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Connecting DNA origami structures using the biotin-streptavidin specific binding

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This work made use of the strong interaction between biotin and streptavidin to connect designed DNA origami structures. The caDNAno software was used to design a 6 layer 3D origami cross-like structure. Selected DNA strands at the engineered attachment sites on the DNA origami structure were biotinylated. After folding of the origami structures, the biotinylated strands stick out of the attachment sites. Purified samples of origami structures were then mixed with streptavidin and the mixture purified. After characterization, we see that attachment only occurs at the biotinylated sites. Agarose gel electrophoresis, UV-vis spectroscopy and TEM were used to characterize the structure.

Key words: DNA origami, interaction, biotin-streptavidin, nanomaterials, TEM.

INTRODUCTION

The specific binding of bases is exploited to self-assemble DNA which gives a large amount of control over nanoscale devices assembly. Seeman (1982, 2003) laid down the theoretical model that allowed the use of DNA as a building material for the construction of devices at the nanoscale. DNA has the capacity to be programmed for self-assembly and has also a high stability making it possible to be used in device construction. There are a large number of materials ranging from metals, semiconductors to biological materials that can chemically be attached to DNA. Researchers have used DNA to construct a large number of composite structures (Chen and Seeman, 1991; Ekani-Nkodo et al., 2004; Fu and Seeman, 1993; Hou et al., 2005; Li et al., 1996; Liu et al., 1999; Winfree et al., 1998). The search continued to build miniaturized structures to design advanced materials with high performance. Rothemund (Rothemund, 2006) came out with the versatile, robust and significant DNA origami method which could be used to construct both 2-D and 3-D structures. The DNA origami method encompasses the folding of a long single-stranded scaffold DNA by shorter single-stranded staple DNA sequences. The mixture is then heated and annealed at room temperature for

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Abbreviations: EDTA, Ethylenediaminetetraacetic acid; **TEM,** Transmission electron microscopy.

several hours or days depending on whether single- or multi-layered structures are involved (Douglas et al., 2009a; Douglas et al., 2009b).

Rothemund was the first to demonstrate the functionalization of DNA origami surfaces (Rothemund, 2006). Since then other researchers have used varying arrays of nanomaterials to functionalize origami surfaces. This is made possible by the use of sticky ends which protrude on the surface. Nanomaterials which are functionalized with complementary sequences are then made to hybridize with these sticky ends. In so doing they attach themselves on the surface. The covalent bond between gold and sulfur is employed in the case of gold. Several groups have made use of this approach (Amoako et al., 2013; Ding et al., 2010; Maune et al., 2010, Pilo-Pais et al., 2011; Shen et al., 2012) to functionalize origami structures. Several groups (Jungmann et al., 2011; Lavella et al., 2012) have made use of the strong biotin-streptavidin interaction to functionalize DNA and DNA origami structures.

Selected staples are modified and extended with biotin making it possible for streptavidin binding with the DNA strand or DNA origami structures. Li et al. (2004) used this interaction to control the templating of two forms of triple crossover molecules through self-assembly. Lyonnais et al. (2008) also used the interaction to conjugate DNA and carbon nanotubes. Eskelinen (Eskelinen et al., 2011) and fellow workers have also used the biotin-streptavidin interaction to assemble carbon nanotubes on DNA origami. In order to reconfigure DNA origami pliers, Kuzuya (Kuzuya et al., 2011) and colleagues used the strong binding biotin-streptavidin interaction.

All these researchers made use of the biotin-streptavidin interaction to functionalize the DNA strand or DNA origami structures. In this work, we demonstrate the use of this interaction to connect two or more DNA origami structures. The square lattice caDNAno (Ke et al., 2009) software was used to design a six layered 3D origami cross-like structure. The connecting sites with DNA strands extended with biotin were designed at the two ends of the long axis of the cross-like origami structures. By means of streptavidin addition to the DNA origami solution, the origami structures are extended.

