JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

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Polycondensation of Polymer Brushes via DNA Hybridization

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Supporting Information

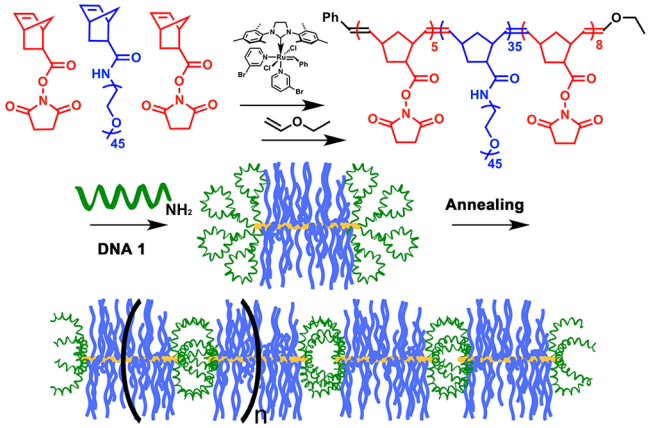
ABSTRACT: Triblock copolymer brushes were functionalized with nucleic acid sequences, which allowed the polymers to connect head-to-tail and form supramolecular nanostructures. Two approaches were designed and implemented, using either a palindromic DNA attached to both ends of the polymer or two different DNA sequences attached regiospecifically. Given appropriate conditions, the DNA-brush conjugates self-assembled to form either nanoworms with length up to several microns or cross-linked networks. This process is analogous to the step-growth polymerization of small molecule monomers.

Recently DNA has been explored as a structure component of a variety of nanomaterials, owing to its tailorability and programmability.[1−5] For instance, origami structures of arbitrary shapes consisting of pure DNA have been created using multiple bespoke DNA sequences.[6−10] DNA has also been used to mediate the assembly of various inorganic nanoparticles to form novel, complex crystals for which analogous atomic structures are oftentimes absent in nature.[11−13] DNA-polymer conjugates are another important class of materials, having both biological properties of the DNA and the architectural and physiochemical properties of the polymer.[14−17] These properties have rendered such conjugates useful in a broad range of applications, spanning drug delivery,[18−21] gene therapy,[22−24] and detection/sensing.[25,26] Herein, we explore the use of DNA in the "polycondensation" of brush copolymers into much larger structures (in one or three dimensions), where the DNA serves as the functional group equivalent in step-growth polymerization.[27]

The self-assembly strategy involves the synthesis of triblock bottle brush polymers as "macromonomers",[28−30] which can be selectively functionalized by amine- or thiol-modified oligonucleotides at the regions near the α- and ω-ends of the brush's linear backbone. Given appropriate conditions, the hybridization between the nucleic acid strands should allow the monomers to self-assemble head-to-tail, connecting them either linearly or with branching, to form higher order assemblies (Scheme 1).

To create these assemblies, several design parameters must be established. First, the polymer backbone should be rigid enough such that it is energetically unfavorable for monomers to cyclize. This hurdle can be overcome by synthesizing a brush copolymer with sufficient side-chain length, which acts as a barrier to prevent intramolecular DNA hybridization, allowing the multivalent conjugate to behave as a divalent structure. Second, there must be at least one DNA strand per block, but not too many as to open up space near the termini to allow more than one brush to form a connection, which will be important in the formation of linear structures where limiting the degree of branching is important. This will require the synthesis of oligomeric blocks having no more than a few repeating units for DNA conjugation. Recent advances in ring-opening metathesis polymerization (ROMP) have paved the way for the synthesis of the required building blocks.[31,32]

Scheme 1. Synthesis of the Hairpin DNA-Polymer Conjugate and Formation of Worm-Like Nanostructures via DNA Hybridization



Our first approach is to utilize a palindromic hairpin DNA sequence, which undergoes a hairpin-to-self-dimer transition when the temperature is raised above the T_m of the hairpin structure. We designed and synthesized an amine-modified DNA sequence (DNA-1, 5′-NH_2-TTT TTA ATC CGT AGC GCT AGC CAT TF-3′) for polymer conjugation. A fluorescein tag was incorporated at the 3′ to enable tracking and quantification of the resulting DNA-polymer conjugate. The calculated Gibbs free energy for the melting of the hairpin (−3.2 kJ/mol, T_m = 36.2 °C) is higher than that of the self-dimer (−62.8 kJ/mol, T_m = 57.0 °C), which allows us to control the polycondensation process by changing the temperature.

