Self-Assembly Hot Paper

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Molecularly Regulated Reversible DNA Polymerization

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Abstract: *Natural polymers are synthesized and decomposed under physiological conditions. However, it is challenging to develop synthetic polymers whose formation and reversibility can be both controlled under physiological conditions. Here we show that both linear and branched DNA polymers can be synthesized via molecular hybridization in aqueous solutions, on the particle surface, and in the extracellular matrix (ECM) without the involvement of any harsh conditions. More importantly, these polymers can be effectively reversed to dissociate under the control of molecular triggers. Since nucleic acids can be conjugated with various molecules or materials, we anticipate that molecularly regulated reversible DNA polymerization holds potential for broad biological and biomedical applications.*

Synthetic polymers are broadly used in various applications such as medicine, cloth, devices, and tools. However, their synthesis and decomposition usually require harsh conditions (e.g., high temperature or organic solvent). In contrast, natural polymers (e.g., proteins) could be synthesized or decomposed with the aid of enzymes under mild physiological conditions.[1] Thus, great efforts have been made to synthesize dynamic polymers with reversible covalent bonds and non-covalent interactions via diverse mechanisms.[2] For instance, units of 2-ureido-4-pyrimidone can form a self-complementary array of four hydrogen bonds for synthesis of unidirectional polymers with the reversibility of formation and decomposition.[3] These polymers not only provide a way of understanding the major processes in nature but also hold potential for broad applications.[4] However, synthetic monomers have been mostly studied in organic solvents and their polymers usually do not have biocompatibility;[5] moreover, these polymers do not exhibit regulatable reversibility under physiological conditions.[6]

The purpose of this study was to demonstrate the ability to develop dynamic polymers whose synthesis and reversibility can be both controlled at the molecular level under physiological conditions (Figure 1), which has not been reported before. The polymers were synthesized using the principle of hybridization chain reaction;[7] the polymers were reversed using the principle of branch displacement.[8] The synthesis of the linear polymer (LP, Figure 1 a, upper panel) involves three molecules including a DNA initiator (DI) and two DNA monomers (DMs). The DI is a linear structure with one functional domain as labeled with i (Figure 1 b and Table S1 a in the Supporting Information). The DMs are hairpin structures. DM_1s_1 has three domains including i*, j and s_1; and DM_2 has two domains including i and j*. During the polymerization (Figure S1 a), DI opens the hairpin structure of DM_1s_1 to form an i-i* double helix with j and s_1 left as a linear segment. The linear j domain further reacts with the j* domain of DM_2 to form a j-j* double helix and a linear segment i. The linear i segment functions as a new initiator to induce the reactions of DM_1s_1 and DM_2 in new cycles for the synthesis of a LP. Notably, the domain s_1 of DM_1s_1 does not participate in the linear polymerization and is a functional side group of LP. Thus, s_1 can hybridize with a molecule carrying a complementary sequence domain s_1* (Figures 1 b and S1 b). The molecular trigger T_1 has two functional domains including s_1* and j*. With the aid of s_1-s_1* and j-j* hybridization, T_1 hybridizes with the DM_1s_1 unit of LP and displaces DM_2. Resultantly, LP is reversed without the involvement of any non-physiological factors.

Similar to DM_1s_1, DM_2 can be designed with a functional side group (named as DM_2k, Figure 1 c and Table S1 a) to bear a total of three domains including k, i and j*. Through the polymerization of DM_1s_1 and DM_2k, LP acquires two functional side groups, s_1 and k (Figure 1 c). Since k can be designed as an initiator for DM_3s_2 and DM_4, these two DMs react with the side group k of LP to form side chains along the backbone of LP (Figures 1 c and S2). Resultantly, a branched polymer (BP) is synthesized with LP and two DMs (Figure 1 a, lower panel). Because both DM_1s_1 and DM_3s_2 have the side groups, that is, s_1 and s_2, respectively, the use of two corresponding molecular triggers T_1 and T_2 can induce the reversible polymerization of BP (Figures 1 c and S2).

