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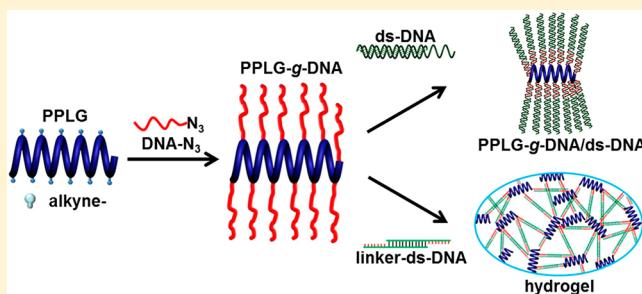
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Supporting Information

ABSTRACT: A new type of DNA grafted polypeptide molecular brush was synthesized via a combination of ring-opening polymerization (ROP) and click chemistry. This conjugation method provides an easy and efficient approach to obtain a hybrid DNA-grafted polypeptide molecular bottlebrush. The structure and assembly behaviors of this hybrid brush were investigated using electrophoresis, UV-vis spectroscopy, transmission electron microscopy (TEM), and atomic force microscopy (AFM). Hierarchical supramolecular assemblies can be obtained through hybridization of two kinds of polypeptide-g-DNA molecular bottlebrushes containing complementary DNA side chains. We further demonstrated that such polypeptide-g-DNA can be hybridized with ds-DNA and DNA-grafted gold nanoparticles to form a supermolecular bottlebrush and hybrid bottlebrush, respectively. In addition, DNA-polypeptide hydrogel can be prepared by hybridization of polypeptide-g-DNA with a linker-ds-DNA, which contains the complementary “sticky ends” to serve as cross-linkers.



INTRODUCTION

DNA molecules have been used to build highly sophisticated supramolecular assemblies with great precision because of their unique structural features and remarkable molecular recognition properties.^{1–3} Using specific DNA as a building unit, various fascinating nanostructures have been constructed in a close mimic to biological systems.^{4,5} Moreover, these supramolecular structures can serve as templates to pattern proteins, inorganic materials, and nanoparticles with great precision.^{6–8} In particular, DNA/polymer hybrids, which combine functional and structural properties of biomacromolecules with synthetic polymers, emerge as new functional materials with promising applications.⁹ Various polymers such as poly(ethylene glycol),^{10,11} poly(*N*-isopropylacrylamide),^{12,13} polystyrene^{12,14} and poly(propylene oxide)¹⁵ have been covalently linked with DNA to form hybrid copolymers. These bioconjugates were employed to construct supramolecular structures and nanodevices with unprecedented properties.^{16–20} In the meantime, the molecular bottlebrush as a unique macromolecular architecture attracts broad research attention because of their interesting properties and potential applications.^{21–23} Recently, a great amount of research interest has been focused on attaching DNA as side chains to a synthetic polymer backbone. These particular hybrid materials have been utilized in DNA detection applications,^{24–26} DNA cross-linked hydrogel systems,^{27–31} and stimuli-responsive micelle assemblies.³² Several strategies have been reported to prepare DNA/polymer hybrids

with DNA as a side chain. For example, a DNA chain was firstly linked to acrylate to form a macromonomer, which was polymerized to give polymer-g-DNA bottlebrushes.³³ Also, an end-functionalized DNA chain was directly linked to polymers via a graft-onto strategy.³⁴ On the other hand, a synthetic polymer can also be conjugated to DNA via solid supported synthesis.^{35,36} However, most reported examples have a nonbiodegradable carbon–carbon backbone, which could seriously limit their biomedical applications. Hence, designing a new strategy to conjugate DNA onto biodegradable biopolymers would be greatly desirable.

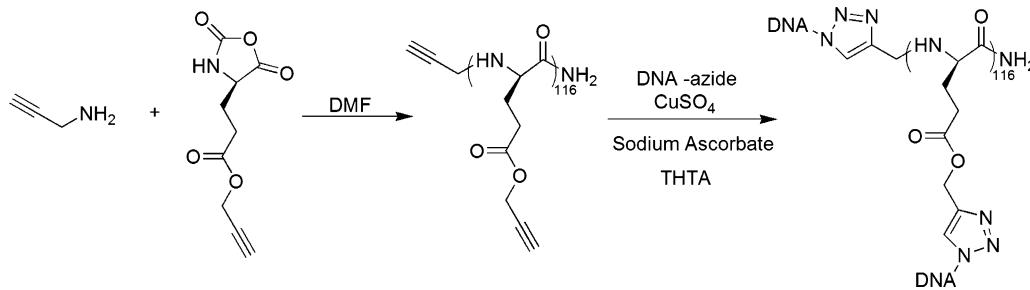
Polypeptides as promising biomaterials have received great interest because of their well-defined secondary structures and biocompatibility.^{37,38} Moreover, molecular bottlebrushes using these biomimetic polypeptides as a backbone have been prepared by atom transfer radical polymerization (“grafting from” method)^{39,40} or click chemistry (“grafting onto” method).^{41–45} Recently, we also reported a new type of molecular bottlebrush containing a polypeptide as the backbone via ring-opening polymerization (ROP) of amino acid *N*-carboxyanhydride (NCA) followed by ATRP⁴⁶ or click chemistry.⁴⁷ Meanwhile, examples of involving polypeptides as backbone attached with nucleic acid were still rare.⁴⁸ Herein,

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Scheme 1. Synthetic Routes to PPLG-g-DNA Molecular Bottlebrush



we demonstrate a convenient route to synthesize DNA grafted polypeptide molecular bottlebrushes via a combination of ROP of γ -propargyl-L-glutamate N-carboxyanhydride (PLG-NCA) followed by copper(I) catalyzed Huisgen [3 + 2] cycloaddition with azide-functionalized DNA strands (Scheme 1). This new synthetic strategy represents a facile and efficient approach of grafting DNA onto polypeptides.

