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DNA-Templated Covalent Coupling of G4 PAMAM Dendrimers

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Abstract: Generation-4 polyamidoamine (PAMAM) dendrimers were surface-functionalized with azides or alkynes and conjugated to one DNA strand. DNA-controlled self-assembly of alternating azide and alkyne dendrimers on a DNA template enabled the coupling of the dendrimers by the azide−alkyne "click" reaction to form covalently coupled dimers, trimers, and tetramers. Polymerization of the DNA−dendrimer conjugates was also demonstrated, as well as assembly in a circular structure on DNA origami and imaging by atomic force microscopy.

One of the ultimate goals of nanotechnology is to construct functional nanodevices that are built from specific components and have precise geometries and functions. A fundamental step toward this goal is the controlled assembly and fusion of nanosized species with nanoscale accuracy.[1] One promising strategy to reach this goal is to exploit the visionary prospects of DNA nanotechnology,[2] since DNA is programmable and can be used to design and form nanoscale structures with predictable geometry. Significant progress has been made in developing 1-, 2-, and 3D DNA nanostructures by self-assembly of genomic and/or synthetic DNA strands.[3] Such DNA nanostructures can also serve as templates for positioning of nanosized species.[4] The distance between attached species can be precisely controlled by adjusting the length of the DNA strands. Both inorganic nanoparticles[4a−c] and proteins[4d,e] have successfully been assembled on various DNA templates. We recently demonstrated that it is also possible to modify DNA nanostructures after their assembly by chemical removal or coupling of single particles at specific positions on a 2D DNA origami template.[5a] However, with few exceptions,[6] the reported nanoassemblies cannot be separated from the DNA scaffold without loss of their relative positioning because they are not covalently interlinked, and this is one of the main limitations of the application of such assemblies.

Herein, we report a new strategy to overcome this limitation by covalently connecting the assembled nanobuilding blocks on a DNA template to obtain a macromolecular nanostructure. To demonstrate this strategy, we used polyamidoamine (PAMAM) dendrimers[7] as model nano-objects to be assembled on various 1D duplex DNA templates and then covalently interlinked using the Huisgen−Meldal−Sharpless azide−alkyne "click" reaction,[8] resulting in the controlled formation of dendrimer oligomers. Furthermore, we explored the assembly of dendrimer−DNA conjugates on a 2D DNA origami template.

As shown in Figure 1a, a PAMAM dendrimer is a repeatedly branched macromolecule with a dendritic architecture and globular shape. Its size and number of surface groups depend on the dendrimer's generation.[7] Dendrimers have attracted much interest in applications such as drug delivery,[9] diagnostics,[10] gene transfection,[11] and catalysis.[12] Recently, polymerization of dendrimers to form linear dendronized polymers has also been reported.[13]

Herein, we used a DNA-template-based scheme to form dendrimer oligomers. A generation-4 PAMAM−succinamic acid dendrimer (G4-COOH) was used (Figure 1a). The dendrimer has 64 carboxylic acid surface groups and a diameter of ∼5 nm. At neutral pH, the carboxylic acid groups are deprotonated, and the negative surface charges circumvent electrostatic interactions with DNA strands.[14]

The dendrimer was first modified at the 64 carboxylic acid groups to generate all-azide dendrimers (G4-azide) or all-alkyne dendrimers (G4-alkyne) to enable the coupling reaction depicted in Figure 1b. The final estimated diameters of G4-azide and G4-alkyne were ∼6.5 nm. Therefore, the interval length of 20 bases (6.8 nm) was used in the DNA template. To prepare 1:1 DNA−dendrimer conjugates, two different 20 nucleotide (nt) single-stranded DNA (ssDNA) strands were coupled to either the G4-alkyne or G4-azide dendrimers via the "click" reaction. The G4-alkyne dendrimer was coupled with azide-modified DNA (DNAa), and the G4-azide dendrimer was coupled with alkyne-modified DNA (DNAb). The 1:1 DNA−G4 conjugates could easily be separated from 2:1 DNA−G4 conjugate byproducts and unreacted starting materials using gel electrophoresis (see Figure S5 and the Supporting Information for further details). The remaining 63 surface groups on these conjugates remained as azide or alkyne groups, enabling further reactions of the dendrimers to form oligomers or polymers.

