Assembly of Heterogeneous Functional Nanomaterials on DNA Origami Scaffolds**
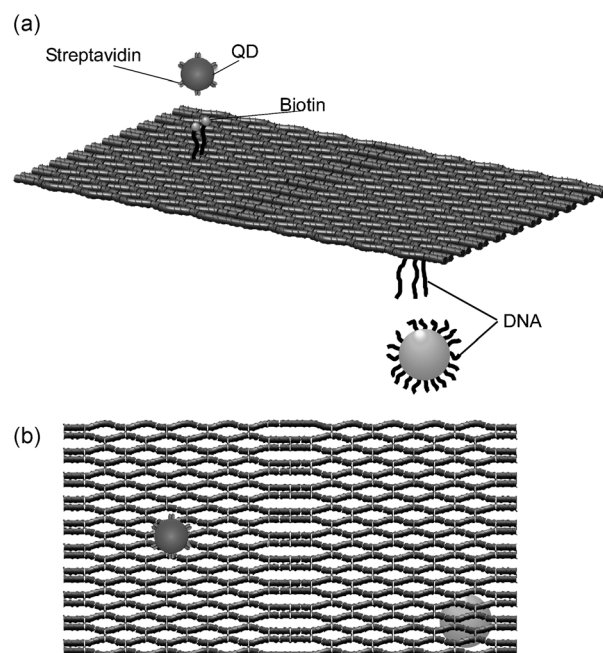
Risheng Wang,* Colin Nuckolls, and Shalom J. Wind*

Hybrid nanomaterial systems comprising different functional components have begun to receive significant attention in recent years.[1–9] The goal of such multicomponent systems is primarily to combine the functionalities of their constituents, which are typically metallic nanoparticles and semiconducting and/or magnetic quantum dots (QDs). These multicomponent systems may also display emergent properties that cannot be realized in homogeneous systems as a result of specific interactions between the components. To date, several different approaches have been taken to assemble multicomponent systems, including colloidal assembly;[2,6,9] thermal decomposition;[1] selective growth;[4,9] and DNA-mediated assembly,[3,8] including the formation of 3D superlattices.[7]

The distinctive programmability and versatility of DNA make it particularly attractive for coupling heterogeneous nanostructures. Indeed, binary complexes of Au nanoparticles (AuNPs) and QDs bound by double-stranded DNA have displayed unique photonic and optoelectronic properties that can be tuned by varying the length of the DNA duplex linker between them.[8,10] Thus, DNA-based assembly opens up tremendous possibilities for material design and new applications. However, the binding schemes used to date for this purpose rely on DNA duplexes, which lack mechanical rigidity. Furthermore, additional steps (e.g., gel electrophoresis) are required to manage the ratio between the particles. These issues will become even more difficult to manage with increasing heterogeneity and number of particles.

In this work, we demonstrate, for the first time, the organization of disparate functional nanomaterials, namely, semiconducting QDs and metallic nanoparticles, on a single DNA origami[11] template. The rigidity of the origami scaffold, along with the specific spatial addressability through defined binding sites of staple strands, provides precise control over the interparticle spacing. We also show that two different binding reactions on DNA origami can be accomplished using orthogonal reaction methods, and we optimize the conditions for such an approach.

DNA origami scaffolds[11] are a general platform for DNA nanotechnology. These structures are formed from a long single-stranded M13mp18 genomic (M13) DNA, which is folded into nearly any predefined forms with the help of short staple strands. Owing to the unique sequence of each staple strand, DNA origami is a fully addressable nanostructure that has been used as a molecular breadboard to spatially organize single nanoparticles.[12–16] Figure 1 shows our strategy for organizing both the quantum dot and the gold nanoparticle on opposite sides of a DNA origami structure. We used a rectangular DNA origami structure, approximately 120 nm × 60 nm on a side, and installed two types of anchors. One anchor consists of three DNA sticky ends (15 adenines) protruding from selected staple strands on one side of the origami to capture ssDNA-coated 10 nm AuNPs through DNA hybridization. The second anchor consists of two DNA staple strands that are biotinylated on the 5′ end and protrude

[*] Dr. R. Wang, Prof. S. J. Wind
Department of Applied Physics & Applied Mathematics
Columbia University
New York, NY 10027 (USA)
E-mail: rw2399@columbia.edu
      sw2128@columbia.edu

Dr. R. Wang, Prof. C. Nuckolls
Department of Chemistry, Columbia University
New York, NY 10027 (USA)

Figure 1. Schematic drawings of the assembly of CdSe QDs and AuNPs on opposite sides of a DNA origami scaffold. a) Streptavidin-coated CdSe QDs were attached to biotin-modified DNA anchors on one side, while ssDNA-wrapped 10 nm AuNPs were assembled to the other side through DNA hybridization. b) Schematic showing the binding position of QDs and AuNPs on the DNA origami scaffold.

with six random oligonucleotides from the opposite side of the origami template to capture streptavidin-coated CdSe QDs (the core size is ca. 5 nm)[12, 15] through the streptavidin–biotin interaction. These two anchors allow us to organize different types of differentially functionalized nanoscale objects by selective binding at the corresponding locations. Notably, this design enables the formation of sandwich-like three-dimensional DNA origami nanostructures. This strategy not only proves the functionality and versatility of a DNA origami scaffold as a two-dimensional structure, but also offers flexibility in varying the interparticle distance without regard to steric hindrance effects that can arise when closely spaced particles are placed on the same side.

