DNA Nanotechnology

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Site-Specific Synthesis and In Situ Immobilization of Fluorescent Silver Nanoclusters on DNA Nanoscaffolds by Use of the Tollens Reaction**

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Fluorescent silver nanoclusters (AgNCs) of less than 2 nm in diameter have emerged as a new class of nanomaterials with potential for application in the nanosciences and nanotechnology.[1] A variety of methods have been demonstrated for the synthesis of fluorescent AgNCs,[2–14] among which the DNA-templated synthesis of AgNCs[13,15,16] is particularly attractive owing to the low toxicity, good biocompatibility, and unique optical properties of the AgNCs obtained. DNA nanostructures have also been envisioned as templates for metallization, for example, with gold or silver, to create nanowires with desired patterns or junctions for nanoelectronics.[17] However, site specificity and the uniform distribution of the metal NCs along the DNA templates remain a challenge, and these features are crucial for the homogeneity and efficiency of the subsequent metallization.

Herein we describe a new DNA-based method for the synthesis of water-soluble fluorescent AgNCs with a narrow size distribution by use of the well-known Tollens reaction, which is commonly employed in carbohydrate chemistry to test for the aldehyde functionality in reducing sugars.[17f] We covalently incorporated a small number of sugar moieties into a DNA sequence at adjacent positions and hoped that they would enable the synthesis of AgNCs by the specific stoichiometry of the Tollens reaction: that is, one aldehyde sugar molecule can reduce two Ag^+ ions to Ag^0_2. These Ag clusters could then act as nucleation sites for further Ag deposition under mild reductive conditions. Tethering of the sugar functional groups to DNA offers stabilization of the AgNCs synthesized,[15] and the DNA strands could serve as addressable points for further sequence-specific DNA hybridization. "DNA origami"[18] structures have become superior nanoscale scaffolds for the organization of various classes of functional materials.[19] Herein we demonstrate the site-specific synthesis and in situ immobilization of AgNCs on a triangular DNA origami[18] scaffold (Figure 1). The addressability of DNA origami enables the site-specific synthesis and in situ incorporation of fluorescent AgNCs on the predefined DNA scaffolds with nanometer-scale spatial resolution.

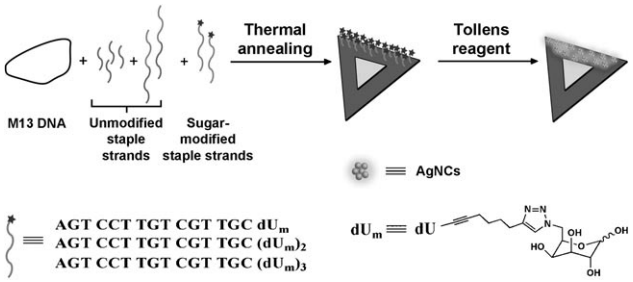

Figure 1. Schematic representation of the site-specific immobilization of fluorescent AgNCs on a triangular DNA origami scaffold and the corresponding sequences of the probe DNA strands used in this study (sequences are shown from the 3′ end (left) to the 5′ end (right)).

By a reported synthetic strategy,[20] we first synthesized the sugar(galactose)-modified DNA strands **DNA1**, **DNA2**, and **DNA3**, each of which contains 15 nucleotides and one, two, or three consecutive modified deoxyurinidine (dU_m) units, respectively; each dU_m unit carries a sugar unit. Details of the synthesis and structural characterization of **DNA1**–**DNA3** are given in the Supporting Information.

The Tollens reagent, $[Ag(NH_3)_2^+]$, was first prepared by adding NH_4OH (28%) to a solution of $AgNO_3$ in 1×TAE–Mg^{2+} buffer (40 mM tris(hydroxymethyl)aminomethane (Tris), 20 mM acetic acid, 2 mM ethylenediaminetetraacetic acid, and 12.5 mM $Mg(OAc)_2$) in the dark and then adding excess NH_4OH to dissolve the precipitated AgOH. The Tollens reagent was filtered and then added to the sugar-modified DNA (**DNA1**, **DNA2**, or **DNA3**) in 1×TAE–Mg^{2+} buffer, and the mixture was incubated overnight in the dark at room temperature. The reaction product with **DNA1** exhibited a fluorescence emission maximum at 412 nm and an excitation maximum at 337 nm. The corresponding products with **DNA2** and **DNA3** exhibited similar emission maxima at 411 ($\lambda_{ex,max} = 337$ nm) and 420 nm ($\lambda_{ex,max} = 337$ nm), respectively (Figure 2a). The fluorescence spectra clearly indicate the formation of similar-sized emissive AgNCs with the DNA strands carrying different numbers of sugar units. This

