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Block Copolymers Containing Conjugated Polymer and Polypeptide Sequences: Synthesis and Self-Assembly of Electroactive and Photoactive Nanostructures

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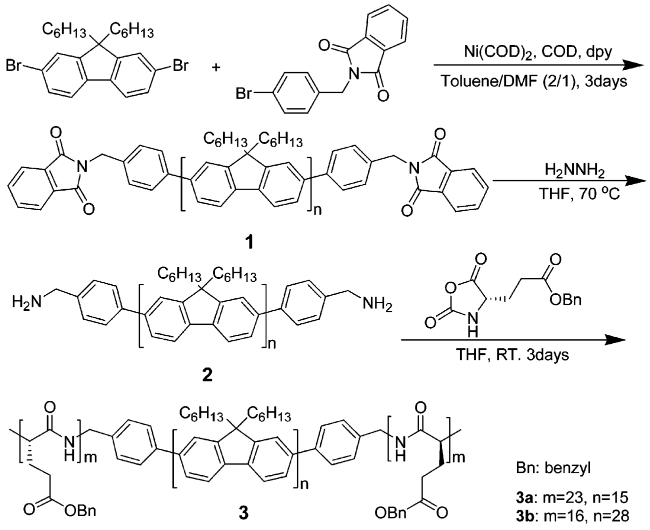
Conjugated polymers in their oxidized, highly conducting, or neutral luminescent and semiconducting forms have recently received much interest as tools in the probe and regulation of biomolecular interactions.[1] Conducting polymers have been explored as biocompatible surfaces or wires for delivering electrical stimuli to influence cell growth and cell morphology[1a] or biointeractions for developing various biosensors.[1b] Luminescent conjugated polymers are also being explored in biosensors.[1c] The rich assemblies of oligopeptides and polypeptides are known to provide biocompatible surfaces and three-dimensional scaffolds for tissue engineering and biomineralization.[2] We describe here the synthesis and self-assembly of the first examples of hybrid block copolymers containing π-conjugated polymer and polypeptide blocks. This novel block copolymer architecture can exhibit both rod−rod and rod−coil conformations by virtue of the known helix (rod)-to-coil transition of polypeptides.[3] Hybrid block copolymers with conjugated polymer and polypeptide sequences can be expected to significantly improve the interaction between the electroactive and photoactive blocks and biomolecules while facilitating self-assemblies with new properties. Such hybrid materials may find applications in biosensors,[1b] scaffolds for tissue engineering,[1a] and nanoelectronics.

The new triblock copolymers containing poly(9,9-dihexylfluorene-2,7-diyl) (PHF) and poly(γ-benzyl-L-glutamate) (PBLG), denoted $\text{BLG}_m\text{-HF}_n\text{-BLG}_m$, were synthesized by the ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (Bn-Glu NCA) using benzylamine end-functionalized polyfluorene macroinitiators (Scheme 1, see Supporting Information for experimental details). The two PHF macroinitiators (**2**) were prepared by Yamamoto coupling polymerization of 2,7-dibromo-9,9-dihexylfluorene[4] and end-capped with *N*-(*p*-bromobenzyl)phthalimide in toluene/dimethylformamide (2/1 v/v) at 70 °C. The bisphthalimide end-capped PHF samples (**1**) were purified by flash chromatography to remove any polyfluorene without end-capping and precipitation from THF into methanol. The ring-opening polymerization of Bn-Glu NCA in THF at room temperature was initiated by the bis(benzylamine) end-capped PHFs (**2a**, **2b**) to give block copolymers **3a** and **3b**. The block copolymer products were purified by stirring in 99% formic acid for 3 h followed by filtration to remove the soluble PBLG oligomers.[5] Both **3a** and **3b** were easily soluble in chloroform and THF, which are good solvents for both blocks.

