JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Open Access on 07/31/2015

Poly(oligonucleotide)

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Supporting Information

ABSTRACT: Here we report the preparation of poly(oligonucleotide) brush polymers and amphiphilic brush copolymers from nucleic acid monomers via graft-through polymerization. We describe the polymerization of PNA-norbornyl monomers to yield poly-PNA (poly(peptide nucleic acid)) via ring-opening metathesis polymerization (ROMP) with the initiator, $(IMesH_2)(C_5H_5N)_2(Cl)_2RuCHPh$.[1] In addition, we present the preparation of poly-PNA nanoparticles from amphiphilic block copolymers and describe their hybridization to a complementary single-stranded DNA (ssDNA) oligonucleotide.

The display of chemical functionality in a multivalent fashion on surfaces and particles and as brushes on polymer backbones is a common theme in nature as well as for synthetic systems.[2−4] Such systems take advantage of the unique properties that arise when monomeric species are incorporated into a densely packed three-dimensional (3D) architecture. Here we describe the preparation of polymeric nucleic acids wherein single-stranded sequences of peptide nucleic acids (PNAs)[5] are incorporated as polymer brushes via graft-through polymerization using the ROMP initiator $(IMesH_2)(C_5H_5N)_2(Cl)_2RuCHPh$ (Figure 1). Nucleic acids, both natural and synthetic, standout as the quintessential carriers of chemical information stored as specific sequences of bases positioned along a backbone.[4−8] As such, synthetic oligonucleotides and nucleic acid bioconjugates are powerful tools in a range of fields including in biotechnology (e.g., PCR),[9,10] in materials science as programmable structural synthons[11−17] and as aptamers selected by *in vitro* evolution.[18−22] In each application the nucleic acid functions to enrich a chemical system with information, facilitating predictable interactions with complementary sequences,[23] or with other molecules including enzymes, proteins, and small molecules.[19,24−26] We reasoned that the graft-through polymerization of an oligonucleotide sequence would provide a powerful new approach for the multivalent display of chemical information on a synthetic template.

There have been extensive efforts to prepare nucleic acid inspired synthetic polymers involving the direct polymerization of appropriately modified monomers, generating synthetic polymers with single nucleobases as side-chains.[27−32] Although this approach allows the integration of purine and pyrimidine bases onto a synthetic backbone, it does not allow the incorporation of sequences containing multiple bases and thus does not result in informational polymeric systems. In addition to strategies involving directly polymerized nucleobases, there are an increasing number of examples of oligonucleotide-polymer bioconjugates in the literature reliant upon post-polymerization conjugation reactions.[33−35] These approaches, shown in Scheme 1, seek to fix recognition elements natural to DNA and RNA along a synthetic polymer or polymeric nanoparticle template and have found use in an array of arenas including the programmed assembly of nanoparticles,[2,36−38] in delivery vehicles,[6,39−41] and as effective DNA-probes.[35,42,43] Furthermore, the function of these materials is intrinsically governed by the information within the nucleic acid sequence

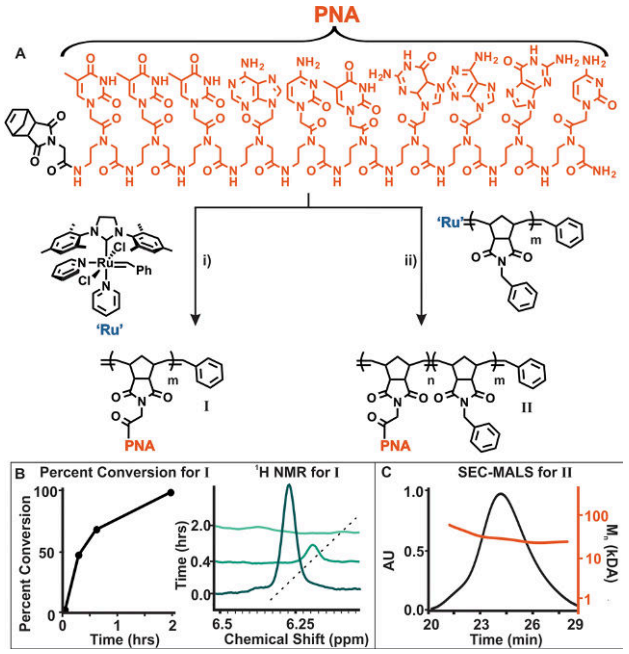
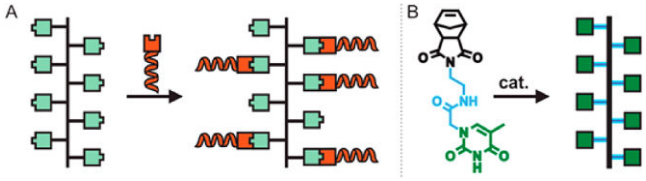


