JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
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Triggering Hairpin-Free Chain-Branching Growth of Fluorescent DNA Dendrimers for Nonlinear Hybridization Chain Reaction

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Supporting Information

ABSTRACT: We present a nonlinear hybridization chain reaction (HCR) system in which a trigger DNA initiates self-sustained assembly of quenched double-stranded substrates into fluorescent dendritic nanostructures. During the process, an increasing number of originally sequestered trigger sequences labeled with fluorescent reporters are freed up from quenched substrates, leading to chain-branching growth of the assembled DNA dendrimers and an exponential increase in the fluorescence intensity. The triggered assembly behavior was examined by PAGE analysis, and the morphologies of the grown dendrimers were verified by AFM imaging. The exponential kinetics of the fluorescence accumulation was also confirmed by time-dependent fluorescence spectroscopy. This method adopts a simple sequence design strategy, the concept of which could be adapted to program assembly systems with higher-order growth kinetics.

Recent advances in dynamic DNA nanotechnology enable programming of the kinetically controlled assembly of DNA nanostructures through a cascade of strand-displacement reactions.[1−6] The hybridization chain reaction (HCR) introduced by Dirks and Pierce,[3] which provides a general principle to initiate the successive assembly of DNA hairpins into nicked polymeric nanowires by the introduction of a triggering sequence, is the most well-known strategy. Theoretically, the concept of HCR is not limited to linear polymerization of DNA hairpins. It can be extended to the programming of more complicated DNA hairpin sets that can assemble into branched or even dendritic nanostructures under a triggered process, realizing nonlinear HCR systems that exhibit exponential growth. However, despite the increased complexity of the sequence design, controlling system leakage (spontaneous initiation) becomes a big challenge because spurious initiation events are also amplified exponentially.[7]

Pierce and co-workers have demonstrated a dynamic assembly system in which DNA hairpins can be triggered to form dendritic nanostructures.[5] However, it requires that new DNA hairpin species be supplied for each generation of dendritic growth, and therefore, the process is not self-sustained and can hardly be considered a nonlinear HCR process. LaBean and coworkers also have reported a nonlinear version of HCR that theoretically can trigger the formation of an exponentially growing dendritic nanostructure by employing two-loop structured hairpins.[6] However, the designed assembly process involves the spontaneous opening of a relatively long hairpin stem (15 base pairs) at a finite rate, which probably gives rise to a linear growing process instead of the argued dendritic growth. No solid evidence such as the morphology of the assembled nanostructure or proof of the exponential growth kinetics has been provided. To date, the goal of constructing a feasible nonlinear HCR system has remained elusive. Here we present a hairpin-free nonlinear HCR system that is realized by kinetically controlled chain-branching growth of fluorescent DNA dendrimers from successive assembly of quenched double-stranded DNA substrates. This system exhibits exponential growth kinetics and well-controlled system leakage. The validity was confirmed by an examination of the molecular weight, morphology, and real-time fluorescence changes of the assembled products.

A mechanistic illustration of our system is shown in Figure 1. It employs two double-stranded substrates (A and B) and two single-stranded assistants. The specifics of the designed sequences are revealed at the bottom of Figure 1. Each of the substrates consists of a quencher-labeled DNA strand (Q strand) and a fluorophore-labeled DNA strand (F strand). The Q strand hybridizes to the complementary region of the F strand, forming an exposed "toehold"[8] at one end and bulge loops in the middle. The fluorophore and the quencher are in close proximity, resulting in a "quenching" state of the substrates. Substrate A possesses two identical sequences [a 7 nucleotide (nt) loop plus the following 16 nt domain). Both of them are complementary to the toehold plus its adjacent 18 nt domain on substrate B, and vice versa. The assistant strands are designed to be complementary to specific regions on the Q strands of their corresponding substrates. The substrates and the assistants are kinetically impeded from "reacting" with each other because all of the binding sites are sequestered in either double-stranded form or bulge loops.[9]