The variation of DM structures would lead to the formation of different forms of LPs (Table S1 b). Four representative LPs were synthesized (Figure S3). The results showed that DMs formed LPs in the presence of DI. Side groups did not apparently influence the LP formation. The apparent molecular weights of LPs mostly fell in the range of 500 to 3000 bp. In contrast, DMs did not form LPs in the absence of DI (Figure 2 a, lane 4). The result also showed that an inhibiting sequence could effectively terminate LP polymerization (Figure S4), suggesting that LPs were formed through sequential hybridization of DMs (Figure S1 a). The increase of the reaction time led to more DM conversion (Figure S5). Specifically, about 60% of DMs were quickly converted into LPs within 0.5–1 h. The polymerization reached plateau at 8 h with a cumulative conversion efficiency of 86%. The successful synthesis of LPs was also confirmed

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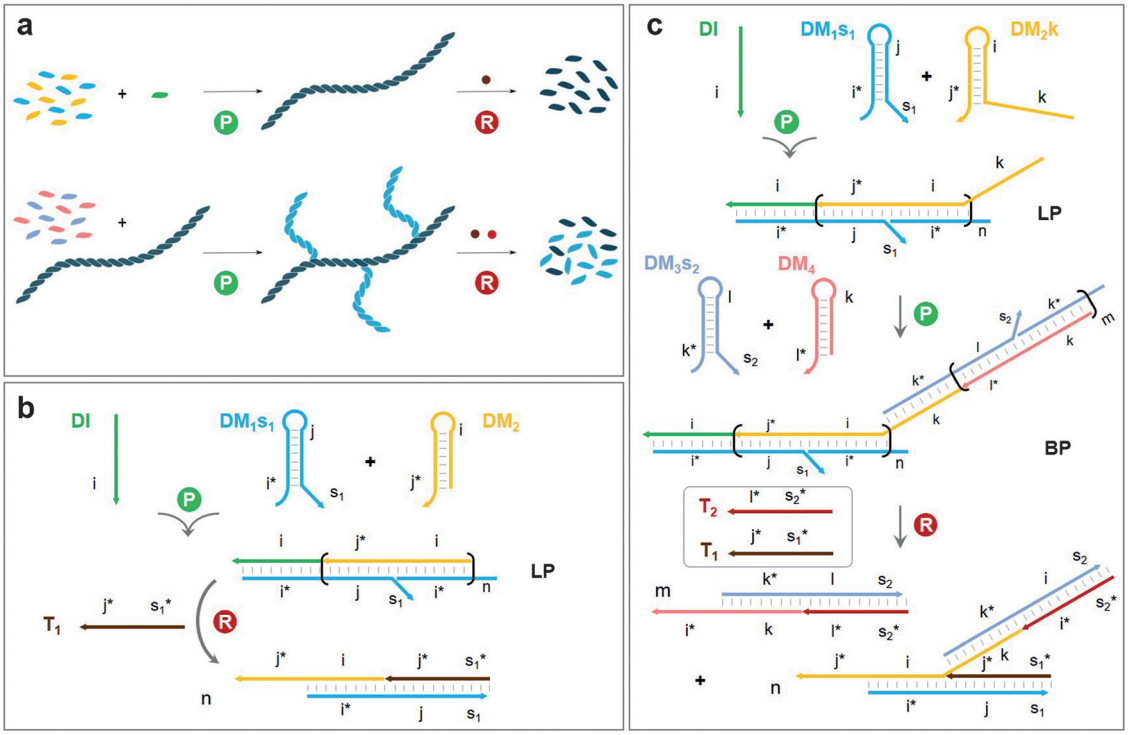

Figure 1. Schematic illustration of the concept. a) Reversible polymerization of LP (upper panel) and BP (lower panel). P: polymerization; R: regulated depolymerization using the reversing trigger molecules (i.e., T). b) and c) Molecular structures of monomer reactants, LP (b) and BP (c) for reversible polymerization. The labeling letters are described in the text. Branches with the leaf-like structure in (a) were drawn for schematic illustration only. In principle, branching can occur from each DM_2k unit as shown in (c).

