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Peptide-polymer bioconjugates: hybrid block copolymers generated *via* living radical polymerizations from resin-supported peptides

Matthew L. Becker†, Jianquan Liu† and Karen L. Wooley*

Washington University, Department of Chemistry, One Brookings Drive, Saint Louis, Missouri 63130-4899, USA. E-mail: klwooley@artsci.wustl.edu; Fax: +1 (314) 935-9844; Tel: +1 (314) 935-7136

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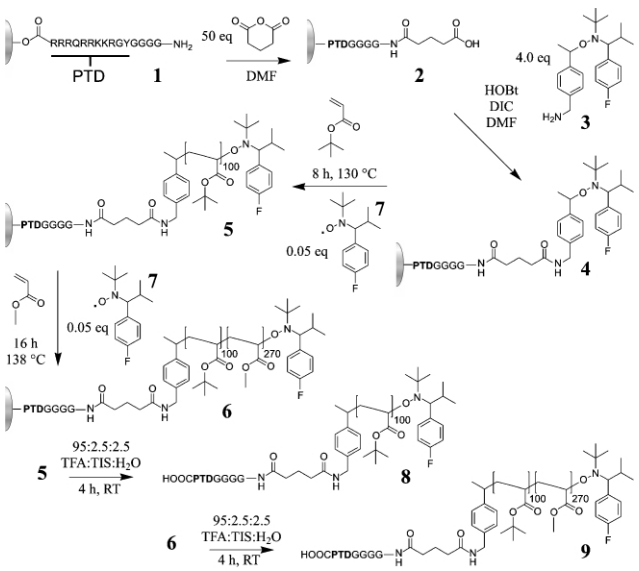
A novel strategy for the preparation of peptidic-synthetic bioconjugate block copolymers is based upon sequential condensation and living radical addition polymerizations, each performed upon a solid support.

Several recent, fundamental advances in peptide biology, polymer chemistry, and nanoscience have emphasized the need for facile routes toward the preparation of well-defined peptide-polymer bioconjugates. A number of peptide sequences have been identified as ligands that facilitate transduction across cell membranes[1] and some have been characterized as specific ligands that direct recognition to cell surface receptors.[2] Such sequences are, therefore, being studied as key components to provide for cell entry by drugs or molecular probes, which are often packaged and transported by a polymer delivery vehicle.[3] Furthermore, the self-assembly of block copolymers has emerged as a popular means by which to produce complex nanostructured materials.[4] Previously, we have demonstrated that conjugation of the protein transduction domain (PTD) of the HIV-1 TAT protein to the surface of shell crosslinked nanocages[5] renders those nanostructures biologically interactive.[6] The peptide sequence was bound to a solid support during the coupling to the nanocage, which limited (to approximately one) the number of peptides that were then presented from the nanocage surface. In this report, we describe the preparation of PTD-functionalized block copolymers by sequential condensation-based peptide growth and living free radical polymerization from a solid support.[7] Standard solid-phase peptide synthesis conditions were employed for the preparation of the peptide chain, from which nitroxide-mediated radical polymerization (NMRP)[8] was conducted to yield the synthetic segment of the bioconjugate. Recent research efforts[9] have focused on the assembly of peptide-based block copolymers as precursors to complex macromolecular assemblies for use in biological applications. The peptide-polymer bioconjugates reported herein and the method of production are expected to be of interest as materials capable of self-assembly[10] into unique nanostructured materials with defined surface localization or internal placement of an accurate number of peptide sequences.

The peptidic-synthetic block copolymers were constructed upon a solid support, as depicted in Scheme 1. The PTD with a four residue glycine extension was assembled on Wang′s resin (0.54 mmol g^{-1}) *via* FMOC solid phase procedures. The N-terminus of this peptide sequence (**1**) was converted to a carboxy functional group (**2**) by coupling glutaric anhydride. Further functionalization by reaction with the benzylic amine of the fluorine-labeled alkoxyamine[11] (**3**) yielded an NMRP initiator tethered to the N-terminus of the peptide, which was bound to a solid support (**4**). Following the cleavage of **1**, **2**, and **4** from the resin, the purities of **1**–**4** were confirmed by MALDI-tof mass spectrometry and HPLC.

The peptide-supported initiator was then used to create block copolymers **5** and **6**, under conditions that allowed for NMRP of *tert*-butyl acrylate and methyl acrylate sequentially. Briefly, a 100 mL Schlenk flask that had been oven dried overnight, flame dried under vacuum, and back filled with argon was charged with dry resin beads **4** (428.4 mg, 2.3×10^{-1} mmol).[12] *tert*-Butyl acrylate (5.00 mL, 34.1 mmol) and 2,2,5-trimethyl-4-(*para*-fluorophenyl)-3-azahexane-3-oxide, **7**, (2.0 mg, 8.4×10^{-3} mmol) were added *via* argon flushed syringes. The reaction mixture was degassed by three cycles of freeze–pump–thaw and, following the final thaw cycle, the mixture was allowed to stir for 10 min before being immersed in an oil bath at 130 °C. After 8 h, the oil bath was removed and the reaction vessel was immersed in liquid nitrogen to quench the polymerization. Residual monomer was removed *in vacuo*, to afford **5**. The chain was further extended by charging a Schlenk flask prepared in the same manner as above with dry resin beads **5** (356.2 mg, 1.9×10^{-1} mmol),[12] adding methyl acrylate (MA) (5.00 mL, 55.5 mmol) and **7** (2.0 mg, 8.4×10^{-3} mmol) *via* argon flushed syringes, degassing the reaction mixture and immersing in an oil bath at 138 °C. After 16 h, the polymerization was quenched in liquid nitrogen and the excess monomer was removed *in vacuo* to afford **6**.

