DNA Nanostructures

DOI: 10.1002/anie.201205862

pH-Responsive Size-Tunable Self-Assembled DNA Dendrimers**

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Dendrimers, since their discovery in the late 1970's, have received increasing attention for their broad applications in chemistry, biology, and medicine, which is due to appealing properties such as highly branched, monodisperse, nanosized, globular, and void-containing structures.[1] However, there are still some unsolved limitations in the development of dendrimers, for example, the size of dendrimers is generally small, mostly below 10 nm in radius.[1a,b] The building blocks of most dendrimers are small molecules, leading to growth of only several angstroms between adjacent generations. Moreover, more than five generations is usually hard to achieve because saturation occurs at a certain layer.[2] Therefore, large monomers are needed to overcome the size limitation of dendrimers. DNA, with programmable sequences, precise recognition, and a persistence length of about 50 nm,[3] has been widely used as building blocks to fabricate static structures on the scale of tens to hundreds of nanometers,[4] as well as molecular machines,[5] and smart materials.[6] In 1997, it was theorized that DNA could be used to fabricate dendritic structures,[2] and in 2004, these dendrimer-like DNA structures were produced using enzymatic ligation.[7] However, enzymatic ligation is a rather slow process, and the preparation of dendrimers by this approach is time-consuming.[8] In addition, the responsiveness of DNA dendrimers to external stimuli has not been explored and still remains a big challenge. Herein, we report an enzyme-free method to swiftly prepare large DNA dendrimers with a high yield, and further to confer size responsiveness by introducing DNA molecular motors into the scaffold.

Our strategy is shown in Scheme 1. A DNA dendrimer was prepared using step-by-step assembly of three-armed DNA dendrimer monomers (named Y-DNA), which contain sticky-end segments and can further hybridize with other Y-DNAs as each generation is added.[2,7a] In previous studies, specifically designed four-base-long sticky ends were used to covalently link Y-DNA in different layers using enzymatic ligation. In general, these four basepair connections could not be maintained as stable assembled structures under room temperature. Our recent studies found that eight-base sticky ends maintained a sufficiently stable assembled structure at room temperature.[8] Herein, we chose 13-base sticky ends for two reasons: 1) the study of size responsiveness would be performed under slightly acidic conditions, in which duplex DNA is not as stable as under neutral or basic conditions, and the extra stability of longer DNA strands is needed;[5b] 2) in general, different sticky-end sequences for the Y-DNAs in each of the dendrimer generations are chosen to achieve precisely assembled structures, and longer sticky ends can provide more variety for sequence design, leading to better distinction of sticky ends and as a result avoiding crosstalk between the different layers.[9]

Considering that the structure of Y-DNA is approximately planar,[10] the lengths of DNA duplex between each layer are carefully designed to make the final dendrimer a three-dimensional structure rather than a two-dimensional one. A normal B-form DNA duplex rotates one full turn in about 10.5 basepairs.[11] In our design, each arm of the Y-DNA contains a 13-basepair duplex (black lines in Scheme 1) and a 13-base single-stranded sticky end (colored lines). Following the assembly of Y-DNA, there are 39 basepairs (approximately 3.75 turns) between each layer, resulting in an approximately 90° rotation when additional Y-DNA hybridizes with the existing core. In principle, this design will lead to a three-dimensional dendritic structure and keep the sticky ends evenly distributed in the outermost layer of each generation.