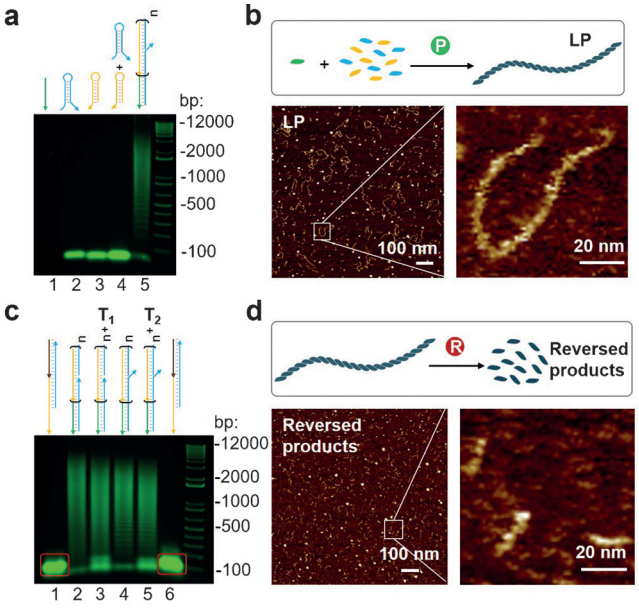

Figure 2. Examination of LP reversibility. a and b), Gel electrophoresis image and AFM images showing LP synthesis. All samples in (a) were incubated for the same time before gel electrophoresis. c) and d), Gel electrophoresis image and AFM images showing the reversibility of LP. Lane 1 of c: DM_1s_1, DM_2 and T_1 were mixed together and annealed to form the hybridized complex; lane 6 of c: LP with the s_1 side group was treated by T_1 after the formation of LP. The red boxes in lanes 1 and 6 demonstrate that LP with the s_1 side group was reversed by T_1. T_2 was used herein as the control of T_1 to show the sequence-specific reversibility of LP.

using AFM (Figure 2b). The average contour length of LPs was about 122.8 nm (Figure S6a).

The side group s_1 had 10 nucleotides (nt). When it was incorporated into the 3′ end of DM_1, the formed LP had periodic side functional groups (Figure S1a). T_1 had 34 nt with 10 nt complementary to s_1 and another 24 nt complementary to the j domain of DM_1s_1. When T_1 and DM_1s_1 hybridize (Figure S7), the formed double-stranded helix has 34 base pairs (bp). In comparison, DM_2 and DM_1s_1 have a helix of 24 bp. The former is thermodynamically more stable than the latter. Thus, T_1 is more competitive than DM_2 in hybridization with DM_1s_1.

As shown in Figure 2c, the LPs bearing the s_1 groups virtually disappeared after reacting with T_1 (lane 6). The major reaction products (lane 6) were located in the same position as the hybridization complex of DM_1s_1, DM_2, and T_1 (lane 1). By comparison, the LP bands were not affected after mixing with the control triggering sequence T_2 (lane 5). When LPs bearing no s_1 groups were treated with T_1, LPs barely changed (lanes 2 and 3). These results clearly demonstrate that T_1 effectively triggered LP depolymerization in a sequence-specific and s_1-dependent manner.

The gel electrophoresis results were confirmed by AFM analysis. The morphologies of LPs with s_1 side groups after polymerization and depolymerization are shown in Figure 2b,d and Figure S8a. Quantitatively, the average length of LPs decreased from 122.8 nm to 16.4 nm (Figure S6). The measured length of depolymerization products is consistent

with the calculated length (Figure S8b). These data further confirm the success of T_1-triggered LP depolymerization.

We also studied the effects of three different hybridization parameters on depolymerization. The reaction time was varied from 10 min to 8 h (Figure S9). The result showed that 95% LPs disappeared within 10 min, suggesting that depolymerization was fast. The depolymerization efficiency increased with the increasing molar ratio of T_1 to DM_1s_1, exhibiting nearly a linear relationship with the molar ratio (Figure S10). The length of T_1 was also varied (Figure S11). Before the hybridization length was reduced to 25 bp, T_1 could effectively trigger LP depolymerization. The efficiency gradually decreased with the hybridization length reduced from 25 to 16 bp. This trend corresponds to the ΔG change of the hybridization helixes (Figure S11d).

BP was synthesized using filtered LP and the mixture of DM_3 and DM_4. The molar ratio of LP monomers to branch monomers was 1:5. LP had two side groups, s_1 and k (Figure S12). The side group k had 29 nt. The 5 nt adjacent to the backbone of LP were designed as a spacer to mitigate potential steric hindrance. The other 24 nt were used as an initiator for reaction with DM_3 and DM_4. The result showed that k was able to induce polymerization of DM_3 and DM_4 (Figure S13a) similar to the DI-induced polymerization of DM_1 and DM_2. We further used k-bearing LP to react with DM_3 (or DM_3s_2) and DM_4 for BP synthesis. A clear band shift is shown in the gel images (lane 4 in Figure 3a and Figure S13b). The results showed that a significant amount of BP molecules had apparent molecular weights over 12,000 bp. In contrast, the reaction of LP without k and the two DMs did not lead to a band shift (lane 3 in Figure 3a and Figure S13b). This difference demonstrates that BP could be synthesized through the k-bearing LP and the two DMs. AFM imaging was performed to confirm the gel electrophoresis data (Figures 3b and S14a). Future work can be further performed to quantify the amount of monomer incorporation.

