Synthesis and Characterization of Polyolefin-*graft*-oligopeptide Polyelectrolytes

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ABSTRACT: Novel polyelectrolytes consisting of hydrophobic polyolefin backbones and charged oligopeptide grafts have been synthesized and characterized. Oligopeptide-containing monomers were prepared by solid-phase peptide synthesis (SPPS) and then polymerized by ring-opening metathesis polymerization (ROMP). Copolymerization of the peptide-functionalized monomers with charge neutral cyclic olefins (i.e., poly(ethylene glycol)-substituted) gave polymers with varying charge density. These graft copolymers were characterized by nuclear magnetic resonance (NMR) spectroscopy, organic and aqueous gel permeation chromatography (GPC), and light scattering. Light scattering indicated that the oligopeptide-functionalized polyolefins could be tailored to form extended, pearl-like, or multimolecular structures, depending on the composition and density of the grafts.

Introduction

Polyelectrolytes are important across a breadth of biological and materials technologies including nucleic acid therapy,[1−3] tissue engineering,[4,5] coatings,[6] membranes,[7,8] and oil recovery.[9,10] Despite extensive synthetic and characterization efforts, open questions remain concerning the correlation between polymer architecture, conformation, and properties. New synthetic polyelectrolytes with tunable structures are needed to expand the range of available properties and applications. Conventional polyelectrolytes are typically vinyl polymers [−($CH_2CH(X)$−)], prepared by anionic[11] or free-radical[12,13] methods. Carboxylate,[14] ammonium,[13,15] sulfonate,[16] and pyridinium moieties are examples of ionic functionality in vinyl-based polyelectrolytes.[17] The close proximity of these charged "X" groups gives rise to synthetic and characterization challenges due to a combination of electrostatic and steric factors. As such, new syntheses that can provide spacing of pendent, charged moieties, and thus variable or tunable charge density, would be valuable.[12]

Because of its functional group tolerance,[18,19] ruthenium benzylidene-catalyzed metathesis polymerization enables the synthesis of wide variety of polyolefins with pendent carboxylate,[20] ammonium,[21] and peptide[22−24] functionality. For this study we have prepared oligopeptide-functionalized poly(cyclooctene), in which backbone rigidity, graft density, and molecular weight are varied. Lysine-functionalized cyclooctene monomers were synthesized by 9-fluorenylmethyloxycarbonyl-(Fmoc-) based solid phase peptide synthesis (SPPS)[25] and then utilized to prepare graft copolymers by ring-opening metathesis polymerization (ROMP), as depicted in Scheme 1. Structure−property relationships were established by varying charge density (i.e., graft length and charge spacing) along the polymer backbone, and through solution characterization by static and dynamic light scattering. The polycationic nature of these graft copolymers, and their tunable structures and properties, make them interesting candidates for applications such as nucleic acid complexation and transfection.

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Results and Discussion

Cyclooctene monomers **1** and **2**, containing pentalysine and lysine pendent groups, respectively, were prepared by Fmoc-based SPPS on a 2-chlorotrityl chloride resin with loading densities ranging from 1.0 to 1.4 mmol/g.[25] Before cleavage from the resin, the *N*-terminus of the peptide sequence was capped with 5-carboxylic acid-1-cyclooctene.[26−28] The oligopeptide-substituted monomers were cleaved from the solid phase under mildly acidic conditions (4:1 dichloromethane:trifluoroethanol (DCM:TFE)), which enabled removal of the macromonomer from the resin without cleaving the protecting groups. Monomers **1**−**3** were characterized by ^1H and ^{13}C NMR spectroscopy, infrared spectrometry, elemental analysis, mass spectrometry, and/or GPC. In the ^1H NMR spectra of **1**−**3**, the cyclic olefin protons were seen at ∼5.6 ppm. In addition, GPC traces of macromonomers **1** and **3** showed them to be monomodal and of very low polydispersity. Because of the affinity of amines for ruthenium, the Boc-protected forms of **1** and **2** were used for ROMP.