To achieve pH responsiveness in a DNA dendrimer, we incorporated a type of DNA molecular motor into the scaffold between the core and the first layer. This DNA molecular motor is composed of a cytosine-rich strand and an optimized complementary sequence X. Under neutral or basic conditions, it forms an extended duplex, however, under slightly acidic conditions, it folds into a constrained four-stranded *i*-motif structure by way of non-Watson–Crick basepairing between cytosine residues.[12] Upon a change in

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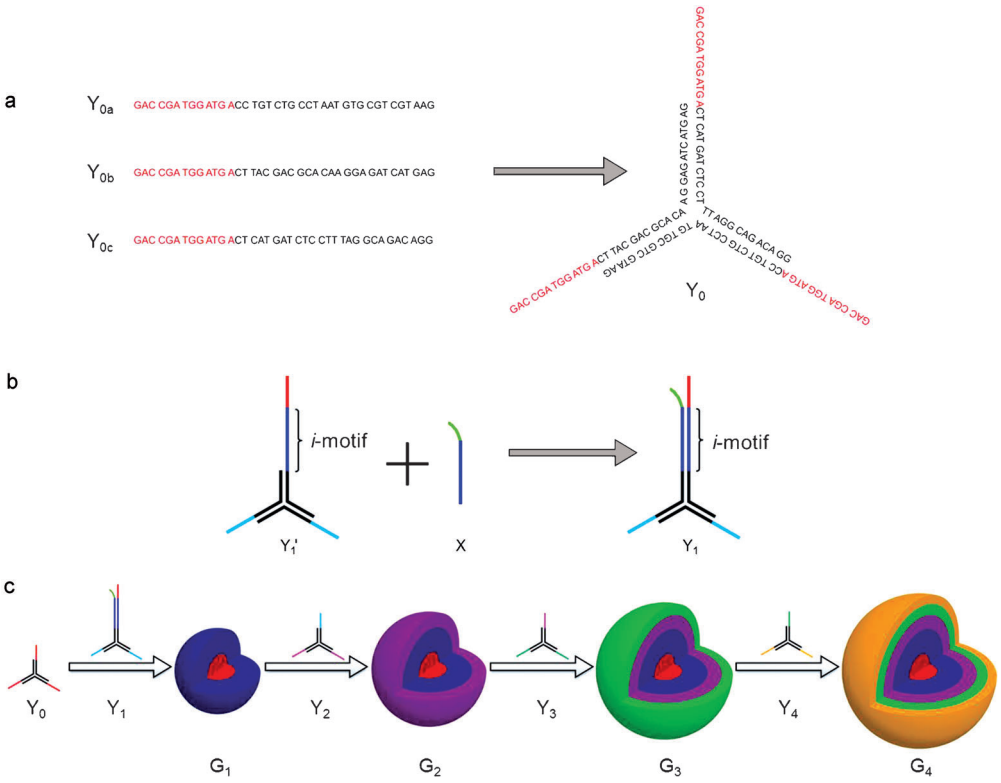
[**] We thank the National Natural Science Foundation of China (No. 20974030, 91027046, 21121004) and the National Basic Research Program of China (973 program, No. 2013CB932803) for financial support.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201205862.



Scheme 1. Strategy for the preparation of size tunable DNA dendrimers. a) Y_0 was assembled from three different single strands, Y_{0a}, Y_{0b}, and Y_{0c}. Other Y-DNA scaffolds were prepared according to same strategy, except for Y_1. b) Y_1 was achieved by the assembly of Y_1' and a single strand X. c) The preparation of the first to fourth generation of DNA dendrimers containing *i*-motif sequences (blue) in the core.

pH value, the *i*-motif DNA undergoes conformational changes and generates several nanometers of linear motion, which we expected to allow for tunable dimensions of the DNA dendrimer.

As shown in Scheme 1c, the DNA dendrimer could be prepared in a step-by-step manner; the three sticky ends of Y_0 were designed to be the same, while other Y-DNAs (Y_n, $n>0$) were designed to have three sticky ends, two of which were the same and the third end complementary to the sticky ends of Y_{n-1}. First, we prepared the Y-DNA scaffolds. In a typical experiment, three oligonucleotide strands (Y_{0a}, Y_{0b}, Y_{0c}) were mixed stoichiometricly in phosphate buffer (50 mM, pH 8.0) with NaCl (100 mM) to give a final concentration of 20 μM for each strand. The mixture was heated to 95 °C for two minutes and then cooled to 4 °C at a rate of 1 °C per minute. All assembled Y-DNAs were characterized by gel electrophoresis (Figure S1). Notably, there was no smear observed, which means the yield of the assembled structure is high, therefore, these Y-DNAs could be used directly for further assembly.