The molecular structures of the macromolecular initiators and block copolymers were confirmed by FT-IR spectra (see Supporting Information for details). The FT-IR peaks at 1773 and 1717 cm^{-1}, which correspond to the phthalimide group in the phthalimide end-capped poly(9,9-dihexylfluorene-2,7-diyl) (**1**), disappeared in the bis(benzylamine) end-capped poly(9,9-dihexylfluorene-2,7-diyl) (**2**) after deprotection. This shows the complete deprotection of **1** to the benzylamine end-capped macroinitiators **2**. A broad peak at 3290 cm^{-1} corresponding to the stretching vibration of N−H and two sharp peaks at 1650 and 1550 cm^{-1} due to the amide I and II bands in PBLG, appeared in triblock copolymers **3a** and **3b**, confirming the structure of the triblock copolymers.

Scheme 1



All the intermediates and the block copolymers structures were also confirmed by the ^{1}H NMR spectra. Incorporation of phthalimide end groups in polyfluorene **1** was proved by the appearance of proton resonance at 4.95 ppm due to the methylene group adjacent to the phthalimide group. The proton resonance of the phthalimide group in the polyfluorene **1** (at 7.8−7.7 ppm) was buried among the peaks of fluorene protons at 7.89−7.48 ppm. The proton resonance in **1** at 4.95 ppm disappeared after removal of the phthalimide protecting groups with excess hydrazine, whereas a new resonance corresponding to the methylene adjacent the amine at 3.95 ppm appeared in the ^{1}H NMR spectra of the deprotected PHFs (**2**). Besides the peaks corresponding to PHF, three broad peaks at 7.3 ppm (Ar−H from benzyl), 5.0 ppm (methylene in benzyl ester), and 2.6−2.12 ppm (methylene in glutamate) corresponding to PBLG blocks were found in the ^{1}H NMR spectra of triblock copolymers **3a** and **3b**.

The degree of polymerization of the macromolecular initiators and the composition of the triblock copolymers were established by end group analysis using ^{1}H NMR spectra. The degree of polymerization (DP) was found by end group analysis (integration of the methylene protons of $-\text{PhC}H_2\text{N}\langle$ in PHF at 4.95 ppm relative to the methylene protons ($6 \times CH_2$) in *n*-hexyl group at 1.20 ppm) to be 15 and 28 for **1a** and **1b**, respectively, which remained the same in **2a** and **2b** after deprotection. Similarly, the repeat units of PBLG in the triblock copolymers were found to be 46 (**3a**) and 32 (**3b**), respectively, by end group analysis (integration of the

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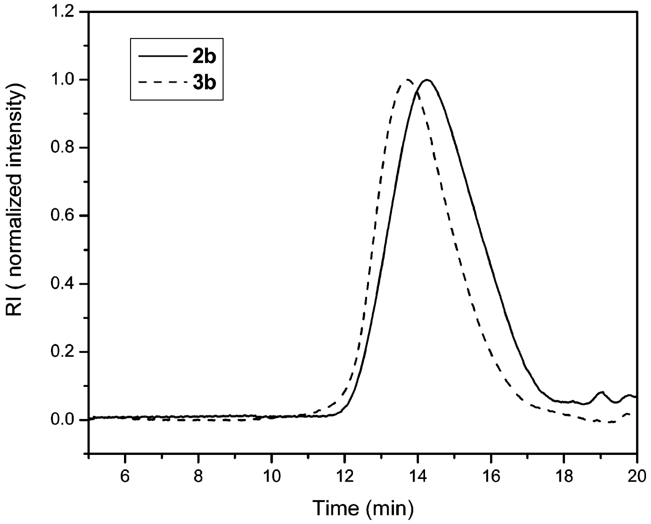

Figure 1. GPC curves of macroinitiator **2b** and triblock copolymer **3b**.