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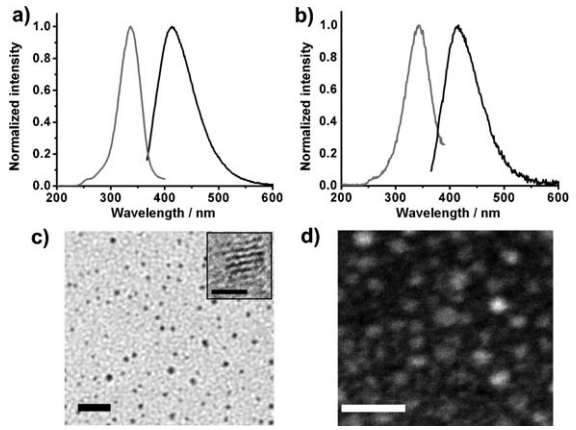

Figure 2. a,b) Excitation (gray) and emission (black) spectra of AgNCs synthesized by the treatment of free **DNA3** (a) and DNA origami (b) with the Tollens reagent. c) TEM and d) STEM images of NCs synthesized by using free **DNA3**. The inset in (c) is a high-resolution TEM image of a nanocluster. Scale bars: 10 nm (c and d) and 2 nm (c, inset).

observation is not as expected on the basis of the simple stoichiometry of the Tollens reaction. We propose that further growth of the silver cluster occurs following the formation of the initial Ag^0_n ($n = 2$, 4, or 6) seed created by the Tollens reaction, and that the particle size finally attained depends on the Ag^+/DNA ratio, irrespective of the number of sugar units present on the DNA.

No significant change in the emission of the NCs was observed even after several days of incubation. Thus, the AgNCs show excellent photostability under these conditions. For all three reaction products, no characteristic Ag plasmon absorption band at around 400–450 nm was observed. The absence of this band indicates that no larger Ag nanoparticles (NPs) were formed. Transmission electron microscope (TEM) and scanning transmission electron microscope (STEM) images (Figure 2c,d) showed that the NCs were nearly monodispersive with an average size of approximately 2 nm. The analysis of TEM images of the NCs obtained with **DNA1** and **DNA2** confirmed that these NCs had similar sizes (see the Supporting Information), in agreement with the similar optical properties observed for the NCs. We speculate that the mechanism for the formation of AgNCs involves the initial reduction of Ag^+ by the sugar units, followed by further reduction of extra Ag^+ ions by Tris in the 1 × TAE buffer solution. The maximum size of the cluster would depend on the initial molar ratio of Ag^+ to the sugar-modified DNA. We obtained kinetic evidence in support of this proposed mechanism by varying the Tris concentration (see the Supporting Information). It is estimated that an AgNC of 2 nm in diameter contains about 200 Ag atoms. In our experiment, we used a 200-fold excess of Ag^+ ions relative to the concentration of DNA. Hence, the expected size of the NCs of about 2 nm is in good agreement with our hypothesis.

We carried out further experiments to immobilize these fluorescent NCs site specifically on a triangular DNA origami structure. The DNA M13mp18 (7249 nucleotides) scaffold strand was mixed with a fivefold quantity of helper strands (total 205 helper strands) and a 650-fold quantity of **DNA3** strands in 1 × TAE–Mg^{2+} buffer. Roughly one third of the total number of helper strands (65 out of 205) located on one arm of the triangle were extended at the 3′ end to carry an additional 15 base segment of the DNA sequence complementary to that of **DNA3** (Figure 1). Therefore, after annealing, the sugar-modified strands hybridized with these probe strands to form DNA duplexes, each of which carried three sugar moieties, which were displayed on the surface of the triangular DNA origami along one of the three arms (see the Supporting Information for details).

We purified the assembled DNA origami by agarose gel electrophoresis to get rid of excess helper strands and excess **DNA3** and thus prevent non-site-specific AgNC formation. AFM analysis of the purified sample showed the formation of the designed triangular shape of the origami, in which each arm has a length of approximately 114 nm, in good agreement with the calculated length (ca. 115 nm) of the design (Figure 3a). The site-specific display of protruding duplexes of

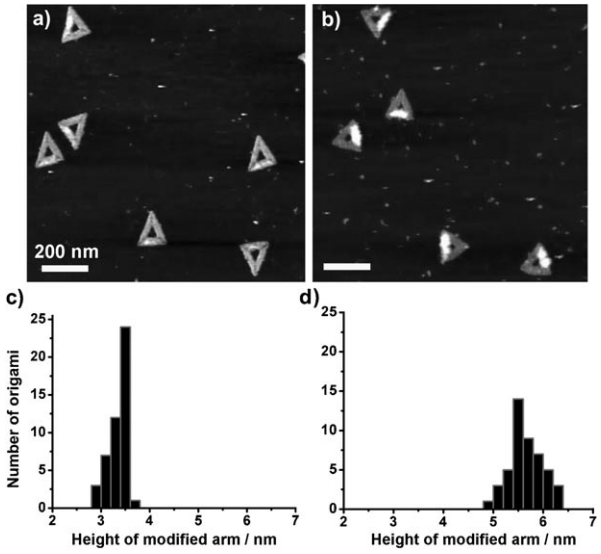

Figure 3. a,b) AFM images showing the site-specific incorporation of **DNA3** and the subsequent in situ synthesis and site-specific immobilization of AgNCs on a particular arm of the triangular DNA origami scaffolds before (a) and after (b) treatment with the Tollens reagent (*z* scale = 10 nm). c,d) Corresponding histograms showing the height of the bright feature on the origami before (c) and after (d) treatment with the Tollens reagent.

helper strands and **DNA3** along one arm of the triangle produces a bright topographical feature in the AFM image; this feature is higher than the other two bare arms (Figure 3a). AFM cross-section analysis showed an average height of approximately 3.4 nm for the modified arm: almost double the height of the bare arms of the triangular origami structure (Figure 3c). This height difference indicates that the duplexes are probably lying flat rather than standing up on the origami surface under tapping-mode AFM.