Second, we prepared different generations of DNA dendrimer (G_n) following the method shown in Scheme 1. The sequences of Y-DNA were specifically designed so that the hybridization of sticky ends could only occur between Y_n and Y_{n+1}. In each step, $3\times2^{n-1}$ molar ratio Y_n was mixed with 1 molar ratio G_{n-1}, resulting in the formation of G_n. For example, one Y_0 (G_0) was combined with three Y_1 by way of hybridization of the sticky ends, forming the first generation of DNA dendrimer (G_1) and leaving six free sticky ends available for further assembly. The mixture was then incubated at room temperature for one hour, allowing hybridization to be completed. By taking advantage of this strategy, DNA dendrimers can be prepared without enzymatic ligation, reducing the time of preparation from days to hours. Finally, we used agarose gel electrophoresis to analyze the efficiency of the assembly process. As shown in Figure 1, G_0 ran the farthest on the gel followed by G_1, G_2, G_3, and G_4 in sequence. This result illustrates that the size of the DNA dendrimer increases with the assembly process. We also note that, without any purification, the prepared DNA dendrimers show only a single band without any smearing from other species. This result demonstrates that our assembly strategy is efficient, and the products in each step can be used for

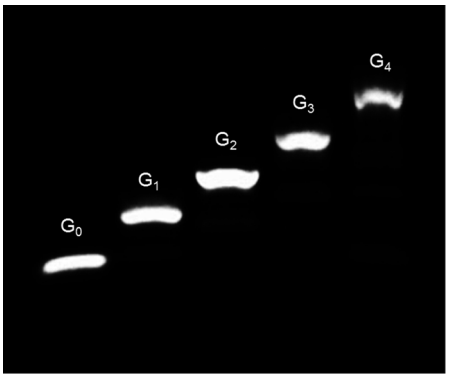


Figure 1. Agarose gel electrophoresis of G_0–G_4. G_0 is a Y-DNA (Y_0); G_1–G_4 are DNA dendrimers.

further assembly without additional purification, which is essential for preparing high generation dendrimers.

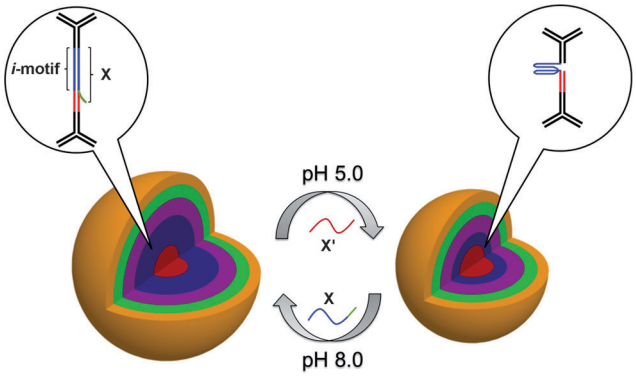
We also investigated the size of the DNA dendrimers by dynamic light scattering (DLS). As shown in Table 1, the average radii of G_1, G_2, G_3, and G_4 were 13.2 nm, 16.1 nm, 21.0 nm, and 28.6 nm, respectively (in 100 mM, pH 8.0 phosphate buffer with 100 mM NaCl), and the size distribution was narrow in all cases (for example, the respective polydispersity indices (PDI) of G_4 and control G_4 were 0.11 and 0.09 at pH 8.0, and 0.12 and 0.03 at pH 5.0). These results demonstrate that the DNA dendrimer from a higher generation is larger in size (Figure S2), in agreement with the gel electrophersis results. Monodispersity is one of the typical characteristics of dendritic structures, suggesting that the formed assembly is a DNA dendrimer. In addition, we also used atomic force microscopy (AFM) to characterize the structure of the assembled DNA dendrimers, which showed a 3D spherical structure (Figure S5).