EXPERIMENTAL SECTION

Materials and Characterizations. The azide-DNA and SH-DNA were purchased from TaKaRa Biotech (Dalian, China) and HPLC purified. All other chemicals were purchased from Sigma-Aldrich. All buffers were prepared with ultrapure Milli-Q water (resistance >18 M Ω ·cm⁻¹). ¹H NMR spectra were recorded on a Bruker AV400 FT-NMR spectrometer (at 400 MHz). Fourier transform infrared spectra were acquired using a Nicolet Avatar 330 FT-IR spectrometer. The solution samples were cast on KBr plates before measurements. Tandem size exclusion chromatography/laser light scattering (SEC/LLS) was performed at 50 °C using an SSI pump connected to Wyatt Optilab DSP and Wyatt DAWN EOS light scattering detectors with 0.02 M LiBr in DMF as the eluent at a flow rate of 1.0 mL/min. The concentration was about 5 mg/mL. UV/vis spectra were recorded on a Varian Cary 100 spectrophotometer equipped with a programmable temperature-control unit. Denaturing polyacrylamide gel electrophoresis (PAGE) (10 wt %, Acr/Bis = 19:1, 7 M Urea) was run at 20 V/cm in 1 × TBE buffer. After the electrophoresis, the gel was stained with Gel-red dye. 3% agarose gel electrophoresis was run at 4 V/cm in 0.5 × TBE buffer. Atomic force microscopy (AFM) measurements were performed on a Veeco MultiMode 8 Scanning Probe Microscope operated in tapping mode. The rheology of DNA hydrogel was carried out on an AR-G2 rheometer (TA Instruments) equipped with a temperature controller.

Synthesis of γ -Propargyl-L-glutamate (PLG). The γ -propargyl-L-glutamate (PLG) was prepared by direct coupling between propargyl alcohol and L-glutamic acid via sulfuric acid catalyzed esterification.⁴² To a solution of L-glutamic acid (20 g) suspended in propargyl alcohol (30 mL) at 0 °C, 8 mL of sulfuric acid were added dropwise over 20 min. The solution was stirred at room temperature overnight before being neutralized with excess 5 wt % NaHCO₃ aqueous solution. A white solid appeared and was collected by centrifugation. The product was then dispersed in methanol, and the mixture was filtered again to remove insoluble byproducts. The filtrate was combined, and the solvent was removed using a rotary evaporator to give a white solid in 38% yield. ¹H NMR (400 MHz, D₂O): δ = 2.17–2.29 (m, 2H, CHCH₂CH₂), 2.55–2.66 (m, 2H, CHCH₂CH₂), 2.93 (t, 1H, C≡CH), 3.81 (t, 1H, CHCH₂CH₂), 4.76 (d, 2H, OCH₂C≡CH).

Synthesis of γ -Propargyl-L-glutamate N-Carboxyanhydride (PLG-NCA). After purification, the PLG monomer was then converted into corresponding α -amino acid N-carboxyanhydride (NCA) using triphosgene in THF at 50 °C. The obtained NCA was viscous oil at room temperature and was redissolved in ethyl acetate, followed by washing with ice-cold water and a 0.5% NaHCO₃ ice-cold aqueous solution sequentially. The organic phase was then dried over anhydrous MgSO₄. The solution was then filtrated, and the solvent was removed via rotary evaporator to give PLG-NCA as a viscous

liquid in 20% yield. ¹H NMR (400 MHz, CDCl₃): δ = 2.17–2.29 (m, 2H, CHCH₂CH₂), 2.51 (t, 1H, C≡CH), 2.60–2.71 (m, 2H, CHCH₂CH₂), 4.41 (t, 1H, CHCH₂CH₂), 4.72 (d, 2H, OCH₂C≡CH), 6.27 (s, br, 1H, NH).

Synthesis of Poly(γ -propargyl-L-glutamate) (PPLG). Poly(γ -propargyl-L-glutamate) (PPLG) was obtained by ROP of PLG-NCA using the propargylamine as the initiator. SEC/LLS characterization gave M_n = 19 500 Da and PDI = 1.07 (Figure S1). The isolated yield is 85% with the degree of polymerization (DP) being 116. ¹H NMR (400 MHz, CDCl₃/CF₃CO₂D (v/v = 10:1)): δ = 2.02–2.86 (br, 5H, CHCH₂CH₂, C≡CH, and CH₂CH₂COO), 3.56 (br, 2H, NCH₂C≡CH), 4.05 (br, 1H, CHCH₂CH₂), 4.72 (br, 2H, OCH₂C≡CH), 8.28 (br, 1H, NH).