Polymer Synthesis. Cyclooctene-functionalized monomers **1**−**3**, shown in Scheme 1, were polymerized by ROMP using the bromopyridine-functionalized Grubbs Generation III catalyst ([$(H_2IMes)(3\text{-Br-pyr})_2(Cl)_2Ru$=CHPh]), to give **poly1**−**3**, as shown in Figure 1 and characterized in Table 1.[29] 5-Pentalysine-(Boc)-1-cyclooctene (**1**) was best homopolymerized as a 0.5 M solution in 9:1 TFE:DCM at room temperature for 40 min. Under these conditions, Boc-protected **poly1** was obtained in ∼80% yield following precipitation in ether. Boc-protected **1** was characterized by ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectrum of **1** showed the expected absence of the cyclic olefin signals, and the appearance of a new signal for the backbone olefins at 5.2 ppm. Molecular weight analysis of boc-protected **1** by GPC was not feasible in THF or chloroform due to limited solubility, nor in DMF due to poor signal-to-noise (low d*n*/d*c*).

The Boc groups of polycyclooctene-*graft*-pentalysine(Boc) were removed by stirring as a solution in HCl/dioxane/methanol

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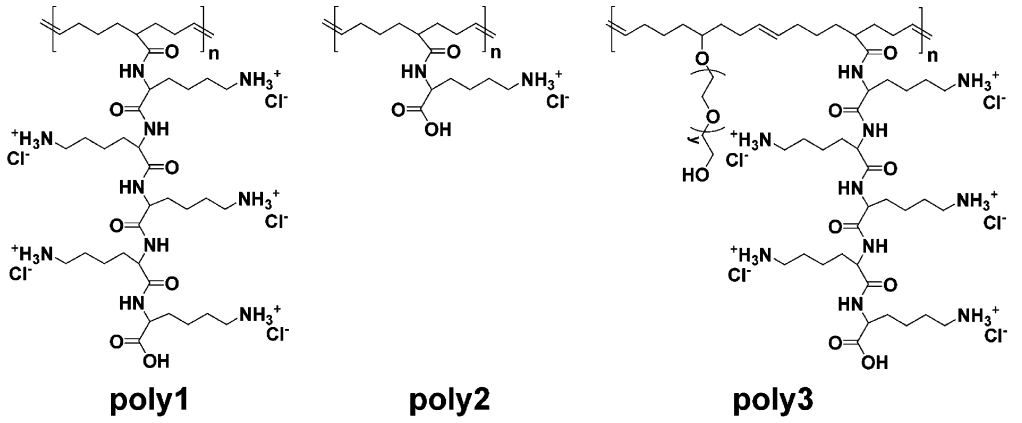

Figure 1. Structures of graft copolymers, **poly1**, **poly2**, and **poly3**.