MATERIALS AND METHODS

Chemicals and supplies

Ethylenediaminetetraacetic acid (EDTA), utrapage purified DNA oligonucleotides, streptavidin and DNA oligonucleotides extended with biotin were purchased from Sangon Biotech (Shanghai, China) Co. Ltd. Tris(hydroxymethyl) aminomethane (Tris), Agarose M, and magnesium acetate tetrahydrate ($(CH_3COO)_2Mg{\cdot}4H_2O$) were obtained from Bio Basic Inc (Markham, Canada). NA-red, and 6X loading buffer were bought from Beyotime Institute of Biotechnology (Haimen, China). Wide range DNA marker was purchased from Takara Biotechnology (Dalian, China) Co. Ltd. The single-stranded viral genomic DNA M13mp18 used in the experiments was purchased from New England Biolabs (Ipswich, UK). We purchased boric acid (H_3BO_3), magnesium chloride ($MgCl_2$), and acetic acid ($C_2H_4O_2$) from Sinopharm Chemical Reagent (Shanghai, China) Co. Ltd. Freeze 'N' Squeeze DNA gel-extraction spin columns were bought from Bio-Rad Laboratories Inc. (Hercules, USA). Carbon copper grids and mica were purchased from Beijing Zhongjingkeyi Technology Co. (China) Ltd. and finally uranyl acetate ($UO_2(CH_3COO)_2{\cdot}2H_2O$) was purchased from Structure Probe, Inc. (Beijing, China).

Folding and purification of DNA origami cross-like structures

Our sample was prepared based on the procedures outlined in (Castro et al., 2011) with slight changes to the annealing process by combining 10 nM scaffold (M13mp18), 100 nM of each of the 178 staple oligonucleotides which were used without further purification, buffer and salts including 5 mM Tris, 1 mM EDTA (PH 7.9 at 20°C), and a magnesium screen covering 7 different concentrations from 12 mM at 2 mM intervals to 24 mM $MgC1_2$. Folding was carried out by rapid heat denaturation to 65°C followed by slow cooling from 65 to 60°C over 50 min, then 60 to 24°C over 72 h. We performed electrophoresis on samples using 2% Agarose gel (0.5X Tris/Borate/EDTA (TBE), 11 mM $MgC1_2$, 10 µL NA-red) at 70 V for 3.5 h in an ice-water bath. Discrete bands were visualized with UV trans-illuminator (Peiqing JS-680B). The desired bands were physically excised, crunched and filtered through a Freeze 'N' Squeeze spin column at 4°C for 10 min at 16000 ×g.

TEM imaging

Transmission electron micrographs were obtained with a HITACHI H-7650B TEM (Hitachi, Japan). A 3 µL DNA origami sample solution was deposited onto the carbon-coated side of the TEM grid and allowed to adsorb for about 5 min. The sample-side of the grid was then immersed in a 2% uranyl acetate stain-solution droplet and incubated for 40 s. Excess liquid was dabbed off with the edge of a filter paper, and the grid allowed to dry completely. Images were taken at 80.0 kV accelerating voltage.

RESULTS AND DISCUSSION

The square lattice based caDNAno software was used to design a 3D DNA origami cross-like structure (since the structure resembled a cross). Our design consisted of an overall 72-helix bundle which was all used to form the structure. The design consists of six layers having a total height of 12 nm. The width of the design consists of 12 helices thus the design is 24 nm wide. We estimated the length of the design from caDNAno to be approximately 38 nm. Therefore, the cross-like origami structure has approximate dimensions of 12 × 24 × 38 nm. Figure 1a shows a model depiction of the cross-like structure as designed. Figure 1b shows the folding scheme of the DNA origami cross-like structure. To prevent base stacking of the structures, we removed all the end sequences. We prepared a magnesium screen covering seven different concentrations from 12 mM at 2 mM intervals to 24 mM $MgCl_2$. The quality of folding was assessed by running a 2% agarose gel electrophoresis of the folded structures. The origami solutions containing the respective $MgCl_2$ salts are shown in Figure 2a. Lanes