Synthesis of (Tris-(1-[3-hydroxypropyl]triazolyl-4-methyl)-amine) (THTA). THTA was synthesized and purified as previously reported.⁴⁹ The 3-bromo-1-propanol (10.0 mmol) and NaN₃ (1.30 g, 20.0 mmol) mixture was stirred in 30 mL of water at 80 °C for 24 h. Then the solution was cooled to rt and extracted twice with diethyl ether. After washing with brine, the organic phase was dried over anhydrous Na₂SO₄ and evaporated to give crude 3-azidopropan-1-ol. It was immediately redissolved in MeCN (15 mL), followed by the addition of triprop-2-nylamine (1.75 mmol), 2,6-lutidine (1.75 mmol), and CuBr (0.158 mmol). The reaction mixture was stirred at room temperature for 64 h under nitrogen. The product was purified using column chromatography (CH₂Cl₂/MeOH, 10:1 → CH₂Cl₂/MeOH, 3:1). ¹H NMR (400 MHz, CD₃OD): δ = 7.99 (s, 3H, C≡CH), 4.51 (t, 6H, NCH₂CH₂CH₂OH), 3.75 (s, 6H, NCH₂C), 3.56 (t, 6H, NCH₂CH₂CH₂OH), 2.10 (qn, 6H, NCH₂CH₂CH₂OH).

Synthesis of PPLG-g-DNA. Typically, PPLG (4.6 mg) was dissolved in DMSO (275 μ L) as a stock solution. 10 μ L of this PPLG/DMSO solution were mixed with 10 μ L of a CuSO₄ solution (200 mM), 7 μ L of THTA (2 M), 24 μ L of an azide-DNA solution (416 μ M), 10 μ L of sodium ascorbate solution (2 M), and 39 μ L of DMSO. After incubation at 50 °C overnight with shaking, 0.4 mL of H₂O was added into the reaction mixture to precipitate the unreacted PPLG. The supernatant was separated by Millipore's Microcon YM-30 filter to remove the unreacted DNA. Three PPLG-g-DNA hybrids, i.e., PPLG-g-S-A, PPLG-g-S-B and PPLG-g-S-C, were synthesized using the above-described strategy. Specifically, the DNA strands in PPLG-g-S-A and PPLG-g-S-B samples are complementary to each other. The detailed sequence information can be found in the Supporting Information. The DNA concentrations of the resulting products were determined by measuring DNA absorbance at 260 nm using a UV-vis spectrophotometer.

Hybridization Assemblies of Azide-S-A/C-S-A, PPLG-g-S-A/C-S-A, and PPLG-g-S-A/PPLG-g-S-B. The DNA strand of C-S-A is complementary to azide-S-A without an azide group. Stoichiometric amounts of DNA or PPLG-g-DNA hybrids were mixed in 100 mM NaCl, 0.5 × TBE to give a final concentration of 1.5 μ M for each DNA strand, respectively. The mixture was heated to 95 °C for 5 min and then cooled to room temperature over 2 h to form DNA hybridizations. The properties of the resulting assemblies were characterized using agarose gel electrophoresis and AFM. AFM samples were prepared by drop-coating sample solution onto freshly cleaned silica surfaces for AFM imaging.

Hybridization between PPLG-g-DNA and Double-Stranded DNA (ds-DNA). DNA strands A and cA were mixed in 10 mM Tris-HCl (pH 8.0) and 100 mM NaCl to give a final concentration of 100 μ M for each DNA strand. The mixture was heated to 95 °C for 5 min and then cooled to room temperature over 2 h to form ds-DNA. Then 4 μ L of ds-DNA (100 μ M) were mixed with 10 μ L of PPLG-g-DNA molecular bottlebrushes (4 μ M in DNA) and kept overnight at room temperature. The mixture was separated by Millipore's Microcon YM-100 filter to remove the excess ds-DNA. AFM samples were prepared by drop casting onto freshly cleaned silica surfaces for AFM imaging. TEM samples were prepared by drop casting solution on carbon coated copper grids and staining with a 1 wt % uranyl acetate aqueous solution.

Hybridization between PPLG-g-DNA and DNA-Grafted Gold Nanoparticles. Gold nanoparticles (5 nm) were prepared and purified according to the published method,⁵⁰ and the concentration was calculated by measuring the UV absorption of the gold nanoparticles at a wavelength of 520 nm. 17.4 μ L of SH-DNA (400 μ M) were added to a 60.9 μ L solution of Au nanoparticles (3.81 μ M). After incubation at room temperature overnight, 13.4 μ L of this DNA modified gold nanoparticle solution (2.96 μ M in Au nanoparticle) were mixed with 6.6 μ L of PPLG-g-DNA (PPLG-g-S-A) molecular bottlebrushes (4 μ M in DNA) and kept overnight. The solution was used directly for TEM characterizations.

Hydrogel Formation via Hybridization of PPLG-g-DNA with Linker-ds-DNA. DNA strands L-1 and L-2 were mixed in 1 \times TBE buffer and 200 mM NaCl to give a final concentration of 2.5 mM for each DNA strand. The mixture was heated to 95 °C for 5 min and then cooled to room temperature over 2 h to form linker-ds-DNA. Then 20.3 μ L of linker-ds-DNA (2.5 mM) were mixed with 16.7 μ L of the PPLG-g-DNA (PPLG-g-S-C) sample (6.8 mM with respect to DNA) in 1 \times TBE buffer and a 200 mM NaCl solution. The mixture changed immediately from the fluidic solution into a transparent gel. The properties of the resulting hydrogel were characterized using oscillatory sheer rheology. Frequency sweep experiments were performed between 0.5 and 50 rad/s at 25 °C with a fixed strain of 1%.