Scheme 1

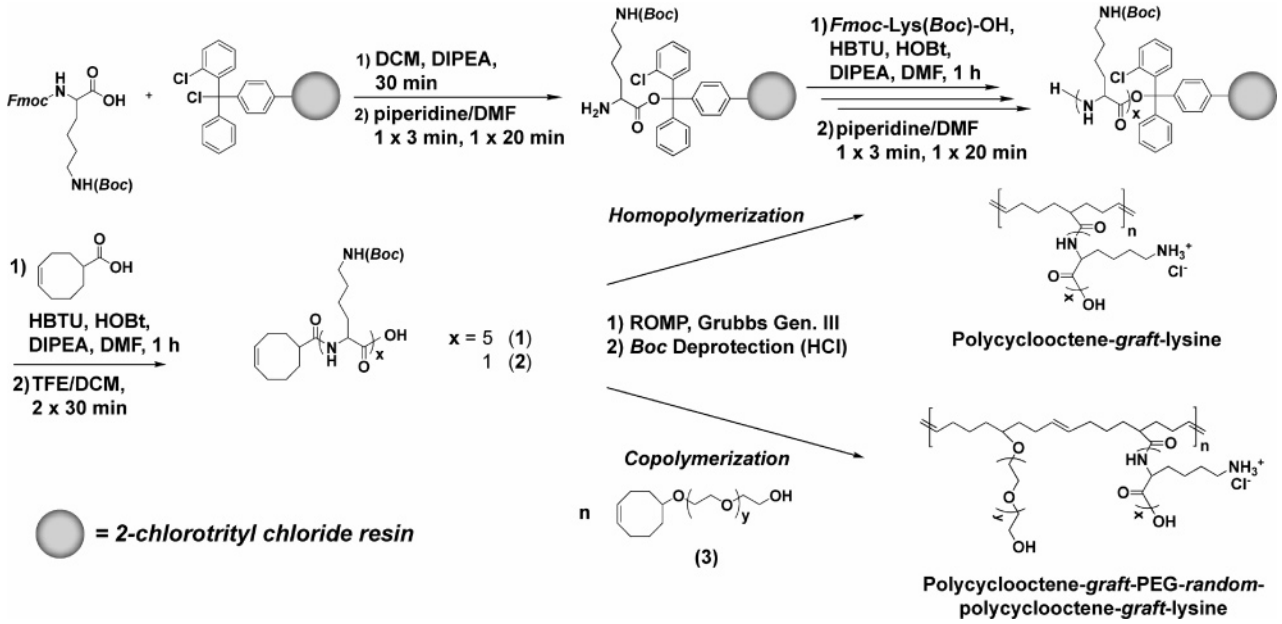

Table 1. Polymerization Conditions and Data for Graft Copolymers 1−3

polymer	solvent	time	M_n	M_w	M_w(abs)	PDI
poly1	0.5 M 10/90 DCM/TFE	40 min	87 000[b]	144 000[b]	48 000[c]	1.7
poly2	1.2 M 50/50 DCM/MeOH	3 h	7500[a]	11 000[a]	36 000[c]	1.5
poly3	0.6 M 50/50 DCM/MeOH	3 h	36 000[a]	61 000[a]	200 000[c]	1.7

[a] Boc-protected analog analyzed by THF GPC relative to PEG standards [b] Determined by aqueous GPC relative to PEG standards (0.5 M acetic acid, 0.3 M sodium sulfate) [c] Absolute M_w determined by static light scattering in 0.1 M NaCl aqueous solution (25 °C)

for 3 h at room temperature. The deprotected polymer, **poly1**, was isolated by precipitation into acetone to give a white powder in 95% yield. Trace macromonomer was removed by centrifugation using a filter device (Millipore Amicon Ultra) with a molecular weight cutoff (MWCO) of 10 000 g/mol. **Poly1** was lyophilized and then characterized by ^{1}H and ^{13}C NMR spectroscopy, GPC, and static and dynamic light scattering. NMR spectra of **poly1** showed the absence of the characteristic Boc peaks, and no change in the olefin signals relative to the protected polymer. GPC was performed at high salt concentration (0.5 M aqueous acetic acid with 0.3 M Na_2SO_4) using ultraviolet (UV) and refractive index (RI) detection. The GPC trace of **poly1** (Figure 2) was nearly monomodal, with only trace residual macromonomer or cyclic oligomer present. **Poly1**, prepared using a 50:1 ratio of Grubbs Generation III catalyst-to-monomer, had a GPC-estimated number-average molecular weight (M_n) of 87 000 g/mol, and a polydispersity index (PDI) of 1.7 (against PEO calibration standards). While this GPC-determined molecular weight is in reasonable agreement with the theoretical value (for DP = 50, M_n of the chloride salt is ~49 000 g/mol), this is of course only an estimated molecular weight based on the PEO calibration standards. Thus, additional characterization of the graft copolymers was performed by static and dynamic light scattering, as described later.

Monomer **2** was homopolymerized as a 1.2 M solution in 50/50 DCM/methanol using a 50:1 monomer:catalyst ratio with Grubbs Generation III catalyst. Boc-protected **poly2** was isolated by precipitation from diethyl ether in 89% yield, and was characterized by GPC in THF, relative to PEG standards, giving M_n and M_w values of 7500 and 11 000 g/mol, respectively (PDI 1.5). Using poly(methyl methacrylate) (PMMA) standards, M_n and M_w were estimated as 13 000 and 18 000 g/mol, respectively (PDI 1.4). At a targeted DP of 50, the theoretical M_n of **poly2** was approximately 16 000 g/mol.

PEGylated cyclooctene macromonomer **3** was prepared by anionic polymerization of ethylene oxide from 5-hydroxycy-

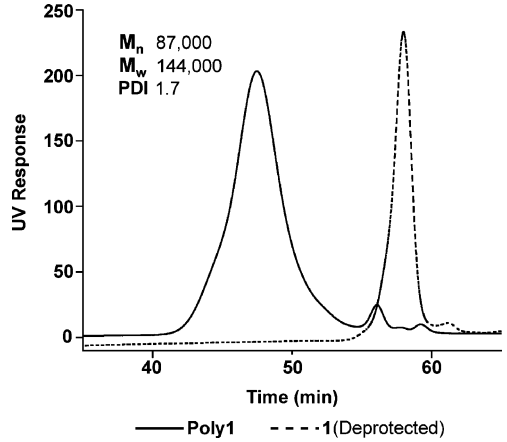

Figure 2. Aqueous GPC chromatograms of deprotected monomer **1** (dashed line) and **poly1** (solid line) in 0.5 M acetic acid, 0.3 M sodium sulfate aqueous buffer at a flow rate of 0.5 mL/min.