When a trigger DNA is introduced, it hybridizes and docks to the exposed toehold of substrate A, leading to a branch migration reaction[10] that displaces part of the Q strand from the F strand and opens the first loop. The opened loop makes the branch migration irreversible and exposes the displaced part of the Q strand as a new toehold. Assistant A then invades by docking to the last six bases of the newly exposed toehold and displaces all but the last two bases of the F strand from the Q strand, subsequently opening the second loop. Displacement is

Received: March 22, 2014
Published: June 26, 2014

dx.doi.org/10.1021/ja502904s | J. Am. Chem. Soc. 2014, 136, 9810−9813

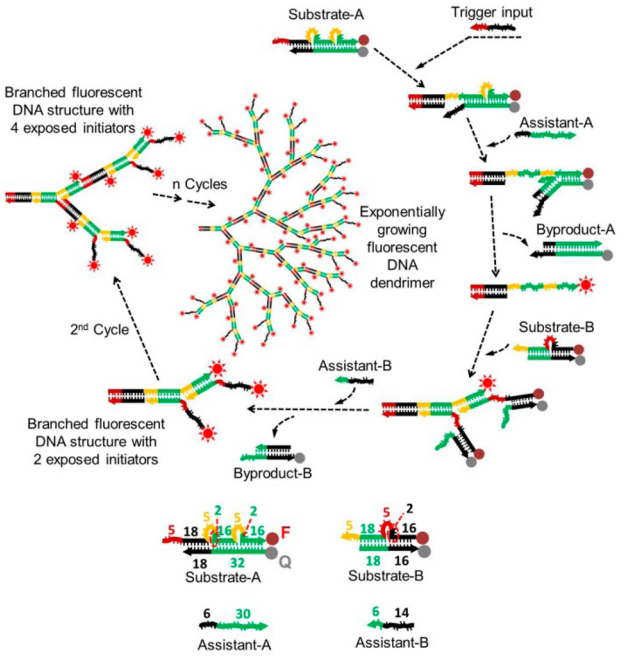

Figure 1. Components and reaction pathway for the triggered chain-branching growth of fluorescent DNA dendrimers. The lengths of different domains are indicated. DNA sequences drawn in the same color are either identical or complementary.

completed by spontaneous dissociation of a double-stranded byproduct A together with removal of the quencher away from the F strand, thus releasing fluorescence. Strategically, now the F strand of substrate A exposes two identical sequences connected in series, which could simultaneously be attacked by and hybridized with toeholds of two substrates B. Soon after assistant B helps to dissociate byproduct B, more fluorescent reporters are freed, and two single-stranded regions consisting of the same sequences as the trigger DNA are exposed and ready to enter a new round of reactions. This crucial step highlights the beginning of a sustainable chain-branching process. Similar to a polymerase chain reaction (PCR) process producing two new child templates from each of the host templates, two originally protected trigger sequences would be exposed in every newly grown branched strand. In the absence of steric effects, theoretically this chain-branching growth of the dendritic structure would continue, and a DNA dendrimer with 2^n exposed trigger DNA could be formed after the nth reaction cycle. Concurrently, the fluorescence intensity of the assembled dendritic structure would also increase exponentially with time during the process.

Native polyacrylamide gel electrophoresis (PAGE) was used to verify the mechanism of the designed system. Experiments were performed in 1× TAE buffer supplemented with 12.5 mM Mg^{2+} (pH 8.0). Substrates A and B were prepared separately by heating mixtures of the F strand (1 μM) and the Q strand (1.5 μM) to 85 °C for 5 min and cooling to room temperature in 20 min. Excess Q strand was added to make sure that all of the F strands were protected by Q strands. The annealed substrates A and B were first mixed with the corresponding assistants (2 μM) separately and incubated for 20 min. Excess single-stranded Q strands in the two substrate solutions were removed by forming byproducts with the assistants, preventing them from assembling together after mixing. The two resulting solutions were then mixed in a ratio of 1:2, giving final concentrations of about 0.15 μM substrate A, 0.23 μM assistant A, 0.3 μM substrate B, and 0.46 μM assistant B in the reaction solution. Finally, different amounts of trigger DNA were introduced. As shown in Figure 2, assembly products with high