Figure 1. Synthesis and characterization of a poly(oligonucleotide). (A) PNA-norbornyl monomer (PNA-Nb) polymerized using ROMP initiator $(IMesH_2)(C_5H_5N)_2(Cl)_2Ru{=}CHPh$ ("Ru") to form poly-PNA homopolymer, **I**, and poly-PNA block copolymer, **II**. (B) Representative percent conversion for **I** determined by the disappearance of the olefin signal associated with PNA-Nb in ^{1}H NMR. (C) Representative SEC-MALS for **II**. M_n = 28,270 indicating a degree of polymerization of 5 for the PNA block.

Received: March 28, 2014
Published: July 31, 2014

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Scheme 1. Known Methods for the Incorporation of Multiple Nucleic Acids or Nucleobases into Polymers[a]



[a](A) Post-polymerization modification of a polymer with a nucleic acid.[52−54] (B) Polymerization of a pyrimidine base as a modified monomer.[45−51]

itself as well as the dense and multivalent 3D array induced by the polymer scaffold. Indeed, function dictated by 3D biomolecular display is not unique to nucleic acids, rather this concept extends to all classes of biomolecules, most effectively demonstrated in the past with peptides and proteins.[3,44−48] Strategies for the polymerization of (graft-through) and polymerization from (graft-from) proteins and peptides have been used to build macromolecules through sequential addition of monomers to a growing chain, taking advantage of catalyst proficiency and avoiding kinetically unfavorable conjugations (graft-to) between multiple large macromolecules.[49−55] However, unlike for other biomolecules (saccharides,[56,58] peptides,[59] and proteins[60,61]), there are no examples of graft-through polymerization of nucleic acids and very few examples of graft-from polymerization off of nucleic acids.[62,63] Therefore, despite their promise, polymer bioconjugates of true nucleic acid sequences have been limited to those prepared via post-polymerization modification and hence are limited in terms of maximum achievable DNA density, are difficult to reproduce, and suffer from incomplete incorporation of the nucleic acid at each position of the polymer (Scheme 1A).

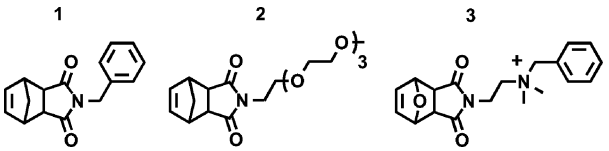


Figure 2. Structures of monomers used for block copolymer preparation.

In order to avoid shortcomings associated with post-polymerization modification reactions, a nucleic acid monomer capable of undergoing direct graft-through polymerization was synthesized. Initial studies attempting direct-polymerization of DNA-based monomers via ROMP were met with limited success. Therefore, a PNA-based monomer was chosen as an ideal target for this study for three key reasons: (1) PNA can be prepared in significant quantities via standard peptide bond-forming reactions on solid support; (2) PNA is soluble in DMF making it readily compatible with $(IMesH_2)(C_5H_5N)_2(Cl)_2$-RuCHPh (Figure 1 for structure); and (3) we hypothesized that the neutral *N*-(2-amino-ethyl)-glycine backbone would be more compatible with the ruthenium-based catalyst than the polyanionic phosphate backbone of DNA.

A 10-base PNA sequence (Figure 1) was designed to discourage self-hybridization while providing a sufficient number of bases for efficient hybridization with a complementary sequence of DNA at room temperature. Moreover, this 10-base sequence represents a sequence encoded with each of the four letters of the genetic alphabet and enough information to communicate specifically with other nucleic acids and proteins, a function not possible via the polymerization of a single purine or pyrimidine monomer (as in Scheme 1B). After the complete PNA sequence was prepared, *N*-(glycine)-*cis*-5-norbornene-*exo*-dicarboximide was coupled to the N-terminus while on solid support. The PNA norbornene monomer (PNA-Nb) was then cleaved and deprotected from the solid support using TFA:cresol (80:20), purified by HPLC, and the mass confirmed by ESI-MS (see Supporting Information (SI)). Following HPLC purification, PNA-Nb was lyophilized to afford a white powder.

