DNA−Block Copolymer Conjugates

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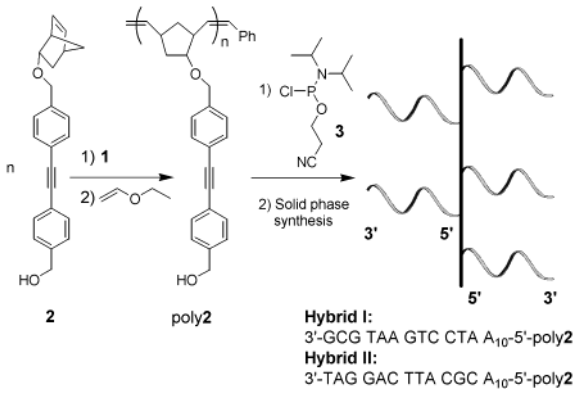
The use of DNA as an interconnect for the synthesis of new materials with preconceived architectural parameters and properties is a field of research that has seen considerable growth over the past several years.[1] The unique and reversible recognition properties of these biomolecules are the key elements through which their utility is derived. Exploring DNA for this purpose already has led to the development of new detection strategies,[2] novel nanostructures,[3−8] and the construction of nanoelectronic structures.[9] In recent years, the coupling of synthetic oligonucleotides to organic polymers has emerged as a promising research area where the combination of properties associated with both the polymer backbone and the attached DNA can be simultaneously addressed, manipulated, and optimized to achieve a particular function. For example, the attachment of DNA to polypyrrole[10−14] and other conducting polymers,[15] either through post-polymerization modification or direct copolymerization, has led to the development of polymer-based amperometric detection methods. While interesting, these DNA/polymer hybrids are limited with respect to their degree of tailorability, ill-defined compositions, and poor solubilities and dispersities, as well as function. The synthesis of well-defined block copolymer hybrids which can overcome these limitations would be an important contribution to this developing technology.

Herein, we report the covalent attachment of DNA to the backbone of a well-defined organic polymer derived from ring-opening metathesis polymerization (ROMP). Given the thorough exploration and optimization of ROMP during the past decade,[16] its use as a template for the construction of DNA/polymer hybrid materials offers several distinct advantages over other polymeric systems. The commercially available catalyst $Cl_2Ru(PPh_3)_2$═CHPh (**1**) has been shown to initiate the polymerization of ring-strained olefins (such as norbornene) in a living manner and to be exceptionally tolerant to a large number of diverse functional groups. These properties have led to the isolation of heretofore unattainable polymers and block copolymers with virtually any functional group covalently attached to the polymer chain, making ROMP an ideal tool for the isolation of novel and useful materials.[17] The combination of such wide ranging functionalities with the unique recognition properties of DNA could lead to the development of new materials with easily programmable parameters.

Scheme 1



In our attempts to incorporate DNA into ROMP polymers, we pursued post-polymerization modification of preformed polymers with DNA. For this task, the norbornenyl-modified alcohol **2**, which is characterized by a diphenylacetylene spacer which separates the alcohol from the polymerizable norbornene, proved to be extremely useful (vide infra). Starting from 5-*exo*-norbornen-2-ol, **2** is isolated in five high-yielding steps. With its strong absorption maximum at 304 nm (ϵ = 26 000 in MeOH), the diphenylacetylene component serves as a convenient UV-tag which can be used to monitor reactivity.

The synthesis of poly**2** and reaction of this polymer with the chlorophosphoramidite **3** resulted in a material with a single resonance in the ^{31}P NMR spectra at 149.2. This result is consistent with that observed for the monomeric analogue. Subsequent coupling to CPG-supported DNA using the syringe technique, followed by deprotection of the DNA and cleavage from the solid support in aqueous ammonia at 60 °C, yielded the desired hybrid product. The DNA is connected to the polymer at the 5′-end. Purification of the hybrid product from failure strands was achieved using ultrafiltration (see Supporting Information).

As a first demonstration, we used this general experimental strategy to isolate two polymers modified with complementary 12-mers of DNA with A_{10} spacers (Scheme 1, **Hybrid-I** and **Hybrid-II**). The UV-spectra of the purified DNA/polymer hybrids in water provide strong evidence that the DNA is attached to the polymer backbone (Figure 1A). The absorption maximum at 310 nm demonstrates that the diphenylacetylene backbone is present, which suggests that the water-soluble oligonucleotides are covalently linked to the hydrophobic polymer structure. Using experimental and calculated extinction coefficients for the polymer and the oligonucleotides (Supporting Information), and assuming a repeat unit of the polymer consistent with the stoichiometry of its synthesis, we estimate that there are, on average, five DNA strands attached to each polymer chain. This translates to 30% occupation of the potential polymer attachment sites by DNA strands.

When solutions of **Hybrid-I** and **Hybrid-II** are mixed in a PBS buffer solution (PBS = 0.3 M NaCl, 10 mM phosphate, pH 7), hybridization triggers the formation of an extended network

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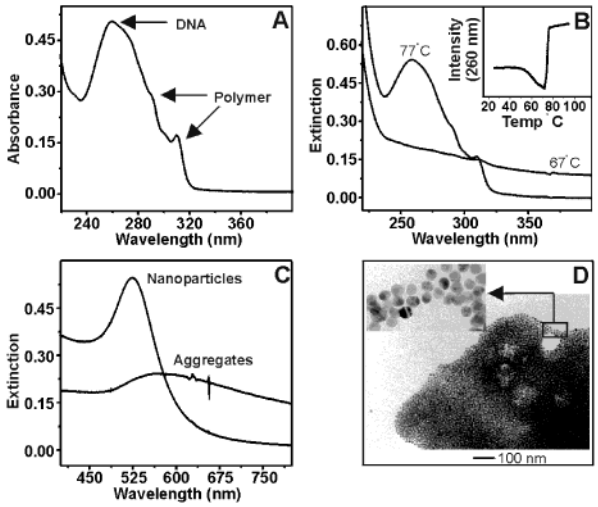


Figure 1. (A) UV−vis spectrum of **Hybrid-I**. (B) UV−vis spectra before and after melting of **Hybrid-I/Hybrid-II** mixture (melting curve inset) (C) UV−vis spectra of DNA-modified 13-nm Au nanoparticles and aggregates of **Hybrid-I** and complementary DNA-modified 13-nm Au nanoparticles. (D) TEM image of the aggregates from C.

