTAGCACATTA	44
D31	GAAGCCCGAGAAGTTTCA	18
E1	GGCCAGCATAAAGTGTAACTGCCCGCTTT	29
E2	ACGCAGCTTGCATGCCTGCCACACAACATACG	32
E3	TAAACGTTAACAATAGGAACGCCATGGGCGC	31
E4	GGTCAAATCCCTGAGAGTCTGGAATTGTATAAGCAAATAAT	41
E5	TGATTCAACACAAAGGCTATCAGATTG	27
E6	ACCTCTTTACCCTGACTTCCCTTATAAATCACCCTTCACCG	41
F1	GTTGCTGCAAGGCGATTCAGTATCGGCCTCAATCGTAACCGTGCATTTT	49
F2	AAGGCGCACTCCAGCCAGCACGTTGGTGTAGATCAA	36
F3	GGAAGATGGGATGTGTAAAACGAC	24
F4	ACAAAGGCCGGAGACAGAGAAGCCTT	26
F5	TCAATATGATACCCTGTAATACTTAGGCAAGGCAA	35
F6	TATTTTTAACATCCAATAACCATTAGATGCTGTCCAG	37
G1	AATAGATTACAAACGAATAATTTGCACGGATG	32

Table S2. (*Continued*)

G2	TTTTGGGCGCCAGGAACAAGATGCTT	26
G3	TTCTTTTACGCGCGTGTGTGACCGGGTATGT	31
G4	TAGACTTAATACATAACGTGGTACCA	26
G5	GCGGTTTGCGTATAGAAGTAT	21
G6	TCGGCCACACCAGTTTGAGTGCGAGAATAGGATTAGTATCGGTTTAT	47
G7	GAAATAATGGATTATACTTCTAATTCGA	28
G8	AGGATCCAATTGTTATCCGCTTAATGAA	28
G9	AAATTAGCGTGTCATAGCCCCCTTATTA	28
G10	TGGGTTGCCCTGACGAGAA	19
G11	TCTGAGAGACAAAGGCCTGTTCCATATTCTGTTTA	35
G12	CGGATTCTCCGTGAAGAGTAATGAACGGTGTACAATAAGGGCGGAA	46
G13	CAATCAACATTAATCAGCGGTAATTAAAGAT	31
G14	ATCGCAAGACTACCTTTTTTAAGAAGATTAAAACA	35
G15	TGCTTATACAGTAGGGCGTCCTGA	24
G16	GTAAATGCTTAGGTAACAATTCAAGAAACATATCA	35
G17	AGAATCGTAGTATCATATGCGAAATCCA	28
G18	TCAAAAGAGGATAAAAATTTTATTCTAC	28
G19	TCCGGTAAAGCCTTCAAAAATGACGGGATAGCCGAAAGAACTGGCAT	47
G20	GTATATTTTGGTCATTCCTT	20
G21	TTGTTCTAAGGAGCATGTAGATAATTAATTG	31
G22	GCTGGCATCATAGAACCGTGTAGGCGTAAAA	31
G23	ATAGCAGCCTTGCGGGATACCGTACACCACCCTCATTTCTCA	42
G24	GACATTTGGTACCGATGTCGCTGAGCAC	28
G25	GTATTTGCGGATGGCTTTAACCTGTGACAACAACCA	36
G26	GAAGGGAAGGTAACGAAACTACAACGCCTGACCCATGGGTT	41
G27	AAACAAAGTACTCCAACGTCAAAAGCCGTC	30
G28	CGGAATCGTCATGGAATTGCGAAT	24
G29	GATTAAGACTCCTTATTACATAAAGGTGGCAACATATA	38
G30	ATAACGGAATCAACAGACGTTAGTAAATGAAGTTAGCCGCATTAGAAA	48
G31	TGTCCCCCTGAATAGACAAAAAAAATCG	28
G32	CGAGAGGCTTTTCGTCCAATACTG	24
G33	AAAGAAACGCAAAGACACCAAAATTCGACATTCGGAAATTCTTG	44
G34	GATAAAGGCATAACCACATAACA	23
G35	AACGCCAAAAGGAATTACGAAACCAAAATAG	31
G36	CCAAAGACAAAAGGGCATATGGTTTACCAGCG	32
G37	TAAAACGAACTAACGGAACTCAACTAATGCAGATACAT	38
G38	TTATCACCGTCACCGAATTCATTAAAGGTGAA	32
G39	AGCTAGCACCTGAAACCATCGATATTTGCCTCAAAC	36
G40	GAATTAGAGGTAAATATTGACAACC	25
G41	TCAACTTTAATCATTGTGAGAAGAAAAATCTACGTTAA	38
G42	CCGGAAACGTAGCGCGTT	18
G43	ATTGGGCTTGAATAAGGCAAGGGCGACCAGGCAAAGCGCCATTCGCCA	48
G44	CTCATTCAGTGAGATGGTTTAATT	24
G45	TTCATCGGCCGGAACCGC	18
G46	TTCGCAGACTGTCACCAAATTACCATTAGCAAGG	34
G47	AGGCTGGCTGACGTAACAAAGCTG	24
G48	CTCCCTCAGCCGCCAGCATTG	21
G49	GAACTTGGCCTGCCAGAATTGATGAGGTCAGTAACC	36
G50	CGGTCAATCGACCAGGCGCAT	21
G51	ACAGGAGGTTGATCTGAATTTACC	24
G52	CAGACGACACCACCAGAGCCGAGCCGCCCCACCACATT	38
G53	CGTGATAAATACAACGCACTCATAAAGAGGCAAAAGAAT	39
G54	ACATGGTACCAAGCGCGAAACAAAGTTGTGTCGAAATCCGCG	42
G55	ATCATCGCCAGGCGCAGA	18
G56	GTTCCAGTAAGCTGCCCGTATAAA	24
G57	ATGGCTTTGGAAAGCGCAGTCGGCAGGT	28
G58	ACACTAAAAGAGATTTGT	18
G59	CAGTTAATGCCCCCTGCCTTCAAGAGAAGGATTAGGAT	38