For polymerization studies, PNA-Nb was resuspended in dry, degassed *N*,*N*-dimethylformamide-d_7 in a J. Young NMR tube in a glovebox. An appropriate amount of ruthenium initiator was added to the solution, and the disappearance of the norbornene olefin resonance was then monitored by ^{1}H NMR. Complete disappearance of the monomeric olefin peak indicated complete polymerization of PNA-Nb into poly-PNA. A series of experiments were carried out to determine reproducibility of polymerization reactions with respect to both the preparation of homopolymers as well as block copolymers (Table 1). For PNA homopolymers, degrees of polymerization

Table 1. Polymers and Copolymers of PNA with Monomers Shown in Figure 2

polymer	mon$_1$[a]	mon$_2$[c]	m[d]	n[d]	% con.[e]
I	PNA-Nb (10:1)[b]	–	10	–	99
II	1 (35:1)	PNA-Nb (5:1)	35	5	97
III	PNA-Nb (5:1)	–	5	–	97
IV	1 (30:1)	PNA-Nb (7.5:1)	30	5	65
V	1 (30:1)	PNA-Nb (7.5:1)	30	5	65
VI	1 (30:1)	PNA-Nb (7.5:1)	30	6	79
VII	1 (36:1)	PNA-Nb (9:1)	35	8	88
VIII	1 (36:1)	PNA-Nb (18:1)	35	16	87
IX	2 (36:1)	PNA-Nb (9:1)	33	7	74
X	3 (36:1)	PNA-Nb (9:1)	41	5	56

[a]Indicates indentity of monomer polymerized first (degree of polymerization, DP = m). [b]Ratios shown indicate monomer to initiator ratio or intended DP. [c]Indicates identity of monomer polymerized second (DP = n). [d]Observed degree of polymerization of mon$_1$ (m) or mon$_2$ (n). [e]Percent conversion of PNA-Nb determined by ^{1}H NMR.

of 10 (polymer **I**) and 5 (polymer **III**) were targeted with complete consumption of PNA-Nb confirmed by ^{1}H NMR for both reactions. In preparation of a PNA-containing block copolymer, a phenyl-functionalized norbornene (**1**) was polymerized as the first block followed by PNA-Nb incorporated as the second block (as shown in polymer **II** (Figure 1, Table 1). To achieve this, PNA-Nb was added to the living phenyl polymer chain, and the disappearance of the norbornene olefin of PNA-Nb was monitored by ^{1}H NMR. The resulting polymeric species were then analyzed by SEC-MALS (see SI). Having established that PNA-Nb could be successfully polymerized into block copolymers, we sought to assess the reproducibility of these reactions by attempting to synthesize block copolymers of identical composition. Using a live ruthenium catalyst on a phenyl homopolymer with a degree of polymerization of 30, three separate but identical reactions were set up in which the attempted degree of polymerization of

dx.doi.org/10.1021/ja503142s | J. Am. Chem. Soc. 2014, 136, 11216−11219

the PNA monomer was 7.5 (Table 1, polymers **IV**–**VI**). The degree of polymerization of the PNA block ranged from 5 to 6 (60–80% completion), indicating a good degree of reproducibility and predictability for these reactions. In addition, higher degrees of polymerization could be achieved for this type of block copolymer as illustrated by the preparation of polymers **VII** and **VIII** (Table 1). To examine the compatibility of PNA-Nb polymerization with other block copolymer systems, an oligoethylene glycol functionalized norbornene (**2**) and a quaternary amine-functionalized norbornene (**3**) were synthesized as monomers for incorporation into block copolymer scaffolds as the initial block. The resulting block polymers (**IX** and **X** in Table 1) showed percent conversions of PNA-Nb comparable to the phenyl-based block copolymers, with the amine-functionalized system demonstrating the lowest percent conversion. Given the slight variation in PNA-Nb percent conversion between these three different block copolymer systems (**VII**, **IX** and **X** in Table 1), the identity of the non-PNA block may dictate PNA conversion efficiency and should be taken into consideration for future studies.

To assess the DNA-binding capability of these systems, block copolymer **II** was chosen. The assembly of **II** to generate spherical nanoparticles was achieved by dissolving **II** in DMSO and then dialyzing into aqueous solution (see SI for details).[64] The resulting nanoparticle (**PNA-NP**) was characterized by DLS and TEM (Figure 3). DLS data support the formation of an aggregated species in solution. TEM reveals the existence of nanoparticles on the order of 20 nm in diameter. The melting temperature (T_m) of **PNA-NP** hybridized with its complementary DNA sequence was determined to be 58.1 °C, an ~8 °C increase over an identical, nonparticulate, unpolymerized PNA sequence. These melting data suggest cooperative binding and accessible PNA forming the shell of the nanoparticles. In support of this model, we conducted a molecular dynamics simulation of **PNA-NP**[65−67] assembled from 60 amphiphiles giving a structure that equilibrated into a spherical particle ~21 nm in diameter. Polynorbornyl chains packed well to form a compact hydrophobic core largely protected from contact with water. The simulations show the hydrophilic PNA chains solvated in water forming the shell of the micellar nanoparticles.

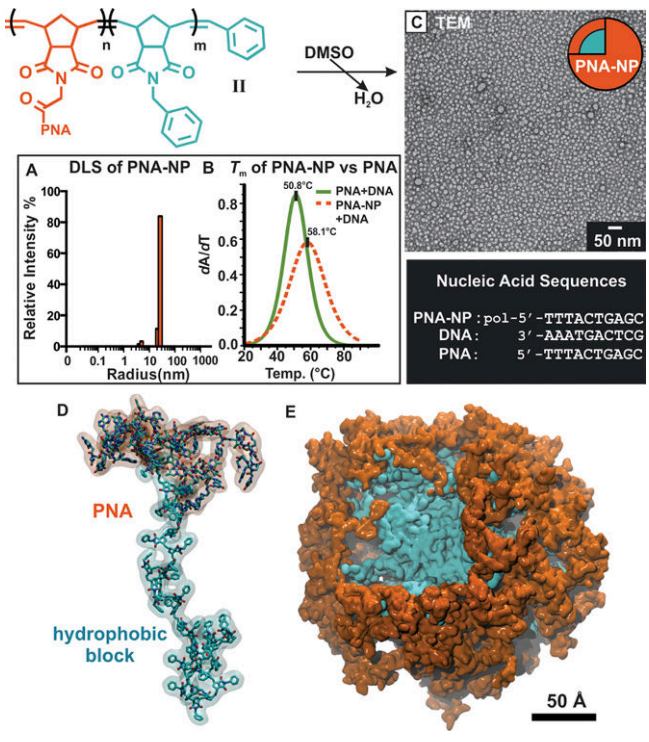


Figure 3. Poly-PNA amphiphile **II** was dialyzed from DMSO into H_2O to generate nanoparticles. (A) DLS data indicating a hydrodynamic radius of 25 nm. (B) T_m of **PNA-NP** with a complementary DNA sequence was found to be 58.1 °C. (C) Negative-stained TEM of **PNA-NP** provided evidence of spherical 20 nm diameter nanoparticles. Atomistic models of (D) **II** and (E) **PNA-NP**. **II** is shown in a conformation present within **PNA-NP**.

In summary, we have shown that one can prepare nucleic acid brush polymers and amphiphilic brush copolymers by direct, graft-through polymerization of an oligonucleotide. To our knowledge, this is the first example of a polymer-nucleic acid bioconjugate generated via direct polymerization of an oligonucleotide monomer. In addition, these materials show cooperative hybridization to complementary DNA oligonucleotides. We believe this type of approach provides an efficient synthetic strategy for the incorporation of nucleic acids into particle and polymer-based materials. The interest in doing so is driven by potential applications including the facile preparation of materials for affinity purification of DNA,[68,69] gene and nucleic acid delivery to cells,[6,39−41,70−73] and in the development of materials capable of programmed self-assembly.[13−17,74−78]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and data. 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 acknowledge support for this work from the AFOSR via a PECASE (FA9550-11-1-0105) and from the ARO (W911NF-11-1-0264 and W911NF-14-1-0169). We acknowledge the UCSD Cryo-Electron Microscopy Facility, supported by NIH grants to T.S.B. and the Agouron Institute. N.C.G acknowledges the Alfred P. Sloan Foundation. Computational work was supported by an ACS PRF grant no. 53062-ND6 to P.K. This research used the Extreme Science and Engineering Discovery Environment (XSEDE): NSF OCI-1053575 to P.K.

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