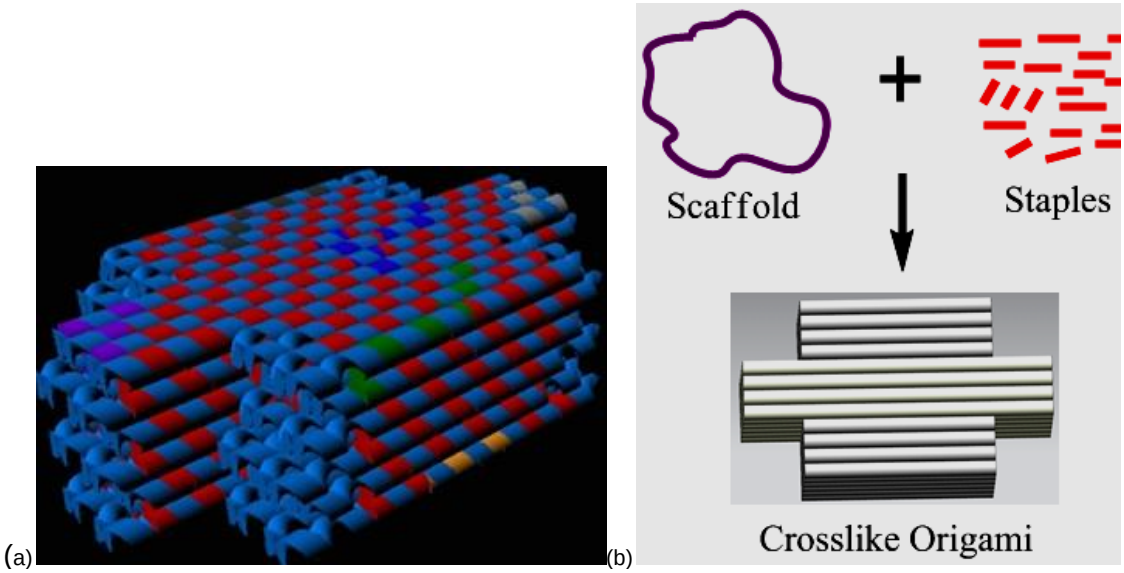

Figure 1. a. Model depiction of the 3D DNA origami cross-like structure. The scaffold strand is shown in light blue color, while the other colors represent the staple strands. **b.** Folding scheme of the DNA origami cross-like structure.

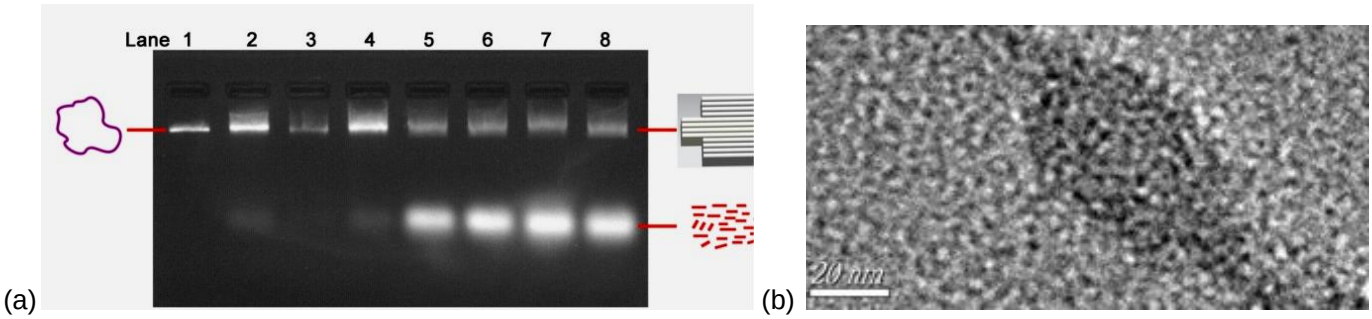

Figure 2. 2% agarose gel electrophoresis of the samples in the magnesium screen. i) Lane 1 contains M13mp18, ii) lanes 2 to 8 contained, respectively solutions from 12 to 24 mM Magnesium salts. The leading bands of lanes 2 to 8 were excised and purified for our objects and under the bands are excess staples. (b) TEM image of the 3D DNA origami cross-like structure with the scale bar indicated. Scale 20 nm.