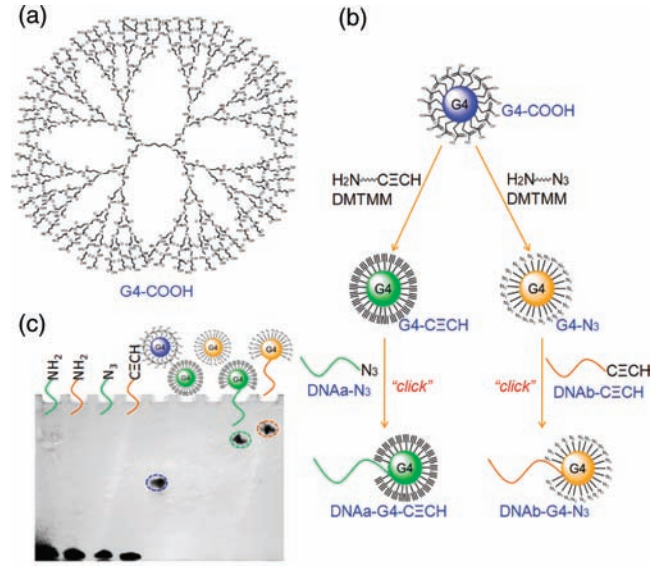

Figure 1. Preparation of DNA−G4 conjugates: (a) structure of generation-4 PAMAM−succinamic acid dendrimer (G4-COOH); (b) preparation of 1:1 DNA−G4-alkyne and DNA−G4-azide conjugates; (c) 10% denaturing PAGE of the conjugates (stained using Stains-all).

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The conjugates were analyzed by polyacrylamide gel electrophoresis (PAGE) (Figure 1c), and their masses were verified by MALDI−TOF mass spectrometry (see the Supporting Information). DNA−G4 conjugates had lower mobilities than G4-COOH, as expected. The G4-azide and G4-alkyne dendrimers could not be identified from the gel since they either did not have negative charges or could not be stained.

To demonstrate controlled oligomerization, a dimerization process was explored, as shown in Figure 2a. The DNAa−G4-alkyne and DNAb−G4-azide conjugates were mixed with a 40 nt ssDNA a′−b′ template in a 1:1:1 manner to form a hybrid of the two 20 nt ssDNA overhangs of the conjugates with the template. Since the diameter of the G4 dendrimers matched well with the interval distance on the DNA template, the two G4 dendrimers were very close to each other, and the effective molarity was high in the local environment. Next, copper(II), a THTA[5] ligand, and the reducing agent ascorbic acid were added to mediate the "click" reaction between the two G4 dendrimers. The reaction was very efficient and fast, reaching completion in half an hour. Similarly, trimerization and tetramerization could also be performed using a′−b′−a′ and a′−b′−a′−b′ templates, respectively. In principle, dendrimer oligomers with any defined length could be prepared by changing the DNA template.

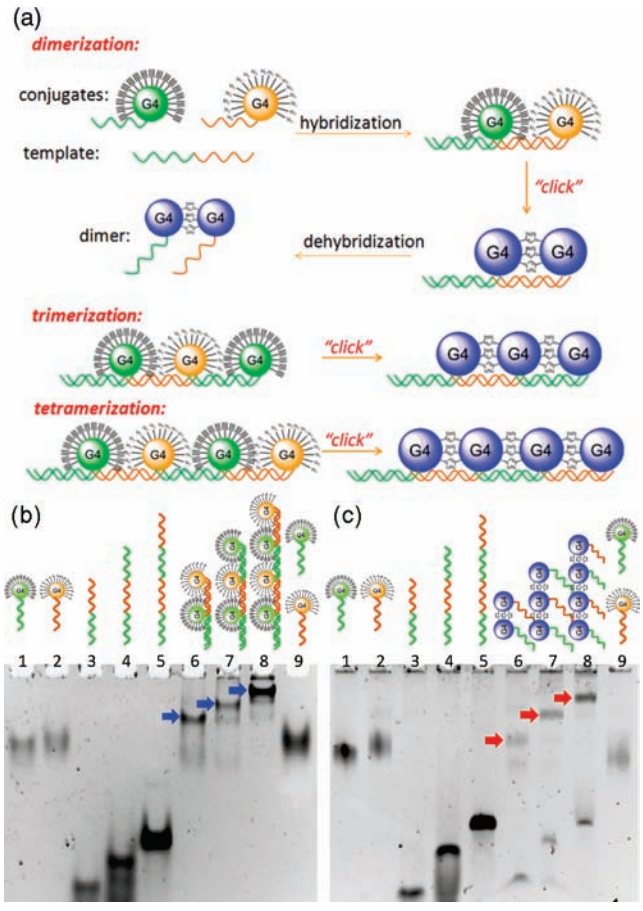

Figure 2. Controlled DNA-templated oligomerization of dendrimers: (a) principle of dimerization, trimerization, and tetramerization; (b) 8% native PAGE before polymerization; (c) 8% denaturing PAGE after polymerization. Gels were stained using SYBR-Gold.