DM_1s_1 and DM_3s_2 both had a side group (s_1 or s_2). They were designed to hybridize with their corresponding molecular triggers T_1 and T_2, respectively. Figure S15 showed that the addition of T_1 into LP_1 solution led to the disappearance of LP_1 band (lane 6 in Figure S15a) and the addition of T_2 into LP_2 solution led to the disappearance of LP_2 band (lane 6 in Figure S15b). These results demonstrate that molecular triggers could reverse LP successfully.

Figure 3c shows that BP treated with different combinations of T_1 and T_2 exhibited a remarkably band shift (lanes 2–4) in comparison to the untreated BP (lane 1). For BP treated with only T_1, the apparent molecular weights of reversed BP products were shifted into the range of 300 to 1,000 bp (lane 2, population i). It suggests that T_1 depolymerized the backbone of BP while not affecting the side chains (Figure S16a). For BP treated with only T_2, the apparent molecular weights of reversed BP fell into two ranges. One was from 500 to 3,000 bp (population ii); the other was at about 100 bp (population iii). It suggests that T_2 reversed the side chains of BP while not affecting the backbone (Figure S16b). For BP treated with the mixture of T_1 and T_2 (lane 4), two bright bands were observed. It suggests that both the backbone and side chains were depolymerized (Figure S2c). By contrast, BP without s_1 and s_2 did not show any change (lanes 5 and 6). These results were confirmed by AFM imaging (Figures 3d and S17).

In addition to demonstrating polymerization and depolymerization of LPs and BPs in the aqueous solution, we further combined these polymeric systems with synthetic microparticle and antibody and validated their working efficiency to demonstrate the potential applications of reversible DNA polymerization.

Polystyrene microparticles were functionalized with DI via streptavidin-biotin interactions and DM_2 was labeled with fluorophore. The flow cytometry analysis showed that the average fluorescence intensity of the microparticles with LP was approximately one order of magnitude higher than that of control with one hybridized DM_1-DM_2 complex (Figure S18). It demonstrates that LP could be synthesized on the microparticle surface. Similarly, the results showed that BP could be synthesized on the microparticle surface (Figures 4a and S19). Experiments were further carried out to examine the reversibility. Microparticles bearing LP were treated with either T_1 or T_2 (Figure S20). T_1 treatment induced a dramatic decrease of fluorescence intensity on microparticles whereas T_2 treatment did not. These data demonstrate that LP was specifically reversed on the microparticle surface. Microparticles bearing BP were also treated with the triggering solution (Figures 4b and S21). Similar to LP, BP was reversed on the microparticle surface.

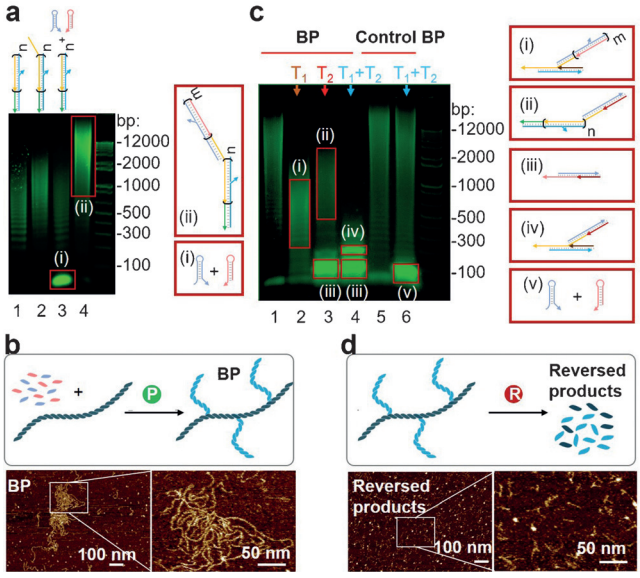

Figure 3. Examination of BP reversibility. a and b), Gel electrophoresis image and AFM images showing BP synthesis. Lanes 1 and 3 in the gel image (a) show the bands of LP without the k side group before and after the treatment of DM_3s_2 and DM_4, respectively; lanes 2 and 4 show the bands of LP with the k side group before and after the treatment of DM_3s_2 and DM_4, respectively. c) and d), Gel electrophoresis image and AFM images showing the reversibility of BP. Lane 1 in the gel image (c): BP with the s_1 and s_2 side groups; lanes 2–4: BP with s_1 and s_2 side groups treated with T_1 (lane 2), T_2 (lane 3) and T_1+T_2 (lane 4), respectively; lane 5: control BP without side groups; lane 6: control BP treated with T_1+T_2. In all cases, triggers were added after the formation of BP.