Table S2. (*Continued*)

G60	TATTATTCTGAAACATAATAAGTTTTAACGGTACAGGA	38
G61	AACGCTTTGACCCCCAGCGATTATACGAAGGCACCAACCAAATACG	46
G62	CCATTTCGGGCCTTGAGTAACAGGTCATAC	30
G63	GAGGCTGGGATAAGTGCCGTCGAGAGACACTGAAAGTATTAA	42
G64	TAATGCCGGCTACAGAGGCTTCGGAACG	28
G65	AGGAAGTTTCCATTAATAAAGACTTTTTCATG	32
G66	TAGCGGGGTTTTGCTCAGTGGTGTATCACCGTACTCAG	38
G67	TATAGCCCCGCCACCCTCAGAACCGCGCCAGGTTGATATAAG	42
G68	AGGGTAGTTGCAGGGAGTTAAATATTCGAGTTGCGATT	38
G69	CCCTCAGCAGCGAAAGTTTTGCGGGATCGTCA	32
G70	GAGGTTTAGTACCGCCACCTCAGGGATAGCA	31
G71	GAACGGAATAACCAGGCAGACT	22
G72	CGCACAGCATTGAGGACACGGGTATAA	27
G73	TCGCCCACGCATAACCGATAGGC	23
G74	AGCCCAATAGGATAGCATTCCACA	24
G75	CAGTACATCTAAAGTTTTGGGTTTAACATAAAAACAGG	38
G76	TCTTAGGAGCCAAATCTCAAGGAACAACT	29
G77	CAGCTTGCTTTCGAGGTGACCGACAATTTA	30
G78	GACAGCCCTCATATTTTCTGTATG	24
G79	TCTTTCCAGTTTCAGCGGAGTGACAAAACGC	31
G80	AATAATTTTTTCACGTTGATTTAATTGAGA	30
G81	GGATTTTGCTAAACAACTTTACCC	24
G82	AAAAAATATTCAGAAAATTGTTCCAGTTTGGGTGGTTT	38
H1	CGAATTCGTAATACACAAAATGCGACAGTTTGCCATCACCG	41
H2	ATTCATTTGAATTACCTTTTTAAACCAGAGACCCTCAGAACCGCCCA	47
H3	TTCATTACATTTTGGGTTATTCATAAAGAGCCACCGAA	38
H4	CTAGCATGTCAAAAAATGCCTGAGTAATCTCA	32
H5	ACAAGAAAAATAGCGCCAACG	21
H6	CCATCCTAATTTACAACGCGAGGCGTTTTAGCGCGATATATC	42
H7	TAAAACAGTTCATTGAATTTAGACTGGA	28
H8	TATTGCTCCCTCCAAAAAACAGCTTGTCAC	30
H9	AATAATGTTAGCAAAAATAGTAAAA	25
H10	ACACTATCATTTGGTCAGTTGAGATTTAGGAATGTAAGAGCA	42
H11	GATTGAGGGAGTCACAATCA	20
H12	TTCAGAAGGCTCATTATACCAGTCAGGTTACAGGTAGAAAGA	42
H13	ACACTATAAGGCTGCGCAACCCGAGCT	27
H14	GATATTCATTACCCAAATCAACCTTCATCGGAA	33
H15	TCAAAATCACCGGACCTCCGGCTGA	25
I1	CTCATGGTCATAGCTGTTTCCGGGAGAG	28
I2	AAATTATGGAAGGGTTAGAAC	21
I3	ATGTACCCCGGTTGATAAATGTGAGCGAGTAACATATATCAT	42
I4	CTCAACAAATTCTTACCAGTAACTATAT	28
I5	TATATTTTAACGAGCTGAAAAG	22
I6	TCCCGACTTTACAGAGAGAATTGATAA	27
I7	AAACCGAGGAAGTCCACTATTAAAGTTGAGGA	32
I8	TAGGCAAAAGAAGTTTTCCAGACGAC	26
I9	TAGAAAAAAGTTTATTAACCCTCGTTTAGCCAGAGGGGGTCG	42
I10	ATAGACGGAATTACATACGCAGT	23
I11	TTAACGTTGGATTACCTTAA	20
I12	ACTGCCCGTAATCAGCAGAACGAGTAGTATGCGATTTTAAGA	42
I13	GCGAATCAAGGCAGCACAGCAAAATCACCAGCATTTGG	38
I14	AGAGGACAGATCTTGACAAGATTTAACCCGT	31
I15	TTACAACTGCTCCATGTTACTTAGCAACCGAACTGACCAACT	42
I16	CACAAACCCTCAGAGCCGCCACACCCTC	28
I17	GTGTACTAAATCCTCATTAAATGATATT	28
I18	CCTCAGAGCACACTGAGTTTCGAGGAACC	29