Figure 2 shows the PAGE results of the dimerization, trimerization, and tetramerization reactions. In both the native (Figure 2b) and denaturing (Figure 2c) gels, lanes 1 and 2 are the two DNA−G4 conjugates. Lanes 3−5 are the ssDNA templates for dimer, trimer, and tetramer formation, respectively. Lanes 6−8 in the native gel show the dimer, trimer, and tetramer constructs before the coupling reaction was performed. The three new main bands (indicated by blue arrows) with lower mobility that appear in these three lanes reveal the formation of duplexes between the DNA−G4 conjugates and the templates. In comparison with the native gel, lanes 6−8 in the denaturing gel show the products after the reaction. The three main products (indicated by red arrows) move more slowly than either the DNA−G4 conjugates or the templates, and there are obvious differences in the mobility of these three products, with the dimer product moving fastest and the tetramer product slowest. As an additional control, a mixture of two DNA−G4 conjugates without any templates was loaded in lane 9. The absence of a distinction between the native gel (before polymerization) and the denaturing gel (after reaction) for the control indicates that there were no side reactions caused by free DNA−G4 conjugates in solution. The dimer was isolated from the denaturing gel and identified by MALDI−TOF mass spectrometry (Figure S6). These results demonstrate the high efficiency and selectivity of this DNA-template-directed covalent coupling of G4 dendrimers.

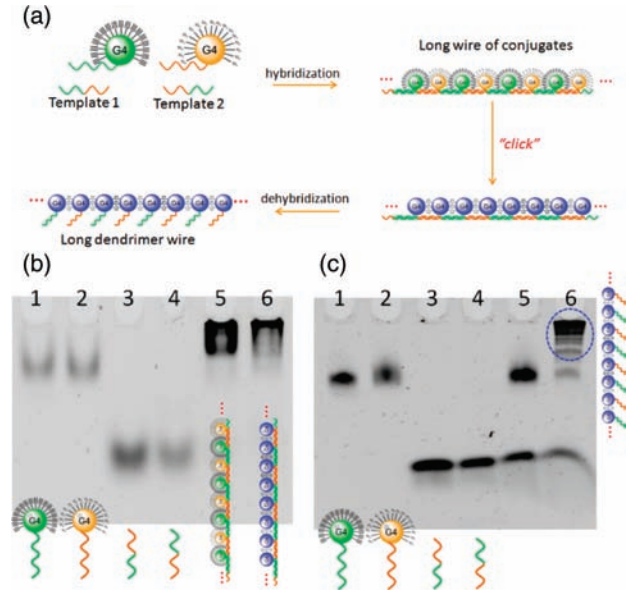

Figure 3. Polymerization of DNA−G4 conjugates on a long linear duplex template: (a) principle of the polymerization; (b) 6% native PAGE gel; (c) 6% denaturing PAGE gel. Gels were stained using SYBR-Gold.

After demonstrating the ability to form dendrimer oligomers with definite lengths, we next pursued the formation of linear polymers of the two DNA−G4 conjugates. As shown in Figure 3a, two DNA templates were used, each of which could hybridize with half of each of the two DNA−G4 conjugates for the formation of long linear or cyclic DNA−G4 polymers. The dendrimers were assembled on the two template strands to form a supramolecular construct and then coupled by the "click" reaction to form a macromolecular polymer. The outcome of the reactions was followed by native and denaturing PAGE (Figure 3b,c). Lanes 1−4 in these two gels are the two monomeric DNA−G4 conjugates and the two DNA templates, respectively, and they have similar mobilities in the two gels. Lane 5 is a mixture of the DNA−G4 conjugates and the two templates, and lane 6 is the mixture after the click reaction. In the native gel, lanes 5 and 6 look very similar, indicating that both of them are very long duplexes. However, in the denaturing gel, the duplex formed before the chemical reaction had dissociated into free DNA−G4 conjugates and templates (lane

5). In contrast, the product after the click reaction shows several slower bands, and the main band (circled in blue in lane 6) did not move in the gel. These results agree well with the design that a long DNA−G4 polymer was prepared.