Cleavage from the solid support allowed for isolation and characterization of the materials. After the preparation of **5**, a portion of the peptide-polymer conjugate was cleaved through treatment of the resin with a solution of 95% trifluoroacetic acid (TFA):2.5% triisopropylsilane (TIS):2.5% water (10 mL) for a minimum of 4 h. These conditions also effected cleavage of the *tert*-butyl esters, to yield PTD-*b*-poly(acrylic acid) (PTD-*b*-PAA) as a hydrophilic block copolymer, **8**. Isolation involved removal of the beads *via* filtration, rinsing with TFA, concentration *in vacuo*, and repeated precipitation into cold ether with collection *via* centrifugation at 3500 rpm for 10 min. Once the pellet was allowed to dry, the PTD-*b*-PAA was suspended in



Scheme 1 The resin-supported assembly of peptidic-synthetic block copolymers was accomplished by coupling an alkoxyamine NMRP initiator to an extended PTD sequence followed by sequential living radical addition polymerizations.

† These authors contributed equally to this work.

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water, and purified by dialysis (1000 MWCO tubing) against water for 48 h. A similar procedure was followed to isolate the amphiphilic triblock copolymer, PTD-*b*-PAA-*b*-PMA, **9**.

The polymers were characterized by ^{1}H and ^{19}F NMR spectroscopies, which confirmed the polymer chain growth and identified the chain ends as being PTD and the ^{19}F-labeled alkoxyamine. The resonances for the protons of the peptide sequence were observed in each of the samples from **1**, **4**, **5** and **6** upon cleavage from the resin (Fig. 1 a, c, d, and e, respectively). The degrees of polymerization were determined to be 100 and 270 for the acrylic acid and methyl acrylate chain segments, respectively, by comparison of the ratios of the aromatic proton intensities to the resonance intensities for the aliphatic protons. The presence of the alkoxyamine chain end was observed by ^{19}F NMR spectroscopy (Fig. 1, insets). The differences in ^{19}F chemical shifts and line widths suggest different solution state interactions[13] for the cationic, zwitterionic and amphiphilic block copolymers. Further studies are needed to determine whether these structures produce well-defined supramolecular assemblies. The zwitterionic and amphiphilic nature of the block copolymers, **8** and **9**, complicated molecular weight and polydispersity determination by traditional methods.[14,15] Further studies are needed to determine these structural features and to assess whether these precursors produce well-defined supramolecular assemblies.

In conclusion, nitroxide mediated radical polymerization has been conducted from initiating sites located on the chain termini of peptides loaded on a solid support. This versatile synthetic strategy provides a route to create peptidic-synthetic block copolymer bioconjugates that are unique in the fact that stoichiometry and regioselectivity of each of the chain segments can be controlled. Although the methodology is demonstrated using PTD and NMRP, virtually any peptide sequence and living radical polymerization conditions can be substituted. This procedure extends the realm of polymer materials that can be produced by well-established solid phase synthesis methods—involving both step-growth and chain-growth polymerization mechanisms. In addition to the study of these unique block copolymers in solution, we are interested in their surface reorganization properties,[16] given the secondary structure that can be programmed into the peptidic component.

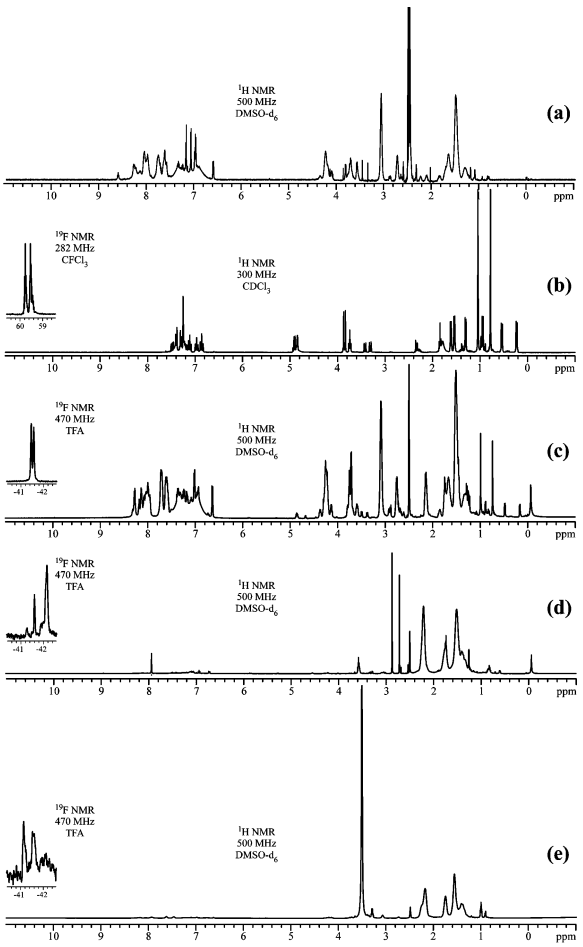

Fig. 1 ^{1}H and ^{19}F NMR spectra of PTD-G_4 (a), ^{19}F-labeled NMRP initiator, **3** (b), PTD conjugated ^{19}F-labeled NMRP initiator (c), PTD-*b*-PAA_{100}, **8** (d), and PTD-*b*-PAA_{100}-*b*-PMA_{270}, **9** (e). For those spectra collected in DMSO-d_6, the solution contained approximately 5% trifluoroacetic acid (TFA). For each ^{19}F spectrum, the internal reference is specified as either $CFCl_3$ or TFA.

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