RESULTS AND DISCUSSION

As illustrated in Scheme 1, PPLG was prepared by ROP of an alkyne-substituted L-glutamate NCA using propargylamine as an initiator in DMF.⁴¹ SEC/LLS characterization gave $M_n = 19\,500$ Da and PDI = 1.07. Subsequently, azide terminated DNA (DNA-N₃) with 16 bases were directly conjugated to the side chain of PPLG via copper(I) catalyzed Huisgen [3 + 2] cycloaddition. The click reactions were conducted in a DMSO/H₂O mixture with the presence of CuSO₄, a THTA ligand,⁴⁹ and a sodium ascorbate solution. After incubation at 50 °C overnight with shaking, the reaction mixtures were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE) (Figure 1a). The conjugation yields can be monitored and identified by imaging of the gel electrophoresis. As shown in Figure 1a (lane 2), several distinct bands with slower mobility are observed in the denaturing PAGE as compared with the control experiment (Figure 1a, lane 1). These discrete ladder-like bands appearing in the same lane suggest a shift of pristine DNA to higher molecular weight species. The increase of molecular weight indicates the successful conjugation of DNA onto PPLG although the exact DNA number per PPLG polypeptide is unknown from available data. The multiple bands with varying mobility indicate that PPLG macromolecules are grafted with different amounts of DNA chains. From an agarose gel image, we can infer that the molecular bottlebrushes with lower grafting density are the major species as indicated in Figure S2, which is typical for the graft-onto strategy to make molecular bottlebrushes.^{21,22} Extension of the reaction time did not solve this problem. There are two possible reasons. One is that the

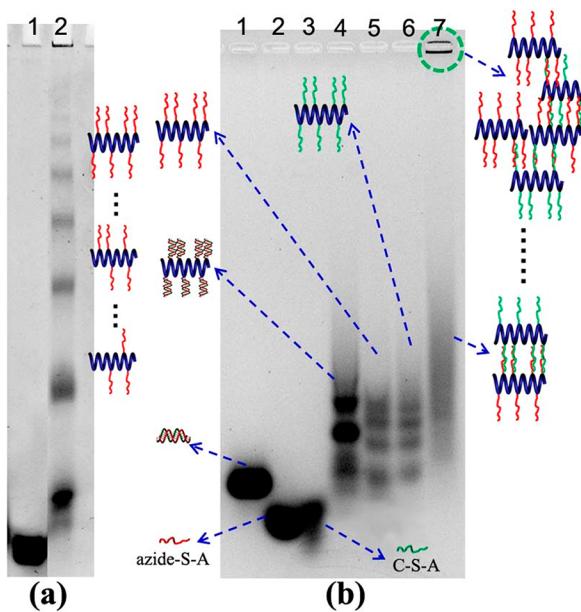


Figure 1. (a) PAGE characterization of azide-DNA sample (Lane 1) and PPLG/azide-DNA hybrids after click reaction (Lane 2). (b) Characterization of the assembly structures by agarose gel: lane 1, azide-S-A/C-S-A; lane 2, azide-S-A; lane 3, C-S-A; lane 4, PPLG-g-S-A/C-S-A; lane 5, PPLG-g-S-A; lane 6, PPLG-g-S-B; lane 7, PPLG-g-S-A/PPLG-g-S-B (C-S-A: complementary DNA strand of azide-S-A without azide modified).

click reaction between PPLG and azide-DNA is not quantitative due to steric hindrance and electrostatic repulsion between DNA molecules. The other reason is that the synthetic PPLG backbone has a certain distribution, which also induces separation of bands in gel electrophoresis. After a click reaction, the excess PPLG was removed by precipitating into water, and the supernatant was isolated by Millipore's Microcon YM-30 filter to remove the unreacted DNA. The identification of PPLG-g-DNA molecular bottlebrushes after purification is confirmed using gel electrophoresis shown in Figures 1b (lanes 5 and 6) and S3. The obtained PPLG-g-DNA hybrids were then characterized using FTIR and UV-vis spectroscopy to verify their structures. Figure 2a shows the FTIR spectra of the PLG-NCA monomer, PPLG homopolypeptide, and PPLG-g-DNA samples. Both PLG-NCA and PPLG show a characteristic $\text{—C}\equiv\text{C—}$ stretching band at 2130 cm^{-1} . After the click reaction, the absorbance of $\text{—C}\equiv\text{C—}$ completely disappears and the appearance of absorptions at 1070 and 1240 cm^{-1} are attributed to symmetric and asymmetric stretching vibrations of DNA phosphodiester groups,¹³ indicating successful conjugation of DNA onto PPLG. Furthermore, the UV-vis spectrum of the PPLG-g-DNA copolymer displays the characteristic absorption of DNA at ~ 260 nm (Figure 2b). These results shown above suggest the successful conjugation of DNA strands onto a PPLG backbone.