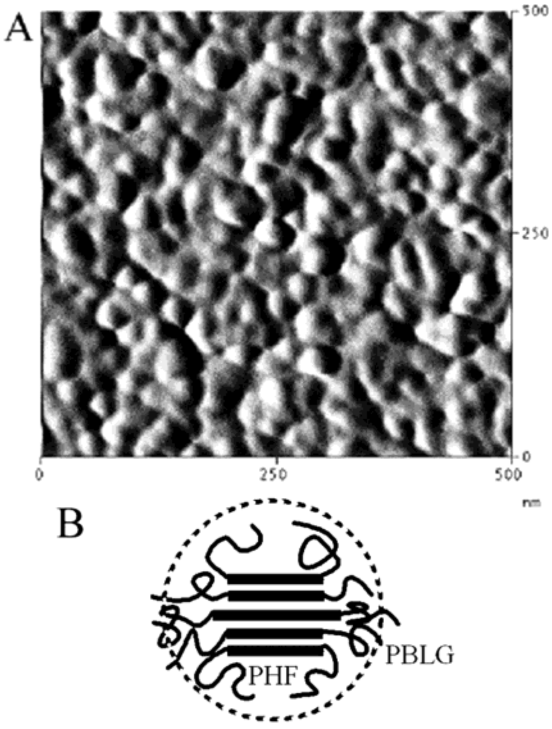

Figure 2. (A) AFM amplitude image of block copolymer **3a** thin film on mica cast from TFA/$CHCl_3$ (30/70, v/v) and annealed in the solvent vapor. (B) Schematic representation of self-organization of coil–rod–coil block copolymers.

methylene protons in $COOCH_2Ph$ of PBLG at 4.49 ppm relative to the methylene protons ($6 \times CH_2$) in *n*-hexyl group at 1.20 ppm). Two BLG_m-HF_n-BLG_m samples with different block lengths were thus prepared: **3a** ($n = 15$, $m = 23$) and **3b** ($n = 28$; $m = 16$).

A direct evidence for block copolymerization and thus the block copolymer structures comes from GPC results. Figure 1 shows the GPC curves of macroinitiator **2b** and block copolymer **3b**. The molecular weight of **3b** shifts to higher molecular weight after block copolymerization, indicating that **3b** is a pure block copolymer without any homopolymer impurity. The number-average molecular weights (M_n) of macroinitiators **2a** and **2b** from the GPC results were 3.69×10^3 and 9.92×10^3, respectively, corresponding to a DP of 11 for **2a** and 29 for **2b**. Both **2a** and **2b** have an identical polydispersity of 2.35. After the block copolymerization, the M_n of triblock copolymers **3a** and **3b** increased to 7.03×10^3 and 1.56×10^4, with polydispersities of 2.32 and 2.10, respectively. As expected, the molecular weights determined from GPC are slightly different from the absolute values obtained from ^1H NMR spectra.

The hybrid block copolymers **3a** and **3b** retained the electroactive and photoactive properties of polyfluorene, as expected. Thin films of **3a** cast from chloroform solution had very similar absorption ($\lambda_{max} = 380$ nm) and emission ($\lambda^{em}_{max} = 424$ nm) properties as those of the conjugated homopolymer.[6] The absorption and emission spectra of **3b** were almost identical to those of **3a**. Preliminary single-layer light-emitting diodes using **3a** or **3b** as the emissive material showed blue electroluminescence (EL) with a brightness comparable to the polyfluorene homopolymer (Supporting Information), indicating that the presence of the polypeptide blocks did not impede charge injection, transport, and photophysical processes essential to EL devices.[6]

The FT-IR spectra of **3a** film cast from chloroform showed amide I band at 1650 and 1550 cm^{-1}, indicating that the PBLG blocks adopt α-helical conformations.[2e] Besides the amide I band, **3b** also showed a shoulder peak at 1630 cm^{-1}, indicating that α-helical and β-sheet conformations coexist. It has been shown that PBLG with a small number of repeat units (<20) prefers the coexistence of both conformations.[2e] This is consistent with our block copolymer **3b**, which has PBLG blocks with 16 repeat units each. The molecular packing in the films was characterized by X-ray diffraction (Supporting Information). Two broad peaks at 2θ of 6.12° and 20°, corresponding to *d*-spacings of 14.4 and 4.23 Å, were observed. The long *d*-spacing is close to the interchain distance in PBLG helical rod[2e,7] ($d = 13.0$ Å) in **3a**. The broad peak with a *d*-spacing of ~4 Å was attributed to a combinations of the helical pitch and the hydrocarbon chain diameter of PHF.[7,8] In addition to the peaks found in copolymer **3a**, copolymer **3b** showed another peak at $2\theta = 6.72$ ($d = 13.1$ Å). This latter *d*-spacing arises from the lateral packing of PHF blocks; we note that poly(9,9-dioctylfluorene) has Bragg spacings of 16 and 4.14 Å.[8] The increase of the PHF block length can account for the improved packing order in **3b**. The broad X-ray diffraction peaks and the lack of higher order diffraction peaks suggest that the size of the crystallites is small.