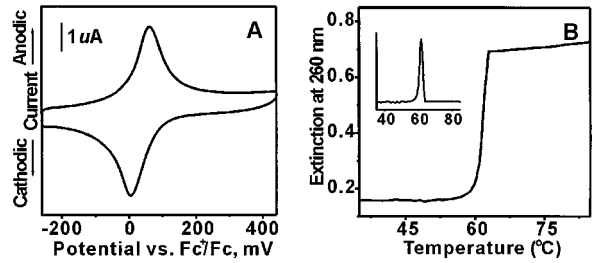


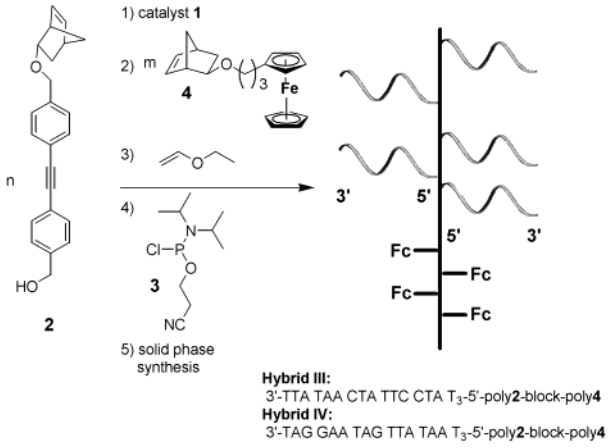
Figure 2. (A) The cyclic voltammogram of **Hybrid IV** in 0.2 M [(*n*-Bu)$_4$N]PF$_6$ in CH_2Cl_2. (B) The melting curve for **Hybrid-III/Hybrid-IV** in a PBS buffer (first derivative inset).

aggregate of linked polymers, which is signaled by the immediate formation of a white precipitate. Presumably, this occurs because each polymer is modified with more than one strand of DNA, which leads to cooperative binding between many DNA functionalized complementary polymer strands, as evidenced by the sharp melting transition (inset of Figure 1B). As expected, this hybridization process is thermally reversible. These studies demonstrate that attachment to the polymer does not hinder the recognition properties of the oligonucleotides.

The DNA/polymer conjugate was utilized to form nanoparticle assemblies. When a PBS buffer solution of **Hybrid-I** (12 μL of 8.3 μM in DNA) is mixed with a PBS buffer solution of 13 nm Au nanoparticles (260 μL of 9.7 nM in nanoparticle) modified with complementary DNA strands,[2] three-dimensional nanoparticle aggregates are formed. Formation of such aggregates is signaled by the diagnostic shift in the surface plasmon resonance of the nanoparticles (from 520 to 570 nm, Figure 1C) and a corresponding change in color (from red to purple).[2,3] Transmission electron microscopy studies reveal a highly networked aggregate (Figure 1D). Control experiments in which a solution of the same nanoparticles was mixed with a buffered solution of **Hybrid-II** (which is noncomplementary) resulted in no aggregate formation under nearly identical conditions.

Given the exceptional functional group tolerance of catalyst **1** in ROMP, we hypothesized that this chemistry could be extended

Scheme 2



to block copolymers of **2** with a number of norbornene monomers, thereby imparting tailorable functionality to branched DNA structures. Our strategy for the synthesis of block copolymer branched DNA is outlined in Scheme 2. In these experiments, monomer **2** is mixed with a catalytic amount of **1** in dry THF. After 1 h, the polymerization of the first block was determined to be complete by ^{1}H NMR spectroscopy. Subsequent addition and polymerization of a second norbornene monomer, followed by the injection of ethyl vinyl ether to terminate the reaction, yielded block copolymers with the desired structure. When the norbornenyl-modified ferrocene **4** was used as a second block, post-polymerization modification with **3** and solid-phase DNA synthesis led to the isolation of **Hybrid III** and **Hybrid IV**. Electrochemical measurements confirm the presence of the ferrocenes (Fc) in these hybrids ($E_{1/2} = 33$ mV vs Fc/Fc$^+$, Figure 2A). When PBS-bufferred solutions of these hybrids were mixed, thermally reversible aggregate formation was again observed. Also, a sharp melting transition was observed, consistent with our proposed structure (Figure 2B).

In conclusion, we have demonstrated that post-polymerization modification of ROMP polymers and block copolymers with DNA can lead to DNA/polymer hybrid materials with a number of interesting properties associated with the hybrid structure. The initial experiments described herein reveal that the recognition properties of the DNA strands are not adversely affected by attachment to the polymer. These new structures can be prepared with properties and function that depend on the choice of ROMP monomer and DNA branch sites. Since the synthesis of block copolymers of **2** with other norbornenyl-modified compounds is a facile process, the isolation of other novel and potentially useful macromolecular hybrid materials should be readily accomplished by utilizing variations of the strategy presented herein.

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Supporting Information Available: Details of the synthesis and characterization of **2**, **4**, poly**2**, poly**2**-*block*-poly**4** and other experimental procedures are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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