In order for this DNA sequence to control the assembly of the brush units, it must be conjugated to both chain termini of the brush. Toward this end, we synthesized norbornenyl *N*-hydroxysuccinimidyl ester (N-NHS) as a reactive monomer,[33] which was sequentially copolymerized by ROMP using a modified second-generation Grubbs' catalyst with norbornenyl poly(ethylene glycol) (M_n = 2 kDa, PDI = 1.05, N-PEG) to

Received: May 13, 2014
Published: July 7, 2014

   dx.doi.org/10.1021/ja504790r | J. Am. Chem. Soc. 2014, 136, 10214−10217

yield a triblock copolymer, p(N-NHS)$_5$-*b*-p(N-PEG)$_{35}$-*b*-p(N-NHS)$_8$ (Scheme 1).[34] Due to the short backbone length, the polymer can alternatively be viewed as a star polymer. The NHS groups enable the coupling with amine-modified DNA strands, while the PEG side chains provide water solubility. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) indicates a polymer M_n of 80.4 kDa and a PDI of 1.15 (Figure S1, GPC data see Figure S2). The successful incorporation of the N-NHS groups is confirmed by the stretching vibration modes for NHS carbonyl groups at 1780 and 1807 cm^{-1} as measured by infrared spectroscopy (Figure S3). The number of available NHS groups is determined by allowing the polymer to react with an excess amount of fluorescein 5-thiosemicarbazide. Following purification, optical absorbance is taken, which is used to calculate the number of NHS groups. Approximately 10 NHS units are available for conjugation on each polymer.

The triblock brush is next coupled to DNA-1 by amidation chemistry at 0 °C in a pH = 8.3 sodium bicarbonate buffer. This conjugate is characterized and purified by agarose gel electrophoresis. Fluorescein excitation/emission filters are applied when the gel containing the conjugates and free DNA is imaged (Figure 1A). The gel image clearly shows that a much higher molecular weight species is present after the reaction. The high-MW band correlating to the conjugated product was removed from the gel, and the conjugates were recovered using GenElute agarose spin column. The number of DNA-1 strands per polymer was determined by fluorescence measurements to be ∼4.2.

In order to initiate the self-assembly process, we subjected the DNA-polymer conjugate to an elevated temperature (80 °C) in the presence of 0.15 M NaCl, to fully dehybridize the duplexes. The solution was then allowed to cool down to room temperature over a period of 10 h. This annealing process should produce thermodynamic DNA duplexes in predominant proportions, which are the self-dimers as opposed to hairpins. As a result, it is expected that the macromolecules will connect in a head-to-tail fashion, to form linear supramolecular chains of polymer brushes. Before the self-assembly, the DNA-polymer conjugates are of a sphere-like morphology as shown by transmission electron microscopy (TEM), and has a dry-state diameter of ∼15 ± 3 nm (Figure 1C). This is expected from the relative length of the PEG side chain (45 repeat units) and the brush backbone (48 repeat units) and is consistent with solution-state observations by dynamic light scattering (DLS), which indicates that the conjugates have a number-average hydrodynamic diameter of 16 ± 5 nm. After the thermal treatment, the hydrodynamic diameter increases to 127 ± 29 nm (Figure 1B). TEM shows that the spherical polymer molecules have been assembled into one-dimensional, worm-like nanostructures. The worms have a cross-section diameter of 13 ± 3 nm, which is consistent with the width of the brush polymer. The lengths of the worms vary significantly, ranging from hundreds of nanometers to several microns, as expected from polycondensation reactions (Figures 1D and S4). The micron-sized worms have a degree of polymerization of several hundred, suggesting that the DNA-mediated self-assembly process is highly efficient and not prone to errors. Nonetheless, we observed a small amount of defects in the assembled product. For example, branching (Figure 1E) and displacement, which creates a point that could lead to branching (Figure 1F), have been observed, and there are a small amount of free brush monomers that are not incorporated into the worm (Figure 1D,