In organic solvents such as chloroform, PBLG adopts a rodlike α-helical conformation, whereas it undergoes helix-to-coil transition upon addition of significant amount (25–30% v/v) of trifluoroacetic acid (TFA) to the solution.[3] Addition of 3 vol % TFA to PBLG solutions preserves the α-helical rodlike conformation while making the chains mobile.[3] This suggests that the conformation of PBLG sequences in a block copolymer and the self-assembly of the block copolymer could be influenced by the acidity of the medium. To test this hypothesis, we investigated thin film morphologies of triblock copolymers **3a** and **3b** cast from different solvents, by tapping mode atomic force microscopy (AFM). Figure 2 shows the AFM amplitude image of **3a** cast from TFA/chloroform (30/70 v/v) onto mica. A globular or spherical morphology was observed with 31.6 ± 4.3 nm average diameter of aggregates; the indicated uncertainty is the standard deviation of 20 measurements. An AFM amplitude image of similarly prepared films of **3b** revealed similar spherical nanostructures with an average diameter of 39.7 ± 4.1 nm. These block copolymer

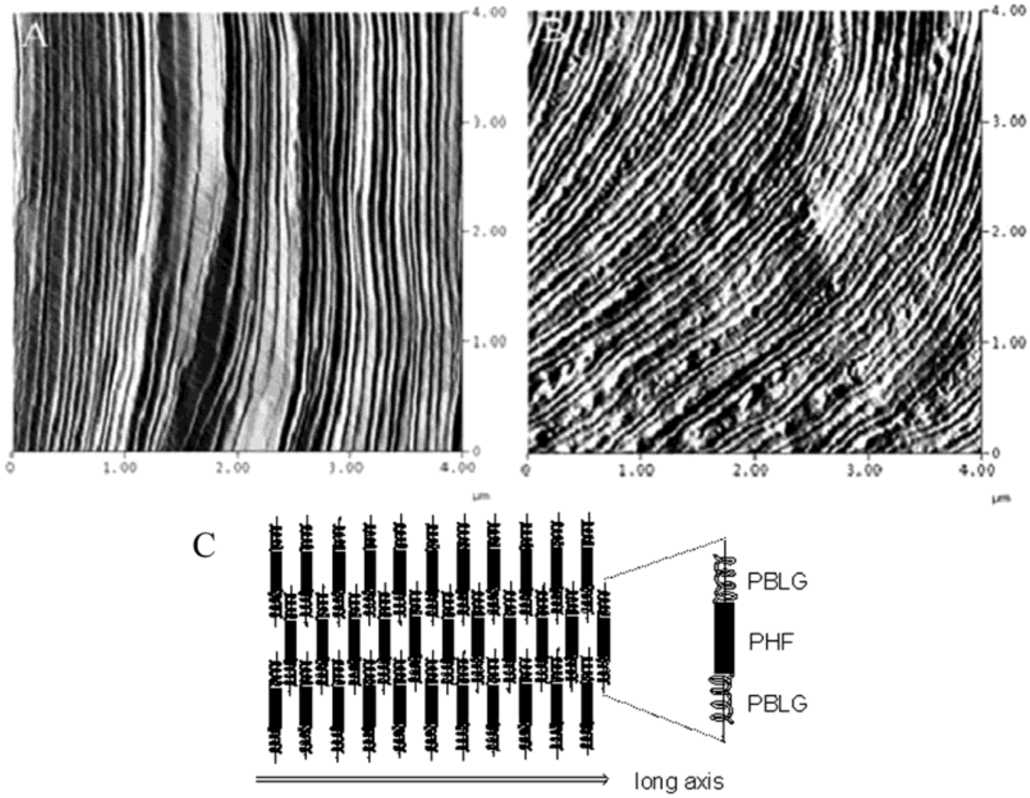

Figure 3. AFM amplitude images of block copolymers **3a** (A) and **3b** (B) cast from TFA/CHCl$_3$ (3/97, v/v) and annealed in the solvent vapor. (C) Possible packing scheme of the block copolymer in thin film.

assemblies in which the polypeptide blocks have a coillike conformation were always discrete spherical aggregates.