To organize the nanoparticles on the DNA origami scaffold, the DNA origami template was first annealed[11] and then purified to remove the extra helper strands (see the Experimental Section). We optimized the binding yield of DNA-coated 10 nm AuNPs (named AuNPs hereafter) to the DNA origami template by varying the annealing protocols. First, the binding efficiency of the AuNPs was optimized as a function of annealing temperature. Freshly purified DNA origami rectangles were mixed with a solution of AuNPs (50 nM) at an elevated temperature, then allowed to slowly cool down to room temperature in a two-liter water bath for 18 h. Three different starting temperatures were used: 47°C, 37°C, and room temperature (that is no temperature control was used). We used atomic force microscopy to gauge the results of each protocol. We found that the binding efficiency of the AuNPs is strongly dependent on the annealing conditions; the efficiencies increased from 6% at room temperature to 98% at 47°C (see Figure S1 in the Supporting Information). We hypothesize that the dependence on annealing conditions is a consequence of the increased diffusion kinetics for both the DNA origami and the AuNPs, thereby increasing the contact probability and hybridization affinity between them; this hypothesis is consistent with previous results.[14]

The second attachment reaction involves the attachment of streptavidin-coated QDs (named QDs hereafter) to the DNA origami. This attachment reaction has been well-studied at room temperature.[12, 15] Figure S2 in the Supporting Information shows an AFM image of the assembly that resulted from first mixing the solution of QDs (50 nM) and freshly purified DNA origami and then incubating it at room temperature over 18 h. The QDs are selectively bound to the designed anchor position with high binding efficiency, thus indicating that room temperature is sufficient for the streptavidin–biotin interaction; this finding is consistent with previous work.[12, 15]

Based on the conditions found for the separate binding of the AuNPs and QDs to the origami scaffold, we tried a two-step annealing procedure for binding both: First, a mixture of rectangular origami with AuNPs was annealed from 47°C to room temperature. Then the DNA origami/AuNP product was mixed with QDs and maintained at room temperature for 18 h. The result, as measured by AFM, is shown in Figure 2a. We use AuNPs and QDs of different size, so they can be distinguished in AFM height scans (Figure 2b). The average center-to-center space between two particles is approximately
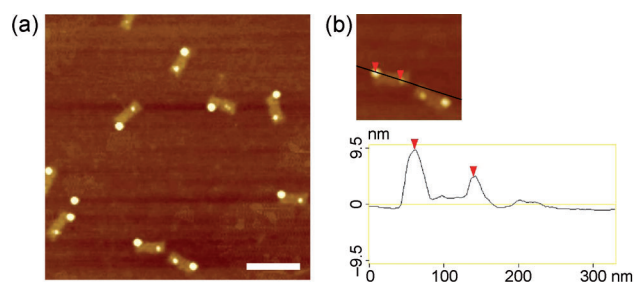

Figure 2. a) AFM image of the assembly of QDs and AuNPs on the rectangular DNA origami template. A two-step annealing process was used: AuNPs self-assembled to the origami template from 47°C to room temperature over 18 h, then the product of AuNP/origami assembly was incubated with QDs at room temperature, and the mixture was kept for another 18 h. The scale bar is 200 nm. b) Cross-section profile analysis showing the height difference between QDs and AuNPs. (Note: Owing to the strong adhesion of the DNA origami to the mica substrate, some deformation may occur; it is therefore difficult to discern in the AFM on which side of the scaffold a particle is located.)

75 nm, which is consistent with the design of the binding sites of the staple strands (this distance was chosen so that the AuNP and QD can be clearly resolved in the AFM).

To simplify the process and reduce overall processing time, we found experimental conditions that support assembly of multiple particles in a single step, that is, a process in which the DNA origami template, AuNPs, and QDs are directly mixed together in the same ratio as in the two-step annealing process. Three different annealing temperatures were tested. 1) When the mixture was annealed at room temperature, almost no AuNPs attached to the origami (Figure 3a). 2) An increase of the annealing temperature to 37°C resulted in an increase of the binding yield of both QDs and AuNPs to approximately 12% (Figure 3b). 3) A slow anneal from 47°C to room temperature increased the yield of the binding of both QDs and AuNPs further up to 83% (Figure 3c and Figure S3 in the Supporting Information). This yield is slightly lower than we found with the two-step annealing process, thus indicating that the mixture of two different moieties can interfere with the binding efficiency of each.

Interestingly, when only one species was bound to the origami, as can be seen for example in Figure 3c, it could be either a single AuNP or a single QD, that is, there was no particular preference for one or the other. Clearly, the low binding efficiency of AuNPs during the annealing processes at 25°C and 37°C (see Figure S1 in the Supporting Information) is the main barrier to achieving a high binding yield of both species together. Further research is needed to fully understand this aspect of the assembly mechanism. However, from this experiment, we demonstrate that the assembly of multiple types of nano-objects on DNA origami can be done in a convenient manner and on a reasonable time scale by optimization of the reaction conditions.