Using the same procedure, we prepared two types of PPLG-g-DNA molecular bottlebrushes, i.e., PPLG-g-S-A and PPLG-g-S-B, which have the identical PPLG backbone. The differentiating factor for them is that they contain complementary DNA as side chains, which can be used to prepare complex aggregates via DNA hybridization. To examine this idea, we mixed PPLG-g-S-A and PPLG-g-S-B in 100 mM NaCl, 10 mM Tris-HCl (pH 8.0) buffer. The mixture was heated to 95 °C for 5 min to destroy any preordered structures of the side chain

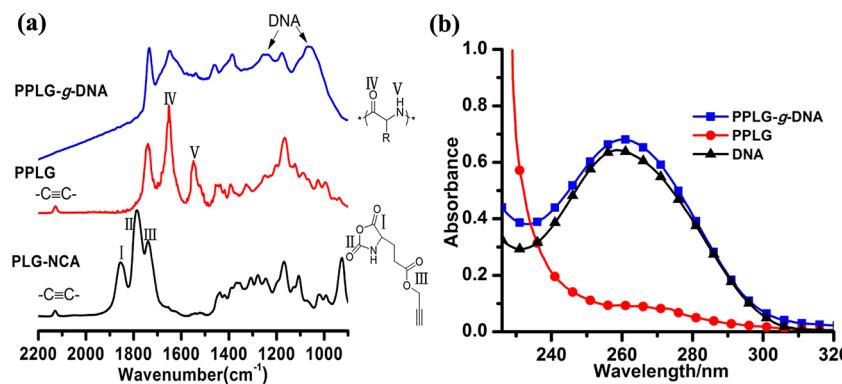


Figure 2. (a) FTIR spectra of PLG-NCA, PPLG, and PPLG-g-DNA. (b) UV-vis spectra of DNA (azide-S-A), PPLG, and PPLG-g-DNA molecular bottlebrushes (PPLG-g-S-A).

Scheme 2. Schematic Illustration of Hybridization between PPLG-g-DNA and ds-DNA

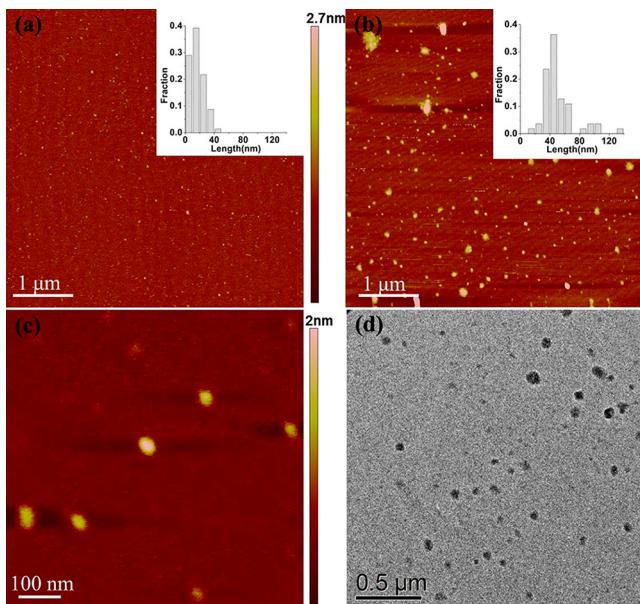
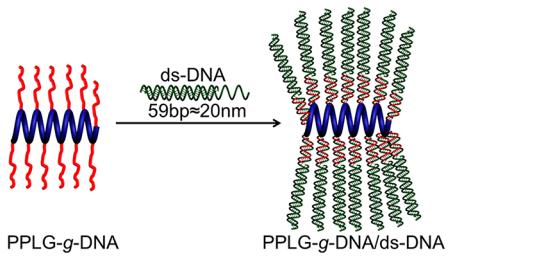


Figure 3. AFM images of PPLG-g-DNA before (a) and after (b–c) hybridization with ds-DNA. Inset: Corresponding histograms showing the size distribution of PPLG-g-DNA before and after hybridization with ds-DNA of randomly counted. (d) TEM image of PPLG-g-DNA hybridization with ds-DNA.

DNA and then cooled to room temperature over 2 h. After the annealing, the mixture was then characterized using gel electrophoresis. Apparently, new bands with slower mobility were observed in the agarose gel electrophoresis (Figure 1b, lane 7), indicating the formation of large aggregates arising most likely from intermolecular DNA-hybridization between PPLG-g-S-A and PPLG-g-S-B. The resulting assemblies were

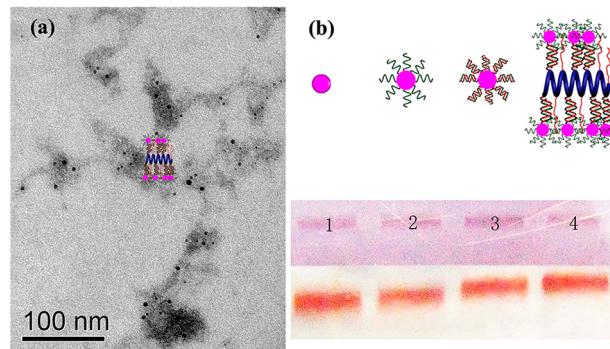
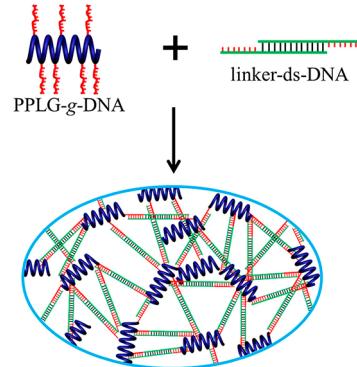


Figure 4. (a) Typical TEM image of PPLG-g-DNA templated assemblies of Au-NP without staining. (b) Agarose gel electrophoresis of different DNA conjugated Au-NP: lane 1, free Au-NP; lane 2, ss-DNA modified Au-NP; lane 3, ds-DNA modified Au-NP; lane 4, PPLG-g-DNA modified Au-NP.