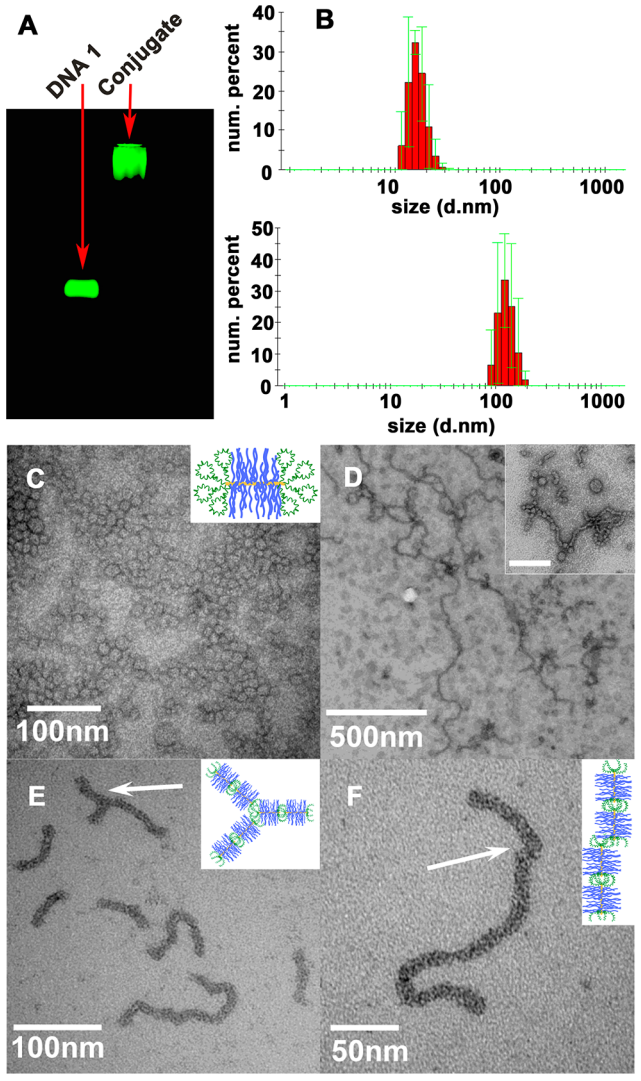


Figure 1. (A) Gel image of the hairpin DNA-polymer conjugate and free DNA-1 (fluorescein filters were applied). (B) DLS data of the conjugate before (top) and after (bottom) annealing. (C) TEM image of the free DNA-polymer conjugate, showing a sphere-like morphology. (D–F) Condensed brushes following annealing. The inset in D shows the worm-like structure as it is being formed; the scale bar is 100 nm. Arrows point to branching (E) and displacement (F) defects.

inset). The DNA-mediated assembly process is fully reversible. Upon removal of NaCl and/or increasing the temperature above the duplex T_m followed by rapid cooling, the worms revert to discrete brush polymers (Figure S5). In addition, in the presence of an excess of a free, complementary strand (chain terminator), the polycondensation process is hindered, and only free "macromonomers" are observed by TEM after annealing (Figure S6).