clooctene and characterized by end group analysis of the ^{1}H NMR spectrum (DP 31 giving 1476 g/mol), and by GPC in THF (M_n 1200 g/mol, PDI 1.1) relative to PEO standards. ROMP copolymerization of **1** and **3**, again using Grubbs Generation III catalyst, was performed at room temperature as a 0.6 M monomer solution in 50/50 DCM/MeOH, and seen to proceed to high conversion. Similar PEGylated cyclic olefins are typically polymerized at higher concentrations to reduce competing cyclization.[30,31] However, due to the high molecular weight of the monomer, and resultant low concentration of cyclic olefin, obtaining higher monomer solution concentrations was not possible. A 1:1 feed ratio of monomers **1** and **3** led to random copolymers composed of approximately 70/30 pentalysine/PEG grafts based on integration of the lysine and PEG resonances in the ^{1}H NMR spectra. The Boc-protected pentalysine/PEG graft copolymers were characterized by aqueous GPC in THF to give M_n 36 000 g/mol and M_w 61 000 g/mol relative to PEO standards (PDI 1.7), and M_n 51 000 g/mol and M_w 75 000 g/mol relative to PMMA standards (PDI 1.5). The GPC-determined molecular weights were in the range of the targeted DP of 50, which puts the theoretical M_n around 60 000−65 000 g/mol depending upon the copolymer composition. At a DP of 50, with a 70/30 pentalysine/PEG ratio of the monomer units, the expected M_n is ∼63 000 g/mol. Boc-protected **poly3** was deprotected according to the same procedure as **poly1** and **poly2** (4 M HCl in dioxane, methanol, 3 h, room temperature). **Poly3** was precipitated into diethyl ether, isolated in 93% yield as an off-white solid, and characterized by NMR spectroscopy and GPC. Dynamic and static light scattering were then used to analyze **poly3** in solution.

Light Scattering Studies of Poly1−3. Static and dynamic light scattering were used to determine the solution properties of **poly1−3** (Figure 1) as summarized in Table 2. Initial studies focused on the effect of salt concentration and pH on the solution behavior of **poly1** (Figure 3 and Table 2), and subsequent experiments on the effect of reducing the graft length (1 vs 5 lysine groups) by comparing **poly2** with **poly1** (Figures 3 and 4, Table 2), and increasing charge spacing (i.e., reducing graft density) while maintaining water solubility as in **poly3** (Figures 3 and 4; Table 2).

Poly1: The Effect of Ionic Strength. The radius of gyration (R_g), second viral coefficient (A_2), and M_w of **poly1** were determined by static light scattering (SLS) by generating Zimm plots under various conditions (Figure 3).[32] While the pK_a of the lysine amines can vary based on the environment (as seen in proteins), the pK_a in its free amino acid form is ∼10.[33] If all the lysine groups release their chloride counterions when dissolved in water, the observed M_w of 48 000 correlates to a DP of ∼60, in good agreement with the targeted DP of 50. Even after considering that some counterions will remain associated with the polymer due to counterion condensation,[34,35] dispersion of individual polyelectrolyte molecules was seen in the concentration range studied. No aggregation of these highly charged, amphiphilic polymers was seen. The positive second viral coefficient (A_2) further indicated this polyelectrolyte to be in the good solvent regime. An R_g of 25 nm for **poly1** was measured in 0.1 M NaCl aqueous solution. This measured R_g is considerably larger than the R_g of a Gaussian chain, calculated[36] to be 6 nm based on a monomer contour length of 1.2 nm and a degree of polymerization of 60. At a fully extended conformation, the estimated R_g would equal 23 nm. This experimentally determined R_g of **poly1** indicated a significantly expanded conformation in low salt solution.

To investigate the effect of solution ionic strength on polymer conformation, M_w, A_2, and R_g of **poly1** were measured in 0.5 M NaCl solutions. The decreased A_2 value of **poly1** observed in this environment indicated that the solvent quality became poorer at elevated ionic strength, and based on the Zimm plot, an M_w value comparable to what was found in low salt solution was observed at this high salt concentration. The slight discrepancy seen in the values of M_w at 0.1 and 0.5 M NaCl can be attributed to the standard practice of ignoring salt effects in Zimm plot analysis. The R_g of **poly1** was significantly reduced, from 25 nm in a 0.1 M NaCl solution to 15 nm in a 0.5 M NaCl solution.