Scheme 3. Schematic Illustration of DNA-Polypeptide Hybrid Hydrogel Formation^a



^aThe PPLG-g-DNA and linker-ds-DNA are designed to crosslink by hybridization of their “sticky ends”.

then characterized using AFM. Figure S4a shows an AFM image of these hierarchical assemblies, which appear to be spherical aggregates with a height of about 15 nm (Figure S4b). The schematic illustration of these hybridization structures is shown in Figure 1b. Upon hybridization between two PPLG-g-DNA molecular bottlebrushes containing complementary side-chain DNA, hierarchical supramolecular assemblies with an extended network are obtained. These results agree well with our design and suggest that each polypeptide backbone has

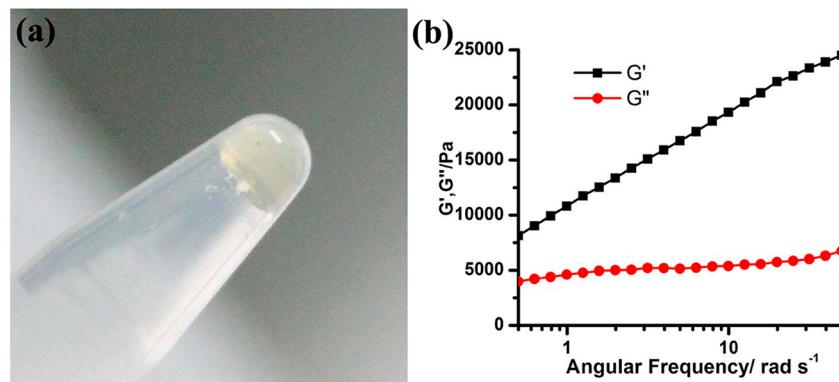


Figure 5. (a) Image of 7.8% (w/v) hydrogel samples in 1 × TBE buffer and 200 mM NaCl. (b) Oscillatory rheological frequency sweep experiments performed between 0.5 and 50 rad/s at 25 °C with a fixed strain of 1%.

linked with more than one DNA strand. These PPLG-g-DNA hybrids offer a new platform to realize controlled self-assembly and supramolecular structures unavailable to linear DNA systems.

To further verify the structures of the PPLG-g-DNA molecular bottlebrush, the DNA side chain was hybridized with “sticky ends” of ds-DNA (Scheme 2). 4 μL of ds-DNA (100 μM) were mixed with 10 μL of the PPLG-g-DNA molecular bottlebrush (4 μM with respect to DNA) in 10 mM Tris-HCl (pH 8.0) and a 100 mM NaCl solution. The mixture was kept overnight at room temperature. The excess ds-DNA was removed using centrifugal ultrafiltration devices. As illustrated in Scheme 2, polypeptide molecular bottlebrushes containing a shell of ds-DNA are expected to form after hybridization. The structure of the resulting super PPLG-g-DNA/ds-DNA complex was then examined using AFM and TEM to check the variation in molecular bottlebrush size and nanostructures. AFM images taken before and after the hybridization are shown in Figure 3a and 3b. Apparently, AFM characterization reveals an increase in sample size after the hybridization process. AFM analysis reveals spherical-like structures before the hybridization (Figure 3a). The length distribution of the PPLG-g-DNA molecular bottlebrush plotted in a histogram shows a number-average length of about 16 nm (inset, Figure 3a), which is consistent with the contour length of PPLG₁₁₆ at the α -helical conformation ($l_0^{\text{helix}} = 0.15 \text{ nm}$, DP = 116). Figure 3b shows the AFM image of the PPLG-g-DNA/ds-DNA complex after the hybridization. The histogram of the analyzed lengths reveals a maximum at about 51 nm (inset, Figure 3b), which is longer than the expected value for the PPLG-g-DNA/ds-DNA complex (~40 nm, assuming 0.34 nm per base pair). The discrepancy may result from the surface absorption. The height of the molecular bottlebrushes adsorbed on mica is about 2 nm (Figure 3c), which correlates with B-DNA (2 nm in diameter). The TEM images of the PPLG-g-DNA/ds-DNA complex also reveal ellipsoidal-like structures (Figure 3d). These dimensions are in good agreement with the assemblies found with AFM and indicate successful hybridization illustrated in Scheme 2.

The PPLG-g-DNA molecular bottlebrushes were also used to hybridize with DNA-grafted gold nanoparticles in terms of controlling their spatial distribution. In a typical experiment, 13.4 μL of a DNA-grafted gold nanoparticle solution (2.96 μM in Au nanoparticle) were mixed with 6.6 μL of PPLG-g-DNA molecular bottlebrushes (4 μM in DNA). It was worth noting that excess gold nanoparticles were used to avoid the

disordered macroscopic aggregation. The mixture was then kept overnight at room temperature. The assembly structure of the gold nanoparticles was characterized by TEM and agarose gel electrophoresis. Clusters of gold nanoparticles are observed as shown in Figure 4a. In contrast, the same DNA-grafted gold nanoparticles were just individual particles as supported from TEM characterization (Figure S7c). The electrophoretic mobility of PPLG-g-DNA modified Au-NP turns out to be the slowest compared with others (Figure 4b), which further supports the hybridization of DNA-grafted gold nanoparticles with PPLG-g-DNA molecular bottlebrushes. Furthermore, the UV-vis spectra of these assemblies show a strong surface plasmon resonance absorption peak at approximately 520 nm (Figure S8). Thus, these DNA-grafted molecular bottlebrushes can be used as a scaffold to direct assembly of functional nanomaterials with tunable space distribution. In addition, it provides a platform for incorporating other functional species into the DNA-grafted polypeptide molecular bottlebrushes with the same strategy.