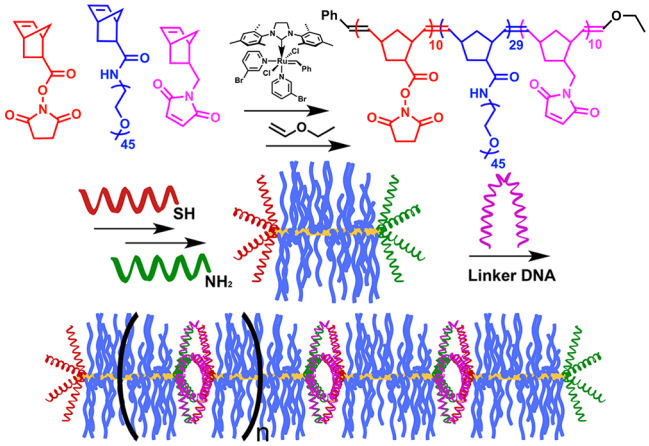
The worm-like morphology is targeted in our study because it has been recognized to exert a profound impact on the behavior of nanomaterials in biological systems.[35−37] For example, filomicelles of several microns in length showed prolonged blood circulation times up to several days.[38] Worm-like polymer micelles that were modified with folate could enter KB cells with nearly 5-fold higher selectivity compared with their spherical counterparts modified with equal amounts of folate.[39] This method of worm-formation, to the best of our

dx.doi.org/10.1021/ja504790r | J. Am. Chem. Soc. 2014, 136, 10214−10217

knowledge, is a new strategy yet unreported, which can potentially lead to programmable assemblies of macromolecules.

In addition to using temperature to initiate the self-assembly process, we were also interested in assembling the brushes with an added DNA sequence as a linker strand. This strategy requires that two different DNA strands to be connected to the two ends of polymer brush in a regioselective fashion. In order to achieve such a structure, we synthesized a thiol-reactive monomer (norbornenyl maleimide, N-MI) and incorporated it into the triblock brush as the third block ($p(N\text{-}NHS)_{10}$-*b*-$p(N\text{-}PEG)_{29}$-*b*-$p(N\text{-}MI)_{10}$, Scheme 2). This "heterotelechilic"

Scheme 2. Synthesis of "Heterotelechelic" DNA-Polymer Conjugate and Induced Self-Assembly upon Addition of a Linker Strand



polymer features NHS ester units which can couple with amine-modified DNA strands on the first block and maleimide groups for reaction with thiol-modified DNA strands on the third block. The successful synthesis of the polymer is verified by MALDI-ToF MS (M_n = 67.5 kDa, PDI = 1.20, Figure S1, for GPC see Figure S2). In addition, the incorporation of the maleimide units was confirmed by ^{1}H NMR (Figure S7), which shows the resonance of the maleimide double bond protons at 6.63 ppm.

The two DNA strands to be conjugated to the polymer are designed to be free of self-dimers or hairpins, but can both hybridize with a common linker strand (DNA-2:5′-Cy3-GAG GGT AAG GAG TTT-SH-3′, DNA-3:5′-NH_2-TTT GGA AAG GTT AGT-F-3′, and linker DNA: 5′-CTC CTT ACC CTC ACT AAC CTT TCC-3′). Again, fluorescent dyes (Cy3 and fluorescein) are incorporated to allow convenient tracking, quantification, and multiplex imaging of the DNA. The regioselective conjugation of the two different DNA strands to the polymer brush proceeds via a two-step process. We first incubated DNA-2 with the polymer at 4 °C and pH 7.0 for 1 h. This consumes the maleimide groups selectively (the reaction rate of thiols to maleimide is 3 orders of magnitude higher than that of amines to maleimides at pH 7.0). The short reaction time and lowered temperature are to preserve the NHS esters from hydrolytic degradation. Thereafter, amine modified DNA-3 was added, and the pH of the solution was increased to 8.3. This step leads to the conjugation of the second DNA strand. The conjugate is again purified by agarose gel electrophoresis. The gel image shows a high-MW band corresponding to the conjugate, which emits both fluorescein and Cy3 fluorescence, indicating that both DNA strands are successfully conjugated (Figure 2A). Quantification by fluorescence shows that ∼3 DNA-2 strands and ∼6 DNA-3 strands are conjugated to each brush.