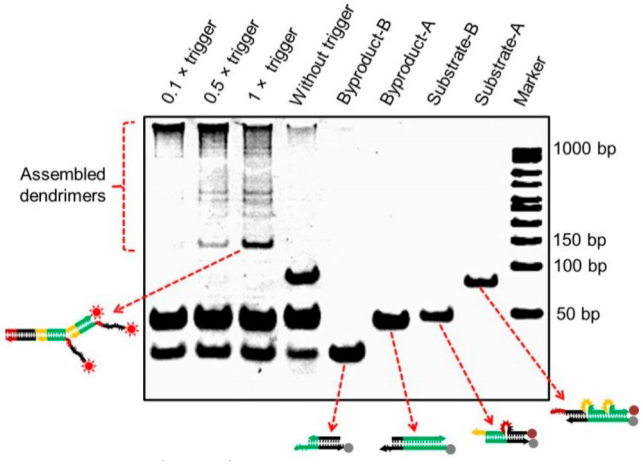

Figure 2. PAGE (8% gel) analysis demonstrating the reaction mechanism and the effect of the concentration of the trigger input on the molecular weights of assembled products. Unless otherwise noted, all of the samples were reacted at room temperature for 30 min. 1× trigger means the concentration of trigger DNA is equal to the concentration of substrate A. The mobilities of assembled products were affected by nicks and branches, preventing direct comparison with DNA markers.

molecular weight were almost invisible in the absence of trigger DNA, showing a very good nonreacting stability between substrates and assistants. After the addition of certain amounts of trigger DNA, the band representing substrate A disappeared and bands with much lower mobility appeared, indicating the assembly of substrate A into products with higher molecular weights. As anticipated, similar to HCR, the assembled products showed a broad distribution of size, and the average molecular weight was inversely related to the concentration of trigger DNA added.

Direct imaging of the assembled products by atomic force microscopy (AFM) further confirmed the PAGE results and revealed the expected dendritic morphology of the assembled products. In the absence of trigger DNA, the AFM image shows only some tiny spots with a thickness of around 2 nm (Figure 3a). These spots represent the unassembled substrates, assistants, and preformed byproducts, showing no sign of the assembly event. In contrast, large dendritic structures of different sizes appear in the presence of trigger DNA (Figure 3b). The observed dendritic morphology of the assembled products is good visual evidence that the chain-branching growth of the DNA dendrimer indeed took place as anticipated. Notably, besides the sizes, the morphologies of the assembled dendrimers were also polydisperse as a result of the random assembly of substrates in two dimensions. In contrast, HCR products are polydisperse only in size because of the one-dimensional growth mechanism.

Time-dependent fluorescence spectroscopy measurements were carried out to quantitatively evaluate the growth kinetics of the above dendrimer assembly system. As shown in Figure 4, in the absence of the trigger DNA (0×), only a negligible fluorescence gain was recorded, which came from system leakage caused by a tiny minority of imperfectly annealed

dx.doi.org/10.1021/ja502904s | J. Am. Chem. Soc. 2014, 136, 9810−9813

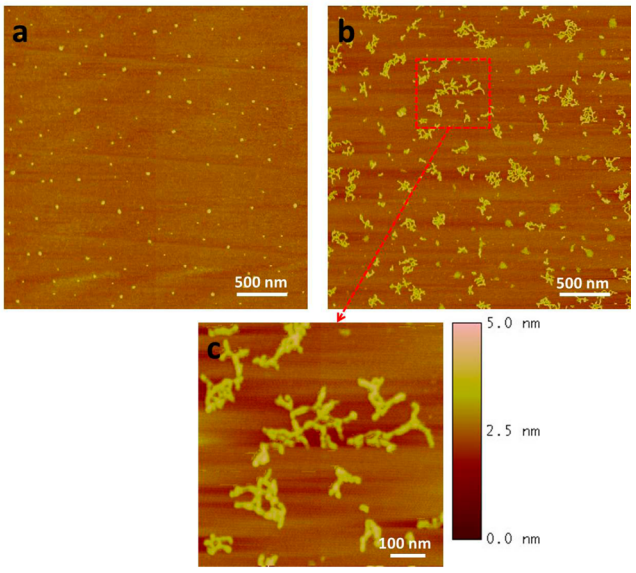

Figure 3. AFM images of the assembled products in (a) the absence and (b) the presence of 0.1× trigger DNA. (c) Partial enlarged detail of (b). Samples were deposited on freshly cleaved mica and imaged in tapping mode in air.

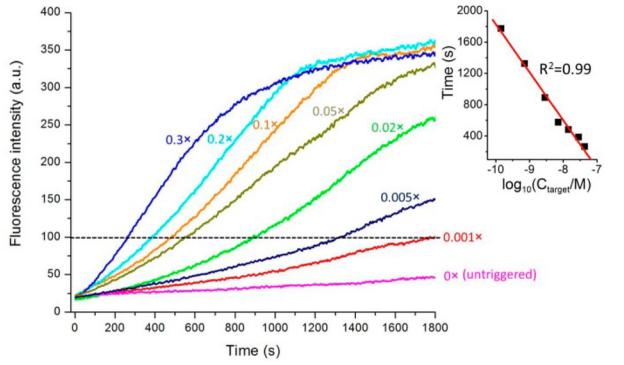

Figure 4. Real-time monitoring of the fluorescence increase when different amounts of trigger DNA were introduced into the reaction system at t = 0. The trigger DNA concentration for 0.3× is 45 nM. The abbreviation a.u. stands for arbitrary units. All of the samples were tested at room temperature. Inset: Linear fit of the plot of the time needed to reach a fluorescence intensity of 100 a.u. against the logarithm of the relative concentration of trigger DNA.

substrates after 30 min of reaction, further confirming the good stability and negligible cross-reactivity between substrates and assistants. Addition of trigger DNA initiated the growth of fluorescent dendrimers and resulted in a large accumulation rate of fluorescence intensity. Substoichiometric quantities (0.3×, 0.2×, 0.1×, and 0.05×) of trigger DNA enabled the fluorescence to increase dramatically and rapidly reach the same plateau due to the depletion of substrate reactants. Overall, a pseudosigmoidal shape of the fluorescence profile could be observed, suggesting exponential growth kinetics of the dendrimers during the process. In a typical exponential growth process such as real-time PCR, the time needed to reach a threshold degree of completion depends logarithmically on the initial concentration of triggering species, and a log−linear trend in a plot of initial concentration of triggering species against time to reach the threshold is indicative.[11] The fluorescence increase during the dendrimer growth process showed this characteristic behavior with a determination coefficient of 0.99 (Figure 4 inset), demonstrating the exponential growth kinetics of the designed system. This result also implies that the growth of fluorescent dendrimer was not substantially affected by steric hindrance under our experimental conditions.

In conclusion, we have demonstrated a hairpin-free kinetically controlled system in which quenched double-stranded DNA substrates successively assemble into fluorescent dendrimers only in the presence of a trigger DNA, resulting in chain-branching growth of the assembled nanostructure and exponentially increasing fluorescence intensity. The exponential reaction kinetics of dendritic growth accompanied by well-controlled system leakage makes it be able to distinguish reliably between 0.001× trigger DNA and 0× trigger DNA within 30 min (Figure 4). In a typical hairpin-based HCR process, the opening of the hairpin structure by strand displacement is reversible to some extent because the displaced strand is tethered in close proximity to the newly formed helix. In our design, the displaced strand forms a double-stranded byproduct with an assistant after opening of the loop. Thus, using double-stranded assembling substrates instead of DNA hairpins not only allows very flexible sequence design but also increases the stability of the assembled products. In fact, the concept of the design principle is very modular and can be extended to the construction of assembly systems with higher-order growth kinetics simply by increasing the number of repeated sequences on substrate A to expose more trigger sequences from substrate B during each reaction cycle. We believe that it has great potential to become a feasible exponential HCR system for a wide range of bioanalysis and sensor applications.[12−19]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Sequence design principle, DNA sequences, and detailed experimental procedures of native PAGE analysis, AFM imaging, and fluorescence measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Research Grants Council of the Hong Kong SAR Government for funding support (RGC 601212).

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