Because of the multifunctional nature of PPLG-g-DNA molecular bottlebrushes, it also can lead to a three-dimensional network through the hybridization of side-chain DNA with linker-DNA to form hydrogel. As illustrated in Scheme 3, the linker-ds-DNA formed by two ss-DNAs contains two “sticky ends”, which can hybridize with side-chain DNA of the PPLG-g-DNA molecular bottlebrush. Given the rigid α -helical conformation of the polypeptide main chain, intermolecular linkage through linker-ds-DNA will predominate. Upon addition of linker-ds-DNA into the PPLG-g-DNA solution, the mixture changed immediately from the fluidic solution into a transparent gel (Figure 5a). The properties of the resulting hydrogel were then characterized using oscillatory shear rheology. Frequency sweep tests were performed between 0.5 and 50 rad/s with a fixed strain of 1% at 25 °C (Figure 5b). In the frequency sweep, the storage modulus (G') is obviously higher than the loss modulus (G'') over the entire frequency range, suggesting the hydrogel status. In addition, G' and G'' show a gradual slope upward with increasing frequency. This behavior is consistent with soft cross-linked elastomers. This hydrogel system will provide a new class of material for potential applications *in vivo* because of its good biocompatibility and biodegradability.

CONCLUSION

In summary, we have synthesized a new type of PPLG-g-DNA molecular bottlebrush via a combination of ROP of NCA

polymerization and a copper(I) catalyzed Huisgen [3 + 2] cycloaddition click reaction. Our research demonstrated a facile and efficient approach to preparing DNA grafted polypeptide molecular bottlebrushes, from which an extended network can be fabricated through hybridization of two kinds of PPLG-g-DNA molecular bottlebrushes containing complementary DNA side chains. Using ds-DNA with specific “stick ends”, we can easily construct core–shell-like supermolecular bottlebrushes via specific DNA hybridization. It also can serve as templates to pattern DNA-grafted gold nanoparticles via hybridization. Furthermore, based on the “sticky ends” of the ds-DNA, a new class of hydrogel can be formed by the hybridization of PPLG-g-DNA molecular bottlebrushes with linker-ds-DNA. Such PPLG-g-DNA hybrid bottlebrushes might show promising applications serving as multifunctional ligands and potential candidates for assembled hierarchical supramolecular nanostructures with unique architecture and properties.

ASSOCIATED CONTENT

Supporting Information

DNA sequences, SEC, additional gel electrophoresis, circular dichroism (CD), TEM images, and UV-vis spectra. 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.