We performed the Tollens reaction on the DNA origami scaffold under the same experimental conditions as those used for the free **DNA3** in solution and with the same molar

ratio of Ag^+ to sugar-modified DNA of 200. The fluorescence spectrum of a sample prepared in this way revealed an emission maximum at 418 nm and an excitation maximum at 340 nm (Figure 2b). This result indicates that the fluorescent AgNCs grown in situ on the origami scaffold had optical characteristics similar to those of the NCs obtained with free **DNA3**. Similarly, no formation of AgNPs with measureable surface plasmon resonance was observed even after several days of incubation. AFM analysis of the DNA origami sample after the Tollens reaction revealed a bright strip with a mean height of about 5.5 nm on one arm of the triangle, but not on the other two arms (Figure 3b). This bright strip is due to the site-specific immobilization of AgNCs along this arm. The approximately 2.1 nm height difference observed for this strip before and after the Tollens reaction could be the apparent diameter of the AgNCs synthesized and deposited in situ (Figure 3d). No deposition of NCs was observed in other regions of the triangular DNA origami scaffold, which demonstrates the excellent site specificity of our DNA-templated approach. The sugar units act as the nucleation sites for AgNC formation. The unmodified DNA scaffolds do not play an active role in the reduction of the Ag^+ ions, but only act as a structural scaffold (see an additional control experiment in the Supporting Information).

TEM analysis further confirmed the site-specific immobilization of AgNCs on the DNA origami nanostructure (Figure 4). The sample was stained with uranyl formate, so

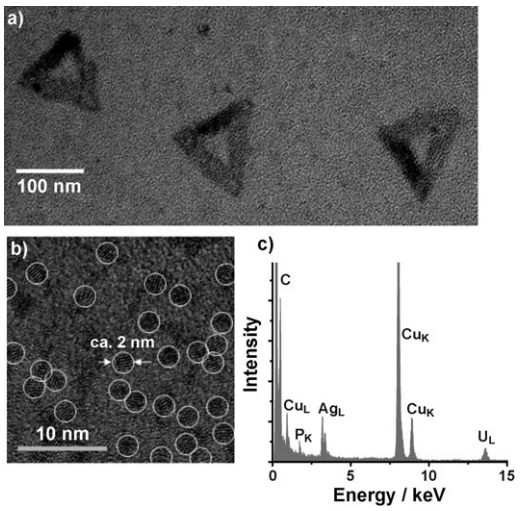

Figure 4. a) TEM image of the origami structure after treatment with the Tollens reagent (the sample was negatively stained with uranyl formate) and b) corresponding high-resolution TEM image of the NCs immobilized on the modified arm. c) EDX spectrum of the AgNCs on the DNA origami structure.

that the DNA origami scaffold was also visible along with the AgNCs in the TEM images. A significant contrast difference was observed for one arm of the triangle in comparison with the other two arms (Figure 4a). As by AFM analysis, no NC deposition was observed in any other part of the triangular structure. A high-resolution TEM image showed a nearly uniform distribution of AgNCs with a diameter of approximately 2 nm (Figure 4b), which is the same as the diameter of NCs obtained with free **DNA3** in solution. The density of the NCs per unit area is consistent with the density of the DNA probe strands distributed along the arm of the DNA origami. Since the estimated yield of the hybridization between the probes and **DNA3** is 100%, this result reveals that each DNA molecule carrying three consecutive sugar moieties in close proximity to one another actually acts as one unique nucleation site for AgNC deposition. Furthermore, energy-dispersive X-ray spectroscopy (EDX) of these structures confirmed the presence of silver on the DNA scaffold (Figure 4c).

In summary, we have demonstrated a new DNA-based method for the synthesis of water-soluble fluorescent AgNCs through the use of the well-known Tollens reaction. DNA of specific sequences with sugar moieties covalently attached have been successfully used for the site-specific incorporation of the sugar units on a triangular DNA origami scaffold to enable the subsequent site-specific synthesis and in situ immobilization of AgNCs at predefined positions on the DNA nanoscaffold. The resulting high-density array of emissive NCs may have potential for application in many fields, such as the fabrication of semiconductor nanostructures.[1b] Our new approach is characterized by excellent site-specific control of NC nucleation and yields uniformly sized high-density arrays of AgNCs. It thus offers a unique platform for the future site-specific deposition of other metals, such as gold, which may lead to advances in DNA-based nanoelectronics.

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