Figure 3 shows the hydrodynamic radius (R_h) measured by dynamic light scattering for **poly1** at different polymer concentrations. The linear dependence of inverse relaxation time on q^2 is characteristic of a diffusive motion for **poly1** in water. It should be noted that in all of the **poly1** solutions studied, only one relaxation mode was observed. As shown in Table 2, R_h changed very little (from 11 to 10 nm) in a 0.1 M vs 0.5 M NaCl aqueous solution, which contrasted the strong salt dependence of R_g. This suggested that even though the size of **poly1** decreased significantly with increasing ionic strength, its hydrodynamic properties were relatively stable despite the solution changes. As a measure of chain extension, the R_g/R_h

Table 2. Summary of Static and Dynamic Light Scattering of Poly1−3

polymer	conditions	M_w (g/mol)	R_g (nm)	R_h (nm)	A_2 ((mol·dm^3)/g^2)
poly1	0.1 M NaCl[a]	48 000	25	11	1.6×10^{-6}
	0.5 M NaCl[a]	53 000	15	10	4.7×10^{-7}
	pH 2[b]	57 000	28	12	2.9×10^{-6}
	pH 12[b]	61 000	20	8.8	6.6×10^{-6}
poly2	0.1 M NaCl[a]	36 000	27	6.5	1.3×10^{-6}
	0.5 M NaCl[a]	28 000	9.0	4.4	-4.2×10^{-6}
poly3	0.1 M NaCl[a]	200 000	35	22	2.2×10^{-7}
	0.5 M NaCl[a]	220 000	39	23	2.7×10^{-7}

[a] pH 7. [b] Salt concentration = 0.1 M NaCl.

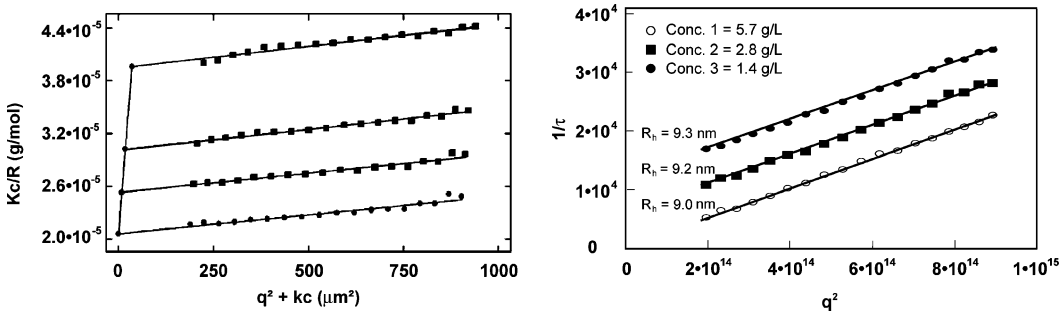

Figure 3. Zimm plot of SLS data (left) and DLS (right) analyses of **poly1** in 0.1 M aqueous NaCl at 25 °C. For the SLS data, Kc/R is proportional to the inverse of scattered intensity, and k is an arbitrary constant to facilitate the display of scattering data. Based on DLS, the inverse of the relaxation time ($1/\tau$) as a function of the scattering vector squared (q^2) is plotted at several concentrations to determine the hydrodynamic radius (R_h) of **poly1**. All $1/\tau$ lines pass through the origin, and the top two curves are shifted vertically upward for clarity.

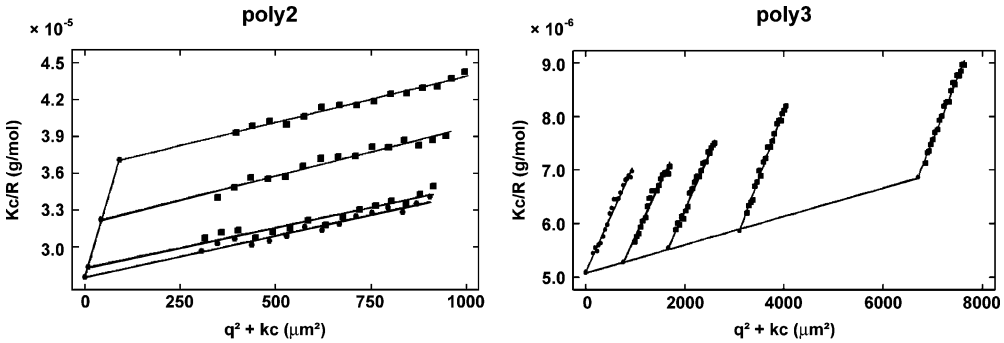

Figure 4. Zimm plot of SLS data for **poly2** (left) and **poly3** (right) in 0.1 M $NaCl_{(aq)}$ at 25 °C.