2 to 8 in Figure 2a contained respectively 12 mM^{-24} mM $MgCl_2$. The band that contained the 12 mM salts was the fastest migrating and also the clearest. We physically excised this band and purified the structures via centrifugation using freeze 'n' squeeze DNA gel extraction spin columns. We performed transmission electron microscopy (TEM) on the purified structures and realized that the structures folded in 12 mM $MgCl_2$ yielded the best results. The TEM image of a single cross-like structure is shown in Figure 2b. In the figure, we see the clearly formed cross-like structure. Since the size of the single structure was very small, it was difficult imaging them. The length and width of the structure were determined from the TEM to be ~36 and ~25 nm. The length and width were caDNAno designed to be ~38 and 24 nm, respectively. The slight deviations could be attributed to the preparations on the TEM grid.

The use of biotin-streptavidin as a linking method has a long history. Biotin is a water soluble B-vitamin and is present in all living things in minute amounts. Biotin is a very small molecule and when used in biotinylation, does not usually alter many properties of the structures (Diamandis and Christopoulos, 1991). Streptavidin is a 52 kDa protein found in *Streptomyces avidinii*. Streptavidin is a symmetric tetramer which forms a brick with dimensions of 6 × 5 × 4 nm and a pair of biotin binding sites per each of the two 6 × 4 nm faces (Ringler and Schulz, 2003). It does have four high affinity binding sites for biotin and the binding of biotin to streptavidin is one of the strongest non-covalent interactions known

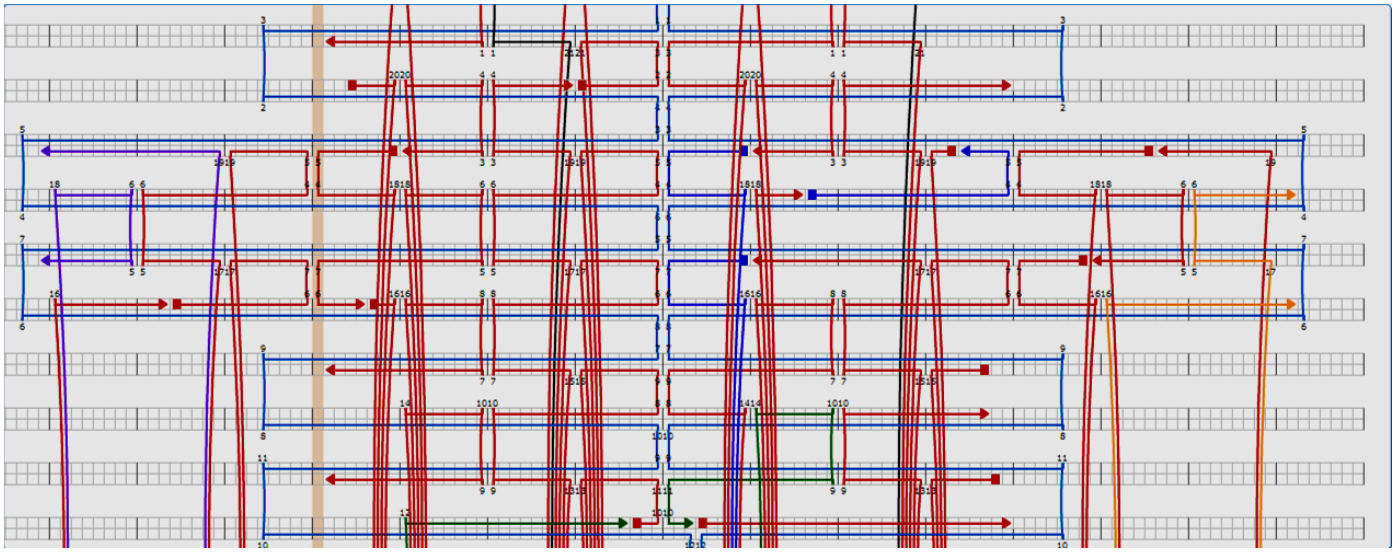

Figure 3. caDNAno interface of a section of the Path panel showing two designed connection sites (shown with violet and yellow colors).