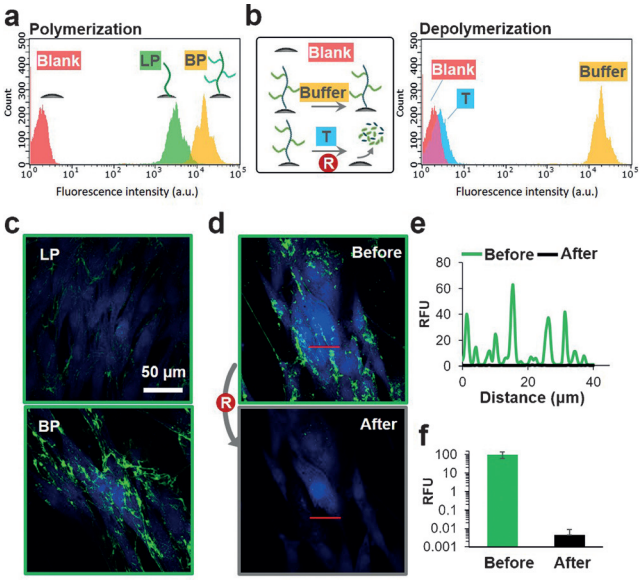

Figure 4. Examination of the reversibility on the microparticle surface and in the extracellular matrix. a) Flow cytometry histograms showing syntheses of LP and BP on the microparticle surface. b) Flow cytometry histograms showing the reversibility of BP treated with molecular triggers. T: triggering solution (containing T1 and T2). c) Confocal images showing LP and BP syntheses in the ECM. Green: DNA polymers; blue: CMAC. d) Images of the ECM with BP before and after treatment with the triggering solution. e) Fluorescence profiles of the lines drew in the images shown in (d). f) Comparison of fluorescence intensities of the entire confocal images shown in d before and after the triggering treatment ($n=3$). For both depolymerization on microparticle or cell surface, triggers were added after the formation of polymers on them.

We also used the ECM of living cells as a substrate to study LP and BP. An antibody-DI conjugate was prepared via the formation of a sandwich structure of biotinylated antibody, streptavidin and biotinylated DI. The antibody-DI conjugate induced the formation of LP in the ECM (Figure S22), and that LP further induced the formation of BP (Figures 4c and S23). When the ECM was treated by triggering solutions, the fluorescence intensities decreased in both cases of LP (Figure S24) and BP (Figures 4d–f and S25) to virtually the same level as the background. While the intensity of the blue background was slightly decreased during the second time imaging of the ECM after the triggering treatment due to photobleaching, Figure S25 demonstrates that triggering depolymerization rather than photobleaching was the dominant reason for the decrease of green fluorescence intensity. Thus, the data show that LP or BP can be polymerized and depolymerized in the ECM.

Owing to high fidelity of hybridization and the water-soluble nature,[9] nucleic acids have been extensively explored in constructing complex structures[10] or nanodevices.[11] These systems have been further studied for numerous applications such as drug delivery,[12] molecular detection[13] and bioimaging.[14] However, previous work has not demonstrated the development of DNA polymers whose formation and reversibility can be both regulated under physiological conditions. This work has successfully demonstrated the ability to synthesize and reverse linear and branched DNA polymers in aqueous solutions, on the microparticle surface, and in the ECM, which further advances the development of nucleic acid-based material systems. We realize that increasing temperature[15] or using DNase[16] can lead to depolymerization of the DNA polymers; however, these methods are not physiologically biocompatible or do not provide selectivity of reversing a certain DNA polymer if multiple polymers exist. Similar to many other self-assembly systems,[3,17] it is also possible to design short self-complementary DNA oligonucleotides that can be spontaneously self-assembled and concomitantly disassembled. However, the spontaneous self-assembly and concomitant disassembly are not stable or molecularly regulatable.[18]

In summary, this work has demonstrated that intermolecular hybridization is an effective mechanism for the synthesis of linear and branched DNA polymers with regulatable reversibility under strict physiological conditions. Since nucleic acids can be conjugated with various molecules or materials, molecularly regulated reversible polymerization and depolymerization are envisioned to hold potential for broad applications.

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