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REFERENCES

- (1) Seeman, N. C. *Nature* **2003**, *421*, 427–431.
- (2) Aldaye, F. A.; Palmer, A. L.; Sleiman, H. F. *Science* **2008**, *321*, 1795–1799.
- (3) Pinheiro, A. V.; Han, D.; Shih, W. M.; Yan, H. *Nat. Nanotechnol.* **2011**, *6*, 763–772.
- (4) Feldkamp, U.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 1856–1876.
- (5) Torring, T.; Voigt, N. V.; Nangreave, J.; Yan, H.; Gothelf, K. V. *Chem. Soc. Rev.* **2011**, *40*, 5636–5646.
- (6) Rajendran, A.; Endo, M.; Sugiyama, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 874–890.
- (7) Samano, E. C.; Pilo-Pais, M.; Goldberg, S.; Vogen, B. N.; Finkelstein, G.; LaBean, T. H. *Soft Matter* **2011**, *7*, 3240–3245.
- (8) Fu, J.; Liu, M.; Liu, Y.; Yan, H. *Acc. Chem. Res.* **2012**, *45*, 1215–1226.
- (9) Kwak, M.; Herrmann, A. *Angew. Chem., Int. Ed.* **2010**, *49*, 8574–8587.
- (10) Alemdaroglu, F. E.; Safak, M.; Wang, J.; Berger, R.; Herrmann, A. *Chem. Commun.* **2007**, 1358–1359.
- (11) Jeong, J. H.; Kim, S. H.; Kim, S. W.; Park, T. G. *Bioconjugate Chem.* **2005**, *16*, 1034–1037.
- (12) Safak, M.; Alemdaroglu, F. E.; Li, Y.; Ergen, E.; Herrmann, A. *Adv. Mater.* **2007**, *19*, 1499–1505.
- (13) Pan, P.; Fujita, M.; Ooi, W.-Y.; Sudesh, K.; Takarada, T.; Goto, A.; Maeda, M. *Polymer* **2011**, *52*, 895–900.
- (14) Li, Z.; Zhang, Y.; Fullhart, P.; Mirkin, C. A. *Nano Lett.* **2004**, *4*, 1055–1058.
- (15) Alemdaroglu, F. E.; Ding, K.; Berger, R.; Herrmann, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4206–4210.
- (16) Jeong, J. H.; Park, T. G. *Bioconjugate Chem.* **2001**, *12*, 917–923.
- (17) Lee, K.; Povlich, L. K.; Kim, J. *Adv. Funct. Mater.* **2007**, *17*, 2580–2587.
- (18) Ding, K.; Alemdaroglu, F. E.; Boersch, M.; Berger, R.; Herrmann, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 1172–1175.
- (19) Alemdaroglu, F. E.; Wang, J.; Boersch, M.; Berger, R.; Herrmann, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 974–976.
- (20) Kwak, M.; Herrmann, A. *Chem. Soc. Rev.* **2011**, *40*, 5745–5755.
- (21) Zhang, M.; Müller, A. H. E. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 3461–3481.
- (22) Sheiko, S. S.; Sumerlin, B. S.; Matyjaszewski, K. *Prog. Polym. Sci.* **2008**, *33*, 759–785.
- (23) Lee, H.-i.; Pietrasik, J.; Sheiko, S. S.; Matyjaszewski, K. *Prog. Polym. Sci.* **2010**, *35*, 24–44.
- (24) Minard-Basquin, C.; Chaix, C.; Pichot, C.; Mandrand, B. *Bioconjugate Chem.* **2000**, *11*, 795–804.
- (25) Gibbs, J. M.; Park, S. J.; Anderson, D. R.; Watson, K. J.; Mirkin, C. A.; Nguyen, S. T. *J. Am. Chem. Soc.* **2005**, *127*, 1170–1178.
- (26) Chien, M.-P.; Thompson, M. P.; Gianneschi, N. C. *Chem. Commun.* **2011**, *47*, 167–169.
- (27) Lin, D. C.; Yurke, B.; Langrana, N. A. *J. Biomech. Eng. Trans. ASME* **2004**, *126*, 104–110.
- (28) Murakami, Y.; Maeda, M. *Macromolecules* **2005**, *38*, 1535–1537.
- (29) Liedl, T.; Dietz, H.; Yurke, B.; Simmel, F. C. *Small* **2007**, *3*, 1688–1693.
- (30) Yang, H.; Liu, H.; Kang, H.; Tan, W. *J. Am. Chem. Soc.* **2008**, *130*, 6320–6321.
- (31) Wei, B.; Cheng, I.; Luo, K. Q.; Mi, Y. *Angew. Chem., Int. Ed.* **2008**, *47*, 331–333.
- (32) Chien, M.-P.; Rush, A. M.; Thompson, M. P.; Gianneschi, N. C. *Angew. Chem., Int. Ed.* **2010**, *49*, 5076–5080.
- (33) Rehman, F. N.; Audeh, M.; Abrams, E. S.; Hammond, P. W.; Kenney, M.; Boles, T. C. *Nucleic Acids Res.* **1999**, *27*, 649–655.
- (34) Nagahara, S.; Matsuda, T. *Polym. Gels Networks* **1996**, *4*, 111–127.
- (35) Watson, K. J.; Park, S. J.; Im, J. H.; Nguyen, S. T.; Mirkin, C. A. *J. Am. Chem. Soc.* **2001**, *123*, 5592–5593.
- (36) Chaix, C.; Minard-Basquin, C.; Delair, T.; Pichot, C.; Mandrand, B. *J. Appl. Polym. Sci.* **1998**, *70*, 2487–2497.
- (37) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. *Chem. Rev.* **2009**, *109*, 5528–5578.
- (38) Deming, T. J. *Prog. Polym. Sci.* **2007**, *32*, 858–875.
- (39) Ding, J.; Xiao, C.; Tang, Z.; Zhuang, X.; Chen, X. *Macromol. Biosci.* **2011**, *11*, 192–198.
- (40) Ding, J.; Xiao, C.; Zhao, L.; Cheng, Y.; Ma, L.; Tang, Z.; Zhuang, X.; Chen, X. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 2665–2676.
- (41) Engler, A. C.; Lee, H.-i.; Hammond, P. T. *Angew. Chem., Int. Ed.* **2009**, *48*, 9334–9338.
- (42) Xiao, C.; Zhao, C.; He, P.; Tang, Z.; Chen, X.; Jing, X. *Macromol. Rapid Commun.* **2010**, *31*, 991–997.
- (43) Tang, H.; Zhang, D. *Biomacromolecules* **2010**, *11*, 1585–1592.
- (44) Tang, H.; Li, Y.; Lahasky, S. H.; Sheiko, S. S.; Zhang, D. *Macromolecules* **2011**, *44*, 1491–1499.
- (45) Chopko, C. M.; Lowden, E. L.; Engler, A. C.; Griffith, L. G.; Hammond, P. T. *ACS Macro Lett.* **2012**, *1*, 727–731.
- (46) Liu, Y.; Chen, P.; Li, Z. *Macromol. Rapid Commun.* **2012**, *33*, 287–295.
- (47) Luo, C.; Chen, C.; Li, Z. *Pure Appl. Chem.* **2012**, *84*, 2569–2578.
- (48) Lemaitre, M.; Bayard, B.; Lebleu, B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 648–652.
- (49) Voigt, N. V.; Torring, T.; Rotaru, A.; Jacobsen, M. F.; Ravnsbaek, J. B.; Subramani, R.; Mamdouh, W.; Kjems, J.; Mokhir, A.; Besenbacher, F.; Gothelf, K. V. *Nat. Nanotechnol.* **2010**, *5*, 200–203.
- (50) Zhang, T.; Chen, P.; Sun, Y.; Xing, Y.; Yang, Y.; Dong, Y.; Xu, L.; Yang, Z.; Liu, D. *Chem. Commun.* **2011**, *47*, 5774–5776.