DNA Packaging Via Combinative Self-Assembly

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Supporting Information

Materials

All chemicals were purchased from Sigma-Aldrich. KWK and CKWK were synthesized using standard Fmoc SPPS procedure with HOBT, HBTU, and DIPEA couplings, followed by N-capping with acetic anhydride. Peptides were analyzed by ESI-MS and ^{1}H NMR before Grafting. Amphiphilic block copolymer of PEG-*b*-PBD was synthesized by the well-established living anionic polymerization. The structure of PEG-*b*-PBD was confirmed by NMR and its polydispersity (PDI) was determined by Gel Permeation Chromatography to be ≤1.05.

λ-phage DNA 250μg/mL stored in 10 mM Tris buffer/0.5 mM EDTA was purchased from New England Biolabs. This DNA stock solution was dialyzed against 10mM sodium cocadylate buffer (pH6.5) containing 0.5 mM EDTA and further diluted to 100 μg/mL before use.

Grafting of Cysteine containing peptide to PEG-b-PBD

CKWK was grafted to PBD_{25}-PEO_{75} according to procedure published elsewhere.[14] Briefly, The reaction flask containing polymer (PBD_{25}-PEO_{75}), peptide (CKWK), and 2,2 azoisobutyronitrile (AIBN) was degassed for 30 minutes and then dry solvent, 1-methyl-2-pyrrolidinone (NMP), was added. Different molar ratios between $[C=C]_0$ and $[-SH]_0$ were used in order to aheive different grafting density. $[C=C]_0/[-SH]_0/[AIBN]_0$ = 1:3:0.33 and 1:5:0.33 were used for **PP4** and **PP8** synthesis respectively. The resulting solution was heated to 70°C and stirred for 48 hours under an argon atmosphere. AIBN was reinjected after 24 hours. After the reaction was complete, NMP was removed under vacuum. The crude product was re-dissolved in water and dialyzed against pure water to remove the unreacted peptides. The product was freeze-dried and collected for ^{1}H NMR and Gel Permeation Chromatography (GPC) analysis.

In ^{1}H NMR analysis on **PP4** and **PP8**, the characteristic signals of the grafted oligopeptide were observed at δ = 6.6 – 7.6 (tryptophan), and 8.1 ppm (NH); the signal of the thioether linkage -CH_2SCH_2- arise at δ ~ 2.7 and 2.9 ppm. Resonances at δ = 4.8-5.6 ppm indicate that the conversion of PBD double bonds did not come to completion. The quantitative analysis of signal intensities relative to that of PEG at δ ~ 3.6 ppm reveals that **PP4** chain contains about 4 KWK units and 21 unreacted butadiene units, whereas **PP8** contains 8 KWK units and 17 unreacted butadiene units. GPC analysis showed single narrow peak for **PP4** and **PP8** respectively, indicating the narrow polydispersity of the PBD-*b*-PEG scaffold has been preserved during the grafting process.

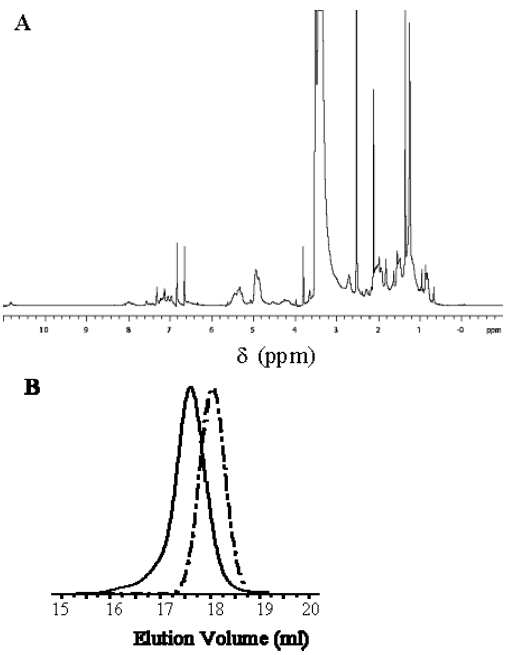

Figure S1. (**A**) Representative ^{1}H NMR spectrum (solvent: DMSO-d2) and (**B**) GPC chromatogram (eluent: NMP; solid line: PBD-PEG precursor, dashed line: PP4) of **PP4**.

Preparation of DNA complexes

DNA-KWK, DNA-PP4, DNA-PP8 complexes were prepared by simply mixing of an equal volume of 100 μg/mL DNA and KWK, PP4, PP8 with desired concentrations in the 10mM sodium cocadylate buffer. The mixture solution was vortexed for 30 seconds and allowed to equilibrate at room temperature for a few hours. The final DNA concentration was set at 50 μg/ml. For comparision purpose, same final stoichiometric KWK concentration at 64 μM was used for the three complex systems: 64 μm free KWK, 16 μM PP4 that contains 16 × 4 = 64 μM KWK, and 8 μM PP8 that contains 8 × 8 = 64 μm KWK.

Characterization of DNA complexes structure by Atomic Force Microscopy

DNA complexes were deposited on freshly cleaved mica and then allowed to air dry. Tapping mode AFM imaging was performed on a Digital Instruments Nanoscope IIIa scanning probe microscope with a multimode head. Silicon probes (VistaProbes T300) with spring constant 40 N/m, resonant frequency, 300 kHz was used to obtain all images.

DNA Melting Studies

DNA melting studies on native DNA and DNA complexes in 10mM sodium cocadylate buffer were performed on a Carey 100 UV-Vis. DNA absorbance at 260nm was monitored with temperature, slowly increasing from 50^{o}C to 95^{o}C at 1^{o}C/min heating rate. For DNA-KWK, DNA-PP4, DNA-PP8 complexes, weak background absorbances from KWK, PP4 and PP8 were directly subtracted from the measurements, by using the corresponding KWK, PP4 and PP8 in 10mM sodium cocadylate buffer as reference cells.