ratio was also calculated at different salt concentrations. The large R_g/R_h value (~2.3) in 0.1 M NaCl aqueous solution suggests a highly extended chain conformation, which collapses at higher salt concentration of 0.5 M, as indicated by the lower R_g/R_h (~1.5). Large R_g/R_h ratios have also been observed for polymers with densely grafted neutral side chains, attributed to the molecules assuming extended conformations in solution.[37−39] The extension of the chain backbone in a so-called molecular bottlebrush[37] originates from a strong excluded volume interaction caused by crowding side chains, which pushes the otherwise flexible polymer backbone into an elongated conformation. Analogous to these bottlebrush molecules, **poly1**, which contains pendent protonated lysines, exhibited an extended conformation due to the combined effects of steric and electrostatic repulsion of the oligopeptide side chains. This electrostatic repulsion was significantly screened when the solution ionic strength was increased from 0.1 to 0.5 M NaCl, as illustrated in the reduced R_g/R_h value in the 0.5 M NaCl solutions. It should also be noted that in high salt solutions, steric crowding alone from relatively short pendent lysine chains is not sufficient to support an extended polymer conformation. An extensive computational modeling study is currently being undertaken to quantify the relative contributions of steric crowding and electrostatic interaction on conformational properties of polymers grafted with charged side chains.

pH Effects. Poly1 was seen to assume an extended conformation under conditions at which the grafts are largely protonated, due to a combination of steric crowding and electrostatic repulsion of the positively charged grafts in 0.1 M NaCl solution. R_g and R_h of **poly1** were also measured at pH 2 and pH 12 (Table 2) (no added NaCl). At pH 2, **poly1** was observed to be slightly more extended than at pH 7, a result of lower salt screening effects. In the high pH environment, the number of protonated amines decreases, and the grafts become more flexible, resulting in a reduction of R_g and R_h. Unscreened electrostatic repulsion among residual charges in the grafts is likely responsible for the conformation that is characterized by a high R_g/R_h ratio at 0.1 M NaCl.

Light Scattering on Poly2. To further explore the effect of the pendent oligolysine grafts on solution properties, **poly2** was studied by static and dynamic light scattering in 0.1 and 0.5 M NaCl aqueous solutions at neutral pH (Figure 4). The R_h of **poly2** was found to be extremely small (on the order of 6.5 and 4.4 nm for 0.1 and 0.5 M NaCl solutions, respectively). The R_g of **poly2** in 0.1 M NaCl solution was found to be larger than would be expected for a globular polymer conformation. The molecular weight indicated individual polymer chains dispersed in water. Based on R_g/R_h, **poly2** is viewed as exhibiting a highly anisotropic conformation, which was even greater than the anisotropic behavior seen in **poly1**. **Poly2** was in a poor solvent regime at high salt concentrations as indicated by the negative A_2 value measured in 0.5 M NaCl solution. Evaluation of these various parameters suggests that **poly2** behaves as a typical hydrophobic polyelectrolyte. Numerous studies[40−44] have determined that when dissolved in water, strongly charged hydrophobic polyelectrolytes adopt a "pearl-necklace" conformation in which the polymer chain consists of multiple mini-aggregates comprised of several monomer units, or "pearls," connected by a string of uncollapsed monomers. Because of the repulsion between pearls, the pearl-necklace conformation becomes stiff. When the solution ionic strength was increased from 0.1 to 0.5 M, the electrostatic repulsion among the pearls was screened, and the entire chain collapsed into a globular structure as evidenced by the decrease in R_g and R_h. The values of R_g and R_h changed from 27 and 6.5 nm at 0.1 M, to 9.0 and 4.4 nm at 0.5 M NaCl. Although these light scattering results for **poly2** are consistent with the pearl-necklace model, more direct observation of "pearls" is desirable. While a direct observation of pearls formed by a single homopolymer molecule continues to be a challenge, **poly2** appears to be an excellent candidate for future experimental verification of theoretical predictions.