The formation of the spherical morphology can be explained by π-stacking of the PHF blocks[9] and the repulsive interactions of the protonated PBLG blocks. The stacked PHF blocks form the spherical core. The positively charged PBLG blocks form the shell around the polyfluorene block core (Figure 2B), resulting in the observed discrete nanospherical assemblies. The largest extended molecular length of **3a** and **3b** calculated by using Chem3D were 29.4 nm (PF block: 12.6 nm; PBLG block: 16.8 nm) and 34.7 nm (PF block: 23.5 nm; PBLG: 11.2 nm), respectively. These sizes are comparable to those observed by AFM.

AFM amplitude image of a thin film of **3a** cast from TFA/chloroform (3/97 v/v) showed parallel fibrillar textures (Figure 3A) in which the self-assembled aggregates had an average width of 79 ± 25 nm and were as long as 4−10 μm. Assemblies in block copolymer **3b** films showed fibers with an average diameter of 85 ± 17 nm in parallel orientation (Figure 3B). Theoretical calculation using Chem3D of the molecular lengths of **3a** and **3b** based on α-helical conformation (one PBLG unit contributes 1.5 Å) gave 19.5 nm (PHF block: 12.6 nm; PBLG blocks: 6.9 nm) and 23.8 nm (PHF block: 23.5 nm; PBLG blocks: 4.8 nm), respectively. The observed 80−85 nm fiber width of the block copolymers is much larger than the largest extended length of the helix−rod−helix triblock copolymer, and hence each fiber width is not a result of single-molecule lateral packing. Since the fiber width is much smaller than the normal pitch (5−200 μm) in the cholesteric phase of PBLG homopolymer,[10] the assemblies are not due to cholesteric liquid crystalline phases. The presence of the PBLG block does not induce the PHF block to form a helical conformation. This is evidenced by the very weak CD spectra which show that the intensity of the CD signal is only 1% of the chiral polyfluorenes.[11] We suggest that the triblock copolymer adopts a packing model shown in Figure 3C, where the PBLG blocks assume side-by-side antiparallel conformation due to the large dipole of the PBLG helix.[12]

The aligned grain texture of **3b** films (Figure 3B) is very different from that of **3a** films. This is obviously caused by the short PBLG blocks in **3b**. It is well-known that the α-helix conformation of PBLG repeats exactly every 18 amino acid residues in 5 turns, and thus there are 3.6 residues per turn of the helix.[7] At short chain lengths ($m < 20$), the α-helical secondary structure becomes significantly less stable, and a large fraction of the polypeptide segments adopt the β-sheet conformation.[2e] The coexistence of α-helical and β-sheet conformations in the PBLG sequences of **3b**,[2e] as discussed above, explains its different fibrillar morphology compared to **3a**. These observed self-assembled nanostructures of **3a** and **3b** are very different from those of conventional rod−coil block copolymers containing π-conjugated polymer blocks.[13]

In summary, we have described the first examples of hybrid block copolymers containing π-conjugated polymer and polypeptide sequences. The block copolymers were synthesized by the ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride using polyfluorene macroinitiators. Different nanostructured assemblies of the block copolymers were observed depending on the copolymer composition and the secondary structure (helix or coil) of the polypeptide blocks. A more detailed study of this class of block copolymers is in progress.

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Supporting Information Available: Synthetic and characterization details of polyfluorene initiators and block copolymers **3a** and **3b**, including FTIR, NMR, GPC, UV/vis, and CD spectra, PL spectra, and XRD diffraction patterns. This material is available free of charge via the Internet at http://pubs.acs.org.

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