Table 1: DLS data of the radius (r) of G_1–G_4 and Control G_1–Control G_4.[a]

Sample	radius at pH 5.0 [nm]	radius at pH 8.0 [nm]	Δr [nm]
G_1	9.6 ± 1.7	13.2 ± 1.4	3.6
Control G_1	11.9 ± 0.9	12.8 ± 1.4	0.9
G_2	12.8 ± 0.9	16.1 ± 0.8	3.3
Control G_2	18.6 ± 0.9	19.8 ± 1.4	1.2
G_3	18.1 ± 0.5	21.0 ± 0.6	2.9
Control G_3	23.4 ± 0.4	23.7 ± 0.5	0.3
G_4	25.2 ± 0.5	28.6 ± 0.4	3.4
Control G_4	29.4 ± 0.4	30.7 ± 0.4	1.3

[a] G_1–G_4 are DNA dendrimers with *i*-motif sequences in the core. Control G_1–Control G_4 are DNA dendrimers containing random sequences instead of the *i*-motif sequences.

Next we sought to determine whether the incorporation of *i*-motif motors could make the size of the DNA dendrimers responsive to pH. A solution of G_n at pH 8.0 was characterized with DLS, and in a parallel experiment, a stoichiometric amount of X′ (Scheme 2) was added to a solution of G_n at pH 8.0 and the pH adjusted to 5.0, before also being characterized with DLS (see Experimental Section for details; Figure S4). As shown in Table 1, the radius of G_1 at pH 8.0 was about 13.2 nm and decreased to 9.6 nm at pH 5.0, shrinking 3.6 nm in size, almost 30%. A similar decrease in radius was also observed for G_2, G_3, and G_4, implying that shrinking of the core containing the DNA motor could pull the outer structure inward, leading to a decrease in the overall size. Note that in different generations, the size decrease was almost the same, around 3 nm. From this, we conclude that the size responsiveness in all generations could be caused by the DNA motor inserted in G_1 between the core and the first layer.



Scheme 2. pH-triggered size responsiveness of a DNA dendrimer.

To further verify that the change in size was caused by the motion of the *i*-motif motors, we designed a series of DNA dendrimers in which the motor sequence was replaced by a random DNA duplex of equal length, named as control G_n. As summarized in Table 1, during the changes in pH value, all generations of control DNA dendrimers showed only about 1 nm change in the radius. This result demonstrates that the radius changes seen with the G_n dendrimers can be primarily attributed to the motion of the DNA motor incorporated between the core and first layer.

We also used circular dichroism (CD) spectroscopy to study the conformational changes of DNA dendrimers at different pH values. We found that: at pH 8.0, the spectral differences between the DNA dendrimers with or without *i*-motifs showed a positive band at 265 nm, which is a standard duplex spectrum; at pH 5.0, the spectral difference showed a positive band at 285 nm, indicating the formation of *i*-motif structures (Figure 2).[13] The curve shows the spectral differences of dendrimer and control dendrimer, not a single signal of either dendrimer or control dendrimer (see Scheme S1 and the text above it for details). These results give additional evidence that the size change of the DNA dendrimers was indeed induced by the motion of the *i*-motif DNA motors, as designed.

In summary, we have developed a new strategy to swiftly prepare DNA dendritic structures based solely on DNA self-assembly. Owing to the precise recognition of DNA sequences, high generation products could be obtained in high yield without any purification. By finely tuning the DNA sequences, the outermost sticky ends of the DNA dendrimers could be evenly distributed in three dimensions. Furthermore, with the introduction of DNA molecular motors, the DNA dendrimer exhibited size responsiveness of about 30% upon pH stimulus. Agarose gel electrophoresis, CD spectroscopy, and DLS were used to characterize the formation and size changes of the prepared DNA dendrimers. In addition, with the development of established modifications of DNA sequences[14] and synthesis of DNA organic/inorganic hybrids,[15] combined with the pH responsive motion, this system can be further designed to construct functional and smart nanostructures, and applied for drug delivery,[1c,16] biosensors,[17] and other fields.[1b,18]