In summary, we have demonstrated, for the first time, the successful organization of quantum dots and gold nano-particles with precisely controlled positions on both sides of a DNA origami template. Both a two-step and single-step
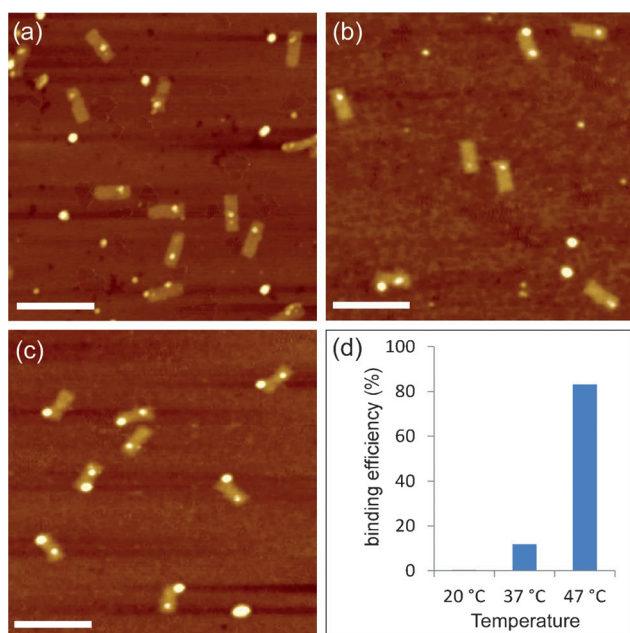
Figure 3. Single-step assembly of QDs and AuNPs on the DNA origami template as a function of annealing temperature. a) The mixture of QDs, AuNPs, and DNA origami was annealed at room temperature (25 °C) over 18 h. b) Annealed from 37 °C to room temperature over 18 h. c) Annealed from 47 °C to room temperature over 18 h. The scale bar is 200 nm. d) Binding efficiency of QDs and AuNPs on both sides of the DNA origami template. These values were calculated for both particles bound to the origami scaffold at the same time.

assembly process resulted in relatively high binding yields when the annealing process was optimized. This method has great promise, not only as a path toward the organization of more complex systems consisting of different materials, but also as a powerful tool to explore interactions between these materials in a site-specific manner.

Experimental Section

Self-Assembly of the rectangular DNA origami template: The DNA origami template was formed according to Rothemund[11]. M13 viral DNA and all the staple strands were mixed together at a 1:10 ratio, in a 1 × TAE buffer solution containing Tris-HCl (40 mM), acetic acid (20 mM), EDTA (2 mM), and magnesium acetate (12.5 mM). The DNA origami solution was slowly cooled from 90 °C to 16 °C with PCR over 1.5 h. The final concentration of M13 mp18 DNA in the solution was 10 nM. DNA origami was then purified to remove the excess DNA helper strands using centrifuge filters with a 100 kDa MWCO. The mixture of AuNPs with/or QD and DNA origami was annealed by incubating the sample in a 2-liter beaker containing heated water (47 °C or 37 °C) and letting it cool down slowly in an insulated (Styrofoam) box to room temperature over the course of 18 h.

Preparation of DNA-coated 10 nm AuNPs: The detailed procedure can be found in reference [13] with slight modification. Briefly, the 10 nm AuNPs were stabilized with adsorption of bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium (BSPP), then sodium chloride was added slowly to this mixture while stirring until the color

changed from deep burgundy to light purple. The resulting mixture was centrifuged and the supernatant was carefully removed. The concentrated AuNPs were resuspended in BSPP solution and concentration was estimated at approximately 520 nm. The thiol-functionalized single-stranded oligonucleotides (5′-TTTTTTTTTTTTTTTT-S) were first reduced by tris(2-carboxyethyl)-phosphine (TCEP) in water and subsequently purified using a G25 column (GE Healthcare) to remove the small molecules. Then thiol-modified oligonucleotides were mixed with phosphinated AuNPs at a 100:1 ratio in 0.5 × TBE buffer containing NaCl (50 mM) for two days at room temperature. AuNP–DNA conjugates were washed with 0.5 × TBE buffer using centrifuge filters with a 100 kDa MWCO to remove the extra oligonucleotides. The concentration of conjugates was estimated from the optical absorbance at approximately 520 nm.

Characterization of the AuNP–QD–DNA origami structure by atomic force microscopy: Sample solution (5 μL) was spotted onto freshly cleaved muscovite mica (Ted Pella inc) and absorbed for approximately three minutes. (Note, the physisorption of QDs on the origami template will happen with long-time incubation). To remove buffer salts, doubly distilled H$_2$O (20–30 μL) was placed on the mica, the drop was wicked off, and the sample was dried with compressed air. Atomic force imaging was done utilizing Nanoscope IV (Digital Instruments) tapping in air, with ultrasharp 14 series (NSC 14) tips purchased from MikroMasch (www.SPMTIPS.com).

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