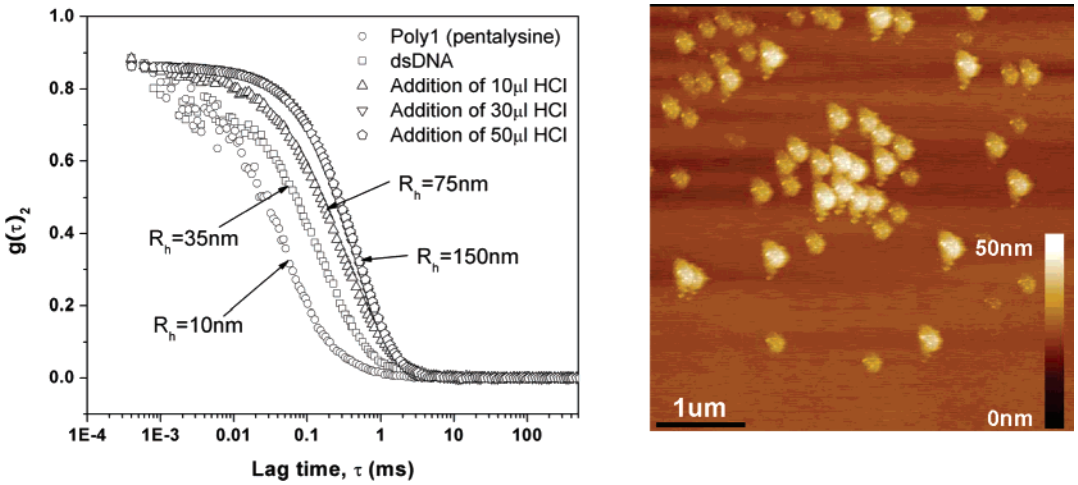

Figure 5. Left: scattering intensity autocorrelation function, $g(\tau)_2$ for **poly1** (open sphere), DNA (open square), DNA + **poly1** solution with 10 μL HCl added (open up triangle), with 30 μL HCl added (open down triangle), and with 50 μL HCl added (open pentagon). The DNA and **poly1** solution was initially held at pH 12, in which **poly1** is neutral, and no complexation was detected by DLS. R_h was calculated from a CONTIN analysis and Stokes−Einstein relationship; Right: tapping mode AFM micrograph of **poly1**-dsDNA complex produced by spin-casting onto freshly cleaved mica.

Light Scattering on Poly3. Aqueous solutions of **poly3** were also studied by light scattering at both low and high ionic strengths to explore the effect of charge spacing by the introduction of nonionic, hydrophilic moieties (Figure 4). Compared to **poly1**, the R_g and R_h of the PEGylated copolymer were substantially higher. Given that the molecular mass of the PEG grafts are comparable to pentalysine, and that the targeted DP of **poly3** is the same as **poly1**, the expansion of **poly3** was unexpected. Incorporating nonionic, hydrophilic PEG grafts was expected to reduce the strong electrostatic repulsion among the pentalysine moieties, which was shown to be responsible for the highly anisotropic conformation of **poly1**. Therefore, based on the dilution of the electrostatic repulsion, **poly3** was expected to be smaller. However, the effective molecular weight of **poly3** based on light scattering was measured to be about three times larger than the value obtained by GPC. Furthermore, the copolymer conformation was stable to drastic changes in ionic strength, in contrast to the behavior of **poly1**. From these observations, the copolymer chains likely formed aggregated structures composed of (on average) three chains. One possible origin of such aggregation could be the hydrophobic nature of the polyolefin backbone. The molecular origin of why such aggregates are comprised of only a few copolymer molecules remains to be fully investigated. The question of the dependence of the unimer-to-multimer transition on the PEG content is a fundamental problem in understanding the molecular origin of multimer formation. This issue will be taken up in a subsequent study.