in nature. The high affinity constant of interaction of biotin with streptavidin is about 10 times greater than the interaction of ligands with specific antibodies which ensures that the complex is stable even in harsh conditions of pH changes and multiple washings. The binding of biotin to streptavidin is very specific such that the binding is directed only to the target. One of the participating components in the biotin-streptavidin system should always be biotinylated (Diamandis and Christopoulos, 1991). Many scientists have utilized this biotin-streptavidin high affinity interaction to direct several processes and applications (Chiruvolu et al., 1994; Eskelinen et al., 2011; Lyonnais et al., 2008; Qi et al., 2005; Ringler and Schulz, 2003). Many of the research associating DNA origami with the biotin-streptavidin interaction has focused on decorating the origami template with other functional materials (Eskelinen et al., 2011; Lyonnais et al., 2008).

In order to connect the DNA origami cross-like structures using biotin-streptavidin interaction, we designed the sites where the connections were to be made. These connection sites were designed at the two extreme ends of the long axis of each of the cross-like structure. We designed two DNA single strands at each connection site. These two single strands were biotinylated at the ends (3' ends) that extend outside the origami structure as shown in figure 3. The connection sites are shown in Figure 3 indicated with violet and yellow colors. The two designed DNA single strands at each of the connection sites were extended with an 8 base sequence ATGCATGC for sufficient flexibility to the DNA strands so that the structure will not be strained. This is significant since it makes it easier for biotinylated strands from two structures to have easy access to the binding streptavidin. To connect the cross-like structure to another cross-like structure at the two positions indicated with violet and yellow colors in figure 3, all the generated sequences from caDNAno were used in addition to the sequences from the two designed connection sites that are extended with biotin.

Figure 4 shows the connecting process of three DNA origami cross-like structures using biotin-streptavidin interaction. After annealing and purification of the folded cross-like structures, we added a 20 µM 20 µl solution of streptavidin to a purified 20 µl solution of the origami cross-like structures and the mixture was incubated overnight at 4°C. After incubation, unbound streptavidin was removed using spin column filtration. The resulting solution was again washed with EDTA. After washing, the solution was mixed with purified 20 µl of the origami solution and the mixture incubated for two days at 4°C. Since streptavidin has four binding sites to biotin, one streptavidin molecule will bind two cross-like structures together. When streptavidin was added to the annealed DNA origami solution, two of its binding sites bonded with the two biotin molecules attached to the DNA origami template at one side. The same process also happens at the opposite side of the DNA origami template. In all the situations, two binding sites on the streptavidin are left free to bind to the biotin attached to the new DNA origami structure. We characterized the structures with TEM.

Figure 5 shows the TEM images of the connected structures at three different resolutions. We see in the images (Figure 5a to 5c) that the structures are only connected at the sites (ends of the long axis) where we have biotinylation. This shows that the connection came about through the biotin-streptavidin interaction and not base stacking interactions since we removed all the end sequences that are responsible for base stacking interactions. Since we biotinylated both lateral ends of the

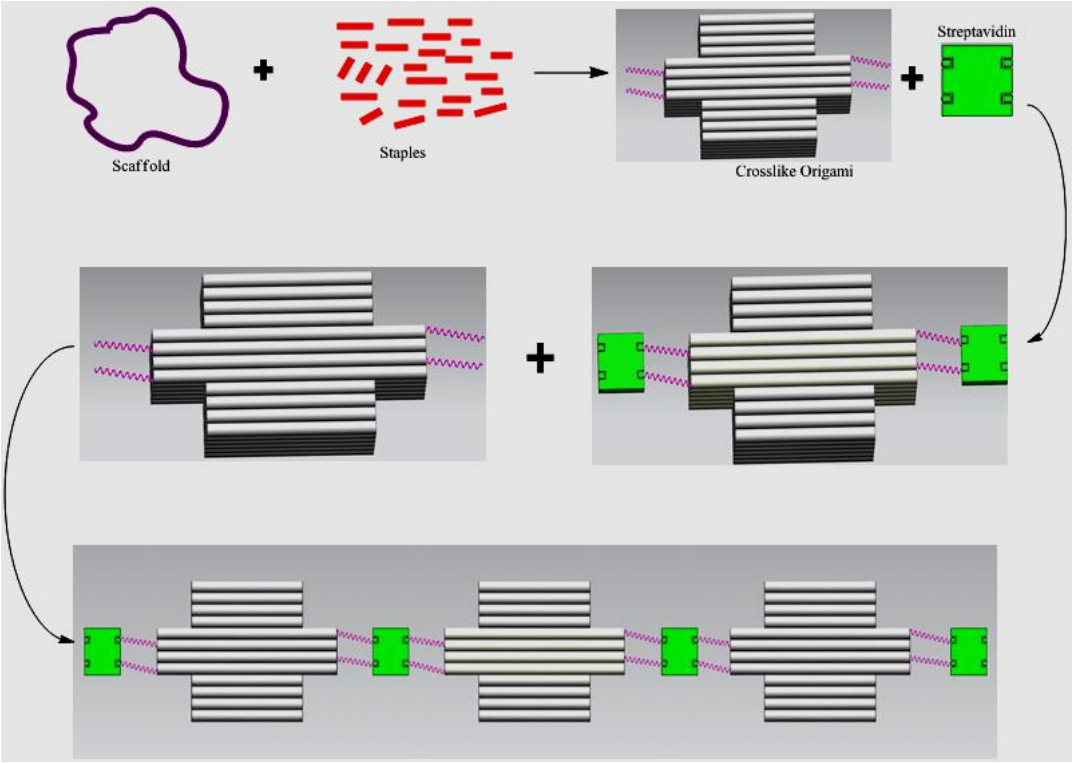

Figure 4. Design schematic of connecting three DNA origami cross-like structures together using the biotin-streptavidin interaction. As a first step, DNA origami cross-like structures (folding of scaffold by staple strands) biotinylated on two opposite sides with four different DNA strands are mixed with streptavidin to form the origami-streptavidin complex. The origami-streptavidin complex is purified to remove excess streptavidin and mixed with biotinylated origami structures to form the connected origami structures.

cross-like structure, it is possible for the connections to continue until both biotinylated strands and attached streptavidins are used up (Figures 5). Figure 5a shows only two DNA origami cross-like structures connected with a total length of approximately 69 nm. Figures 5b and 5c show the connection of five and four origami structures, respectively. The approximate lengths determined from the TEM images are respectively 207 nm for the five cross-like structures in Figure 5b and 145.5 nm for the four structures in Figure 5c. These lengths compare favorably with the length determined from caDNAno which has already been stated. We also observed that longer chains were not formed as we had assumed from the design. This could be that the streptavidin used was not enough. The fact that longer chains were not formed may also be due to the incubation period which might be short.

Conclusion

DNA origami structures can bind end-to-end through sticky end interactions. These interactions are always undesirable and efforts are made to remove them. Even if these interactions are needed, they are not strong enough. We have demonstrated the use of the biotin-streptavidin interaction to connect DNA origami structures even though longer chains were not realized. This interaction is very strong and could be used to form DNA origami networks. Assembly of two different configurations has been shown. These configurations consist, respectively, of two, four, and different cross-like origami structures which are connected together end-to-end. It was difficult quantifying the overall yields of our structures, but it was easy to find many structures on the TEM grids sufficient for our characterization analyses.

Conflict of interests

The authors did not declare any conflict of interest.

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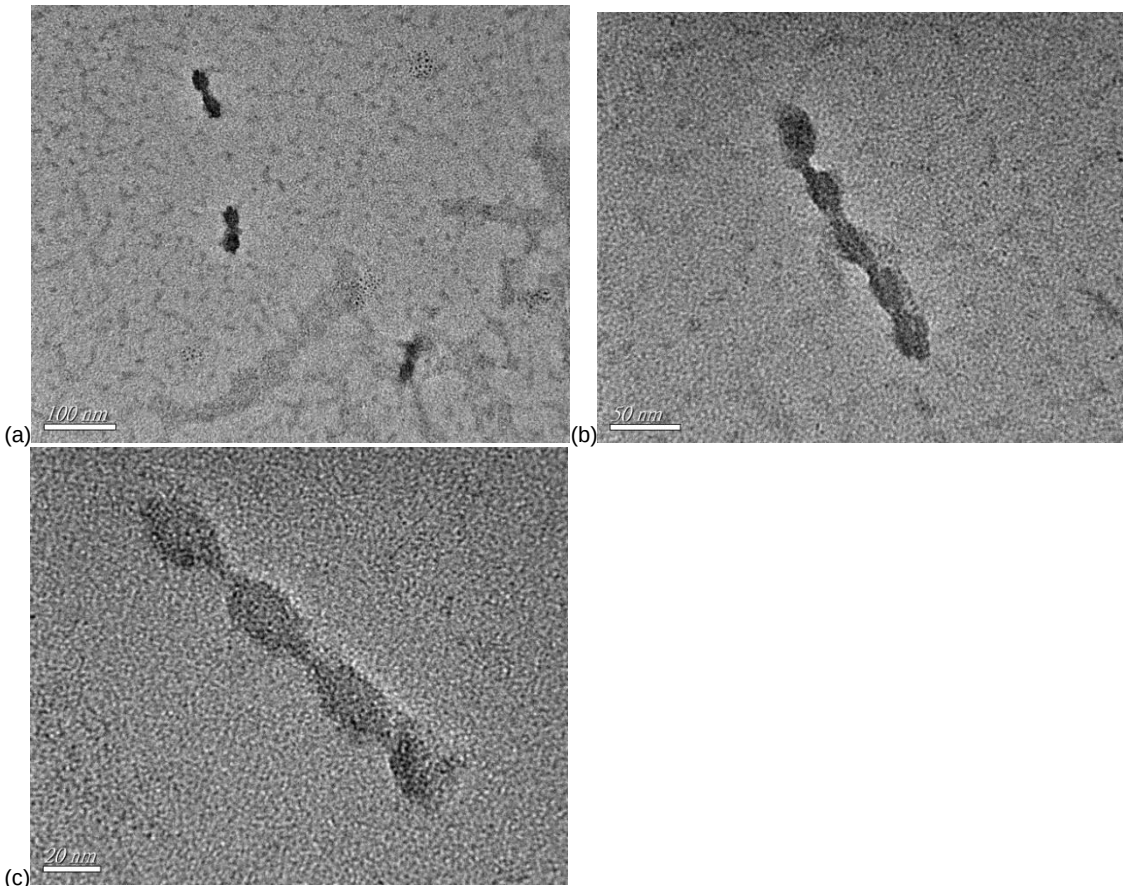

Figure 5. TEM images of the connected origami structures at two opposing connecting sites. Different scales (a) 100 nm, (b) 50 nm, and (c) 20 nm showing the lateral connections of the structures.

REFERENCES

Amoako G, Ye R, Zhuang L, Yang X, Shen Z, Zhou M (2013). DNA origami site-specific arrangement of gold nanoparticles. Nano 8: 13500641-135006411.

Castro CE, Kilchherr F, Kim D-N, Shiao EL, Wauer T, Wortmann P, Bathe M, Dietz H (2011). A primer to scaffolded DNA origami. Nat. Methods 8:221-229.

Chen JH, Seeman NC (1991). Synthesis from DNA of a molecule with the connectivity of a cube. Nature 350: 631–633.

Chiruvolu S, Walker S, Israelachvili J, Schmitt F-J, Leckband D, Zasadzinski JA (1994). Higher order self-assembly of vesicles by site-specific binding. Science 264:1753-1756.

Diamandis EP, Christopoulos TK (1991). The biotin-(strept) avidin system: Principles and applications in biotechnology. Clin. Chem. 37:625-636.

Ding B, Deng Z, Yan H, Cabrini S, Zuckermann RN, Bokor J (2010). Gold nanoparticle self-similar chain structure organized by DNA Origami. J. Am. Chem. Soc. 132:3248-3249.

Douglas SM, Dietz H, Liedl T, Hogberg B, Graf F, Shih WM (2009b). Self-Assembly of DNA into nanoscale three-dimensional shapes. Nature 459:414-418.

Douglas SM, Marblestone AH, Teerapittayanon S, Vazquez A, Church GM, Shih WM (2009a). Rapid prototyping of 3D DNA-origami shapes with caDNAno. Nucleic Acids Res. 37:5001-5006.

Ekani-Nkodo A, Kumar A, Fygenson DK (2004). Joining and Scission in the Self-Assembly of Nanotubes from DNATiles. Phys. Rev. Lett. 93:268301-268304.

Eskelinen A-P, Kuzyk A, Kaltiaisenaho TK, Timmermans MY, Nasibulin AG, Kauppinen EI, Törmä P (2011). Assembly of Single-Walled Carbon Nanotubes on DNA-Origami Templates through Streptavidin–biotin interaction. Small 7:746-750.

Fu TJ, Seeman NC (1993). DNA double-crossover molecules. Biochemistry 32:3211-3220.

Hou S, Wang J, Martin CR (2005). Template-Synthesized DNA Nanotubes. J. Am. Chem. Soc. 127: 8586-8587.

Jungmann R, Scheible M, Kuzyk A, Pardatscher G, Castro CE, Simmel FC (2011). DNA origami-based nanoribbons: assembly, length distribution, and twist. Nanotechnology 22:275301.

Ke Y, Douglas SM, Liu M, Sharma J, Cheng A, Leung A, Liu Y, Shih WM, Yan H (2009). Multilayer DNA Origami Packed on a Square Lattice. J. Am. Chem. Soc. 131:15903-15908.

Kuzuya A, Sakai Y, Yamazaki T, Xu Y, Komiyama M (2011). Nanomechanical DNA origami 'single-molecule beacons' directly imaged by atomic force microscopy. Nat. Commun. 2:449-456.

Lavella GJ, Jadhav AD, Maharbiz MM (2012). A Synthetic Chemomechanical Machine Driven by Ligand–Receptor Bonding. Nano Lett. 12:4983-4987.

Li H, Park SH, Reif JH, LaBean TH, Yan H (2004). DNA templated self-assembly of protein and nanoparticle linear arrays. J. Am. Chem. Soc.126: 418-419.

Li XJ, Yang XP, Qi J, Seeman NC (1996). Antiparallel DNA double crossover molecules as components for nanoconstruction. J. Am. Chem. Soc.118: 6131-6140.

Liu F, Sha R, Seeman NC (1999). Modifying the surface features of two-dimensional DNA crystals. J. Am. Chem. Soc. 121:917-922.

Lyonnais S, Goux-Capes L, Christophe Escude, Cote D, Filoramo A, Bourgoin J-P (2008). DNA–carbon nanotube conjugates prepared

by a Versatile Method Using Streptavidin–Biotin Recognition. Small 4:442-446.
Maune HT, Si-ping H, Barish RD, Bockrath M, Goddard III WA, Rothemund PWK, Winfree E (2010). Self-assembly of carbon nanotubes into two-dimensional geometries using DNA origami templates. Nat. Nano 5(1):61-66.
Pilo-Pais M, Goldberg S, Samano E, LaBean TH, Finkelstein G (2011). Connecting the Nanodots: Programmable Nanofabrication of Fused Metal Shapes on DNA Templates. Nano Lett. 11:3489–3492.
Qi K, Zhou C, Walker AV, Wooley KL, Jhaveri SB, Sogah DY, Malkoch M, Beinhoff M, Carter KR, Hawker CJ (2005). Biotin/streptavidin recognition on polymer brushes and self-assembled monolayers. Polymer Prepr. 46:363.
Ringler P, Schulz GE (2003). Self-Assembly of Proteins into Designed Networks. Science 302:106-109.
Rothemund P (2006). Folding DNA to create nanoscale shapes and patterns. Nature 440:297-302.
Seeman NC (1982). Nucleic acid junctions and lattices. J. Theor. Biol. 99:237–247.
Seeman NC (2003). DNA in a material world. Nature 421:427–431.
Shen X, Song C, Wang J, Shi D, Wang Z, Liu N, Ding B (2012). Rolling up gold nanoparticle-dressed DNA origami into threedimensional plasmonic Chiral nanostructures. J. Am. Chem. Soc.134: 146-149.
Winfree E, Liu F, Wenzler LA, Seeman NC (1998). Design and self-assembly of two-dimensional DNA crystals. Nature 394:539-544.