Experimental Section

For the preparation of Y-DNA (e.g., Y_0), three corresponding oligonucleotide strands (Y_{0a}, Y_{0b}, Y_{0c}) were mixed in phosphate buffer (50 mM phosphate, pH 8.0; 100 mM NaCl) and the final concentration was 20 μM for each strand. The mixture was then heated to 95 °C for 2 min and cooled to 4 °C at a rate of 1 °C min^{-1}.

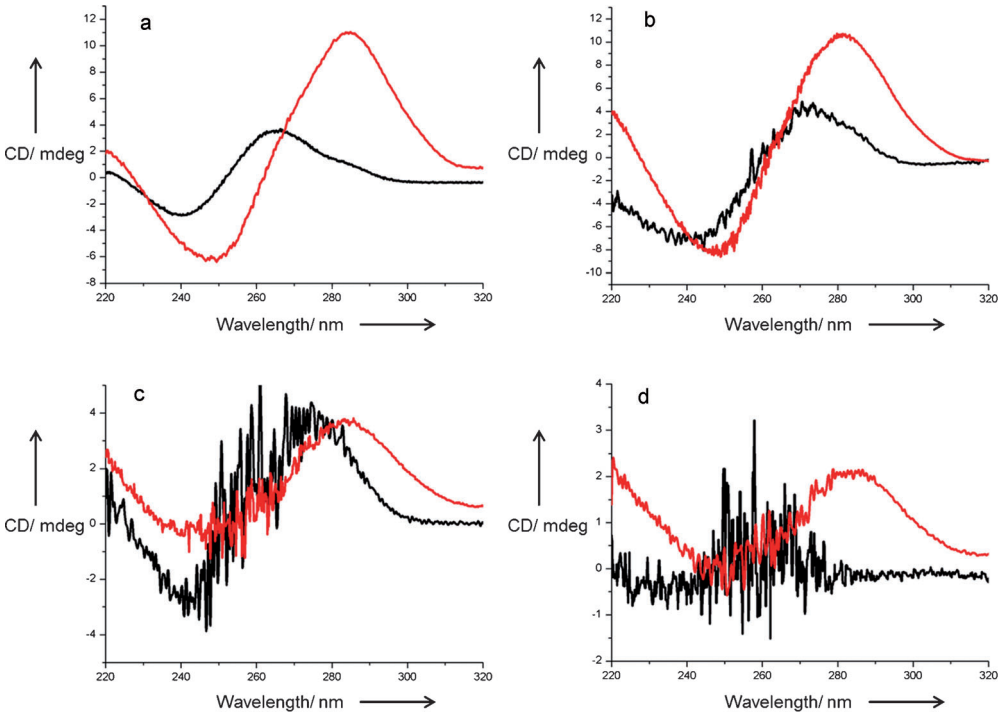


Figure 2. CD spectra of a) G_1, b) G_2, c) G_3, and d) G_4 at both pH 5.0 (red) and pH 8.0 (black).

For the preparation of DNA dendrimers (G_n), Y_0 (G_0) and Y_1 were mixed at a 1:3 molar ratio and the mixture was kept at room temperature for 1 h to prepare G_1. In subsequent steps, G_n ($n > 1$) was prepared by simply mixing G_{n-1} and Y_n at a $1:3\times2^{n-1}$ molar ratio and treated with same procedure.

Dynamic light scattering experiments were carried out on a Brookhaven spectrometer equipped with a BI-200SM Goniometer and a BI-Turbo-Corr Digital Correlator. To keep the scattering signal in a comparable range, the concentration of the samples used was calculated based on all Y-DNA scaffolds.

Received: July 24, 2012
Published online: October 4, 2012

Keywords: dendrimers · DNA · pH responsiveness · self-assembly · size tunable

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