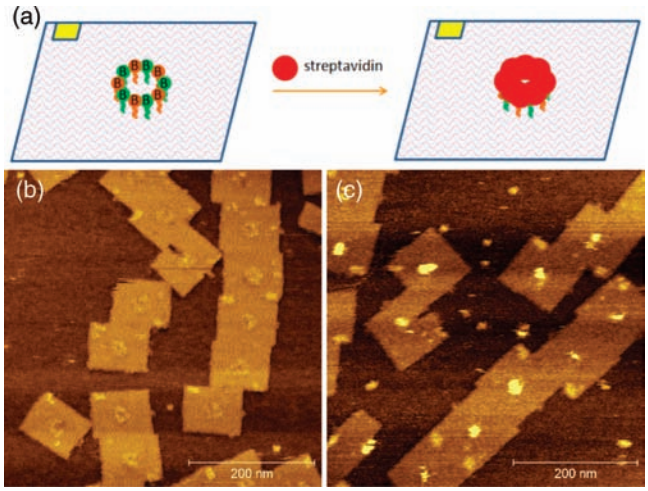

Figure 4. Hybridization of biotin-labeled DNA−G4 conjugates on a DNA origami template and binding of streptavidin: (a) principle of the design; (b) AFM image of the origami template with biotin-modified dendrimers; (c) AFM image of the sample in (b) after streptavidin labeling.

An extension of the dendrimer self-assembly from a 1D linear template to a 2D pattern was also explored by assembling G4 dendrimers on a 2D DNA origami template. The DNA origami method applies the 7.2 kilobase genome of the virus M13mp18, which is folded with the help of 200−250 short synthetic oligonucleotides.[3c,e−g] In our design, Rothemund's rectangular origami[3c] was used as a 100 nm × 70 nm template, and the positions at which ssDNA overhangs could most conveniently be extended from the origami surface were ∼6.4 nm apart. We inserted 10 staple strands with two different 20 nts ssDNA overhangs around the center of this origami to form a "ring" pattern (Figure 4a). These 10 overhangs could hybridize with the two kinds of DNA−G4 conjugates alternately. The atomic force microscopy (AFM) image of this DNA origami adsorbed on mica showed that it was well-formed and revealed a small ring structure in the center of each origami (Figures S7b and S8b). However, after the addition of DNA−G4 conjugates, no distinct difference could be found (Figures S7c and S8c). This result correlates with a previous report showing that the G4 PAMAM dendrimer is too soft for imaging by AFM.[15] Therefore, we synthesized DNA−G4 conjugates containing biotin groups on the G4 surface (see the Supporting Information). The subsequent binding of streptavidin to the dendrimer ring after immobilization on origami was clearly imaged by AFM, and a large height increase (∼4 nm) was measured,[16] providing strong support for the presence of the dendrimers on the surface (Figure 4b,c; for additional AFM images and height profiles, see Figure S7).

Covalent coupling of alternating azide and alkyne G4 dendrimers in a ring pattern on the origami surface using the "click" reaction was also attempted. Denaturation of the origami and subsequent gel analysis showed vague indications of the formation of a higher molecular species by gel electrophoresis (Figure S8).

In conclusion, a scheme for DNA-templated covalent coupling of modified PAMAM dendrimers has been demonstrated. Our results show that this approach is reliable, efficient, and fast. The concept of covalent coupling after assembly on a DNA template could be potentially extended to other kinds of macromolecules or inorganic nanoparticles. Formation of coupled dendrimer structures on more complex DNA templates such as other 2D and 3D origami may also be possible. Since DNA structures provide the most precise addressability at the nanoscale, it is expected that combining more complex DNA templates with functional nanomaterials by this polymerization strategy would lead to new applications. For example, a more complex polymerized dendrimer pattern could be constructed rather than simple linear or 2D dendrimer polymers. Further research based on this strategy will focus on building fused functional multicomponent nanopatterns.

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Supporting Information Available: Materials and methods, DNA sequences, MALDI−TOF spectra, additional AFM images, and agarose gel characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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