We also performed preliminary experiments on the interactions of poly(cyclooctene)-*graft*-pentalysine and DNA, using light scattering and atomic force microscopy as characterization tools. Figure 5 shows dynamic light scattering (DLS) data associated with complexation of the graft copolymer with DNA, and the pH dependence of this complexation. No polymer−DNA complexation was detected at high pH, as expected under conditions at which the lysine residues are predominately neutral. With the addition of HCl to the mixture, and associated charging of the grafts, a progressive increase in solution scattering intensity and hydrodynamic radius (R_h) was seen. The complex size leveled off toward the end of the HCl titration. The size of such polyelectrolyte complexes was found to depend on several experimental parameters, such as polycation-to-polyanion mixing ratio, solution pH, and the order of mixing. In separate experiments, we found that the size of **poly1**−dsDNA complexes to vary from 60 to 200 nm, depending on the mixing ratio of two polymers. Following completion of the titration curve, the solution was spun cast onto a freshly cleaved mica surface, and examined by AFM. The average size of the complexes observed by AFM was smaller than that found by DLS, an expected result of the spinning and drying process.

In summary, by utilizing a combination of solid-phase peptide synthesis and ring-opening metathesis polymerization, new polyelectrolytes were prepared containing hydrophobic backbones with pendent lysine and PEG grafts. The solution behavior of these polyelectrolytes was tailored by changing the peptide graft length and density. Polycyclooctene-*graft*-pentalysine (**poly1**) exhibited an extended conformation in low ionic strength aqueous solutions due to combined influences of steric crowding and electrostatic repulsion. Screening of electrostatic repulsion at high ionic strength resulted in a more condensed structure. The R_g/R_h found for the monolysine derivative **poly2** is consistent with a pearl-necklace structure, in which the hydrophobic backbone collapses on itself in the aqueous environment. However, with the introduction of only 30 mol % PEG grafts to the pentalysine derivative, the resulting random copolymer (**poly3**) no longer adopted an extended conformation based on the R_g and R_h values in solution, instead forming aggregates. Complex formation of **poly1** with DNA suggests potential utility of these polymers to enhance transfection of plasmid DNA, a topic of current experiments.

Experimental Details

Materials. Piperidine (reagent plus, 99%), 2,2,2-trifluoroethanol (TFE) (99.5%), diisopropylethylamine (biotech grade, 99.5%), methanol (anhydrous, 99.8%), 3-bromopyridine (99%), and Grubbs Generation II catalyst ((1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene) dichloro(phenylmethylene)(tricyclohexylphosphine)-ruthenium, $C_{46}H_{65}Cl_2N_2PRu$) were purchased from Aldrich (Saint Louis, MO). *N*,*N*-Dimethylformamide (EM Science, guaranteed reagent, 99.8% and EM Science, anhydrous, 99.8%) and pentane (EM Science, ACS reagent) were purchased from VWR International (West Chester, PA). Sodium chloride (reagent grade), HCl (concentrated), Millipore Amicon Ultra (MWCO 10 000) centrifugal filter devices, and Millipore hydrophilic PVDF membrane filters (0.22 μm diameter) were obtained from Thermo Fisher Scientific Inc. (Waltham, MA). Fmoc−Lys(Boc)−OH, 1-hydroxybenzotriazole (HOBt) hydrate, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetra-

methyluronium hexafluorophosphate (HBTU), and 2-chlorotrityl chloride resin (1.4 mmol/g, 100−200 mesh) were purchased from Advanced ChemTech (Louisville, KY). Deuterated solvents DMSO-d_6 and D_2O were obtained from Cambridge Isotopes (Andover, MA). CH_2Cl_2 was washed then distilled over CaH_2, and TFE was distilled over sodium bicarbonate.[45] All other materials were used without purification.

Equipment. NMR spectra were recorded on Bruker Avance400 (^{1}H) and 100 (^{13}C) MHz NMR spectrometer, using a Bruker BBO5 probe. Mass spectrometry was performed on a JEOL JMS700 MStation high-resolution two-sector mass spectrometer with low-resolution electrospray ionization (ESI). Gel permeation chromatography (GPC) was performed in tetrahydrofuran (THF) and aqueous eluents. For analysis in THF, a KNAUER HPLC Pump K-501, UV detector K-2600 (absorbance measured at 232 and 260 nm), and RI detector K-2301 were utilized with three PLgel 5 μm MIXED-D 300 × 7.5 mm columns (flow rate = 1.0 mL/min). Aqueous GPC was performed in 0.5 M acetic acid and 0.3 M sodium phosphate buffer with a flow rate of 0.5 mL/min. The aqueous system was comprised of a HP Series 1050 Pump, HP 1047A RI detector, and Waters 486 Tunable Absorbance detector (λ = 232 nm) