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Programmable Assembly of Gold Nanoparticle Nanoclusters and Lattices

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Abstract: Deterministic assembly of metallic nanoparticles (e.g. gold nanoparticles) into prescribed configurations have promising applications in many fields such as biosensing and drug delivery. DNA-directed bottom-up assembly has demonstrated unparalleled capability to precisely organize metallic nanoparticles into assemblies of designer configurations. However, the fabrication of assemblies comprising delicate nanoparticle arrangements, especially across large dimensions (e.g. micron size), has remained challenging. In this report, we have designed DNA origami hexagon tiles that are capable of assembling into higher-order networks of honeycomb arrays or tubes with dimensions up to several microns. The versatile addressability of the unit tile enables precise and periodic positioning of nanoparticles onto these higher-order DNA origami frame structures. Overall, we have constructed a series of 9 gold nanoparticle architectures with programmable configurations ranging from nanometer-sized clusters to micrometer-sized lattices. We believe these architectures shall hold great application potentials in numerous biomedical fields.

Keywords: DNA origami, bottom-up assembly, nanoparticle clusters, nanoparticle lattices

Metallic nanoparticle assemblies with well-defined configurations hold great promise in a variety of biomedical fields including drug delivery[1, 2] and biosensing.[3-5] Fabrication of high-quality, cost-effective, and programmable nanoparticle assemblies, however, remains a key challenge in the field of nanotechnology. Standard top-down fabrication methods, such as E-beam lithography, have relatively low throughput and/or limited precision. In contrast, bottom-up assembly of metallic nanoparticles, often directed by molecular ligands, offers large-scale fabrication capability and nanoscale precision. DNA is among the most potent molecular ligands due to its programmability derived from the specificity of Watson-Crick basepairing.[6-10] DNA has been used to construct increasingly complex structures,[11] particularly, the DNA origami technique allows for creation of fully addressable DNA structures with arbitrary sizes and shapes,[12-14] which have been used as frames to guide the assembly of various types of nanoparticles into custom-designed architectures.[15-27] Nevertheless, it is challenging to fabricate nanoparticle assemblies of sophisticated configurations, especially with large dimensions (e.g. micron size), due to the difficulty in assembling micron-sized DNA frames with versatile addressability. Herein, to address this challenge, we employed a DNA origami hexagon tile, which is able to self-assemble into addressable monomers and higher-order networks of micron-sized two-dimensional (2D) arrays and tubes.[21] These DNA origami structures have shown unprecedented

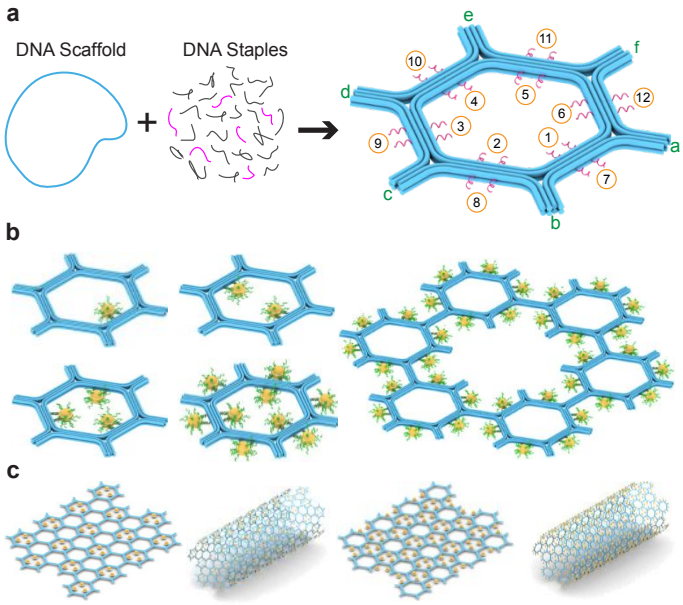

Figure 1. DNA origami design and assembly of gold nanoparticles. (a) Design of DNA origami hexagon tile. A total of 12 binding sites were designed, with 6 on the interior sides and 6 on the exterior sides of the hexagon. (b) Gold nanoparticle clusters assembled on the hexagon monomer and hexamer. (c) Gold nanoparticle lattices assembled on honeycomb arrays and tubes.

capability on directing the assembly of gold nanoparticles (AuNPs) forming a number of designer architectures including clusters and lattices.

The DNA origami hexagon tile (DHT) was designed and modified based on our earlier report[21, 27]. In the current design, both the interior and exterior sides of the hexagon were docked with one nanoparticle binding site, thus yielding a total of 12 per tile (Figure 1a, Figures S1, S2). Each binding site was composed of 2 single-stranded DNA extensions protruding from designated staple strands to capture complementary DNA-functionalized nanoparticles *via* sequence specific DNA hybridization (Figure 1a). AuNPs were anchored onto designated binding sites to form architectures of designer configurations. Connector strands were designed to bridge the tiles to induce hierarchical assembly forming oligomers or 2D arrays and tubes. To demonstrate the versatility of DHT frames on directing the intricate assembly of nanoparticles, we fabricated a group of 9 nanoparticle architectures with sophisticated yet programmable configurations, including clusters (Figure 1b) and lattices (Figure 1c). Comparing to previous reports[21, 27], the configuration complexity of nanoparticle architectures was significantly enhanced in the current work, in terms of number and disposition of nanoparticles within architectures (e.g. DHT hexamer architecture), which shall hold higher application potential with enhanced drug-loading and multiplexing capabilities.

AuNP clusters were programmed by precisely controlling the number, and location of nanoparticles. AuNP clusters of 4 different configurations were assembled on the DHT monomer by anchoring (Figure 2, Figure S3). TEM images unambiguously showed that 1 (Figure 2a), 2 (Figure 2b), 3 (Figure 2c) nanoparticles were anchored precisely inside the hexagon cavity. A number of 9 nanoparticles (Figure 2d) were observed at prescribed locations on the interior and exterior sides of DHT, indicating the successful construction of designed AuNP clusters. Additional TEM images of AuNP clusters on DHT monomer are included in Figures S4-S7. Statistical analysis revealed efficient assembly of AuNPs with 99%, 90%, 90%, and 36% yield for 1-, 2-, 3-, and 9-AuNP clusters, respectively. The relatively low yield of 9-AuNP cluster may attribute to the electrostatic repulsion between two neighboring nanoparticles on opposite sides of one edge.[23] Agarose gel electrophoresis also suggested the successful construction of 1-, 2-, 3-, and 9-AuNP clusters (Figure S3). In addition, DHT monomers can hierarchically assemble into hexamers by using connector strands. For instance, a

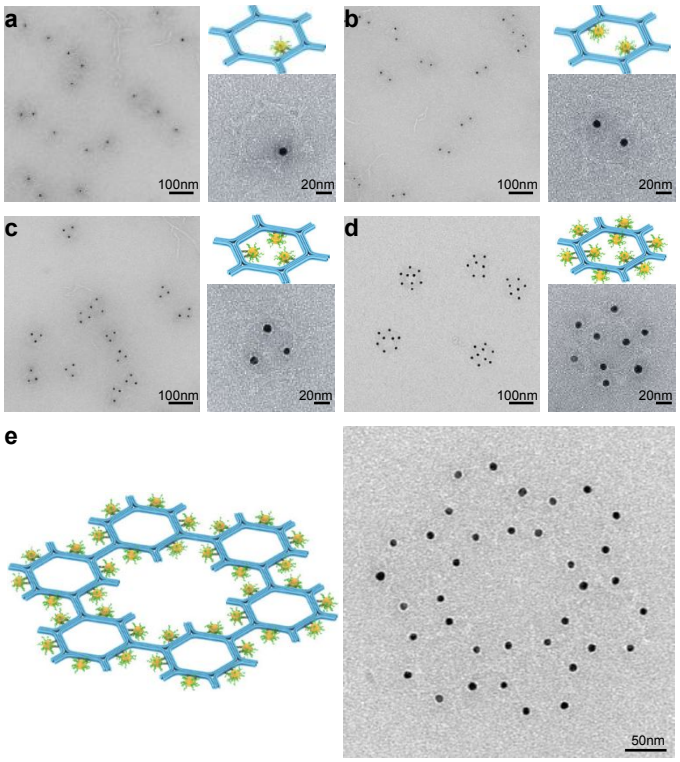

Figure 2. TEM imaging of gold nanoparticle clusters. (a-d) Gold nanoparticle clusters assembled on DHT monomer consisting of 1, 2, 3, and 9 nanoparticles, respectively. (e) Gold nanoparticle clusters assembled on DHT hexamer consisting of 36 nanoparticles.

total of 36 AuNPs can be assembled onto DHT hexamer (Figure 2e, Figure S8). TEM imaging showed that 36 designated number of AuNPs (10 nm in diameter) within the interior cavity or on the exterior sides AuNPs were precisely attached at the desired positions of DHT hexamer. Overall, these AuNP clusters exhibited prescribed configurations, suggesting the robustness and versatility of DHT for assembling nanoparticles.

To produce nanoparticle lattices, higher-order DNA origami frames including micrometer-sized honeycomb arrays and tubes were first assembled. AuNP lattices were assembled on honeycomb arrays and tubes *via* capturing AuNPs within designated cavities of the frames. A group of 4 AuNP lattices were fabricated, with AuNPs docked within either the intra-tile hexagon cavity (type-1 configuration) or the inter-tile hexagon cavity (type-2 configuration). (**Figure 3**, Figures S9-S12). TEM images showed AuNP lattices formed ordered structures on micron scale, with overall length ranged from1 μm to several micrometers. The arrangement of AuNPs in type-1 configuration (Figure 3a, b, Figures S9, S10) was more integrated than that of in type-2 configuration (Figure 3c, d, Figures S11, S12). This may attribute to its higher density of nanoparticle binding sites in type-2 configuration,

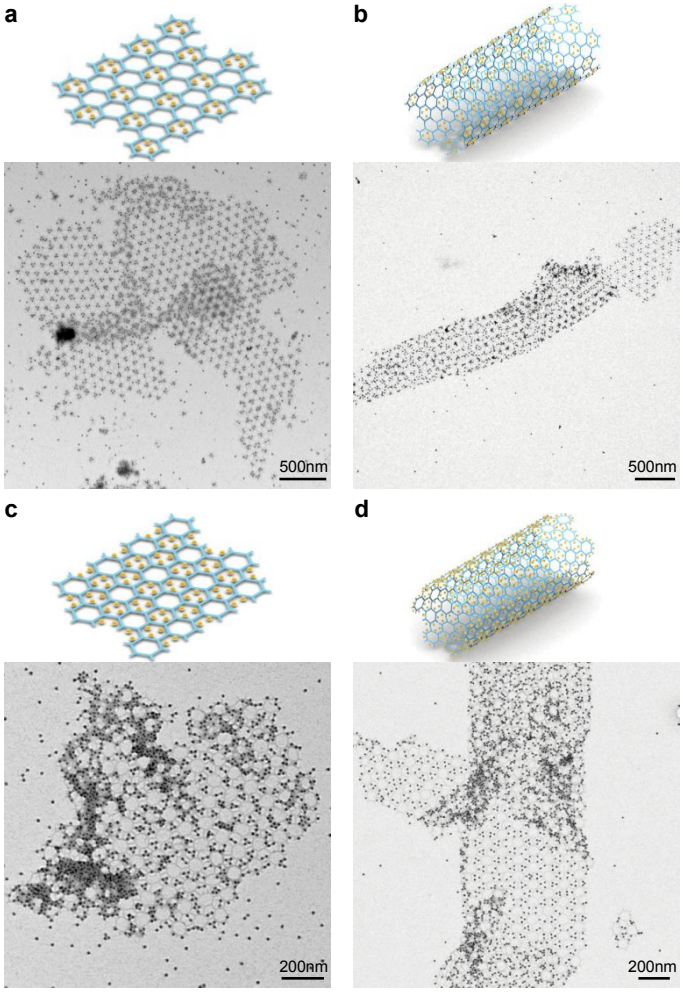

Figure 3. TEM imaging of gold nanoparticle lattices. (a, b) Gold nanoparticle lattices assembled on honeycomb array or tube with type-1 configuration. (c, d) Gold nanoparticle lattices assembled on honeycomb array or tube with type-2 configuration.

which therefore hold higher chance of having nanoparticles occupying more than one binding site to induce aggregation.

To investigate the biomedical application potential of nanoparticle architectures, we examined their stability in physiological buffers and cell culture medium at 37 °C (Figure S13). 9-AuNP cluster was used as the representative architecture for this study, which revealed that nanoparticle architectures could largely remain intact after incubated at 37 °C for 0.5 to 4 hours in TE/Mg^{2+} and PBS. The majority of nanoparticle architectures, however, were damaged even after 0.5 hour of incubation in RPMI-1640 medium supplemented with 10% FBS. The rapid severe damage of nanoparticle architectures in cell culture medium may be attributed to low cationic concentration-induced origami disassembly and DNase degradation of DNA, suggesting that nanoparticle architectures may not be directly administrated to cell culture medium or serum for applications.

Nevertheless, to circumvent this challenge, cations may be supplemented into heat inactivated medium to minimize DNA origami disassembly and degradation, as suggested by Shih and colleagues.[28]

In conclusion, we have demonstrated a versatile strategy to fabricate nanoparticle assemblies with programmable configurations of high fidelity across different scales. These architectures were constructed *via* deterministic assembly of nanoparticles onto DNA origami frames. We designed DNA origami hexagon tile, which were able to self-assemble into monomers, oligomers, arrays and tubes with the monomeric unit fully addressable for decorating nanoparticles. To validate its versatility and generality, we successfully constructed a set of 9 nanoparticle architectures with delicate configurations ranging from nanometer-sized clusters, to micrometer-sized lattices. Overall, these nanoparticle architectures fabricated in this report have easily programmable and yet sophisticated configurations that have enormous application potentials in a variety of biomedical fields including nanomedicine and biosensing.

Conflict of interest
The authors have no competing conflict of interest.

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