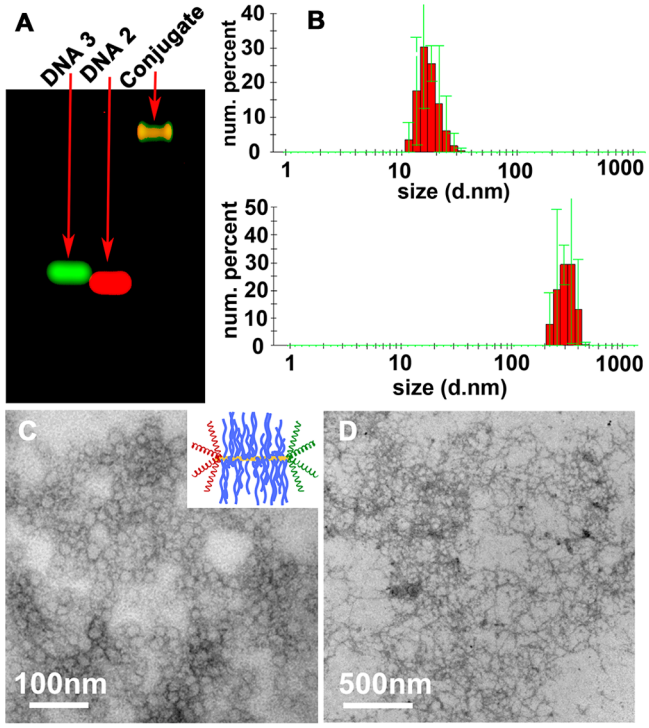


Figure 2. (A) Multiplex gel image of the "heterotelechelic" DNA-polymer conjugate and free DNA-2 and DNA-3 strands (fluorescein and Cy3 channels are overlaid). (B) DLS measurements before (top) and after (bottom) assembly. (C) TEM images of free conjugates and (D) self-assembled structure following addition of the linker strand.

We next investigated the assembly of the DNA-polymer conjugate in the presence of the linker sequence. Before the self-assembly, the conjugates are discrete, spherical particles similar to the hairpin DNA-polymer conjugates (Figure 2C), with a mean hydrodynamic diameter of 17 ± 6 nm as determined by DLS (Figure 2B). The addition of the linker DNA (3.0 equiv to polymer) increases the size to 308 ± 71 nm. TEM reveals that the brushes have formed cross-linked networks (Figures 2D and S8). In contrast, a noncomplementary dummy linker has no effect on the assembly state of the brushes (Figure S9). On closer examination of the networks, it is observed that the degree of branching is much more significant when compared with the self-dimer constructs, with branching every one to two repeat units (Figure S8A,B). This is likely due to the added length of the linker strand, which creates room for more than two brushes to connect at each junction. Such a scenario may be further favored by the rigidity of the duplexes, which creates kinks in the assembly, facilitating branching. A third possibility would be the longer length (10 repeating units) of the oligomeric blocks used for DNA conjugation. These factors restrict the polymer brushes from forming very long linear structures and instead guided them to yield cross-linked networks. Of note, it is important to have a strict stoichiometry of the linker strand. With a large stoichiometric imbalance (i.e., <0.5 equiv or >10 equiv of the linker DNA), assembly is greatly hindered. This is expected as each junction requires at least one linker strand, but excessive

dx.doi.org/10.1021/ja504790r | J. Am. Chem. Soc. 2014, 136, 10214−10217

amounts would quickly populate all of the brush chain ends, which disfavors further assembly of the brushes.

This study opens up new promising possibilities to create tailored polymer assemblies. We expect that with the synthetic availability of various types of polymer architectures, the diversity of the assembled polymer structures should increase significantly. The hybridization-controlled self-assembly also has the potential to be mediated by innate mRNA in an *in vivo* setting and therefore has important implications in nanomedicine.

■ ASSOCIATED CONTENT

Supporting Information

Materials, experimental procedures, DNA synthesis, and supplemental figures. 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

The authors thank William Fowle at the NEU Biology Department for help with the TEM. Financial support from Northeastern University start-up and Tier 1 seed grant (FY2013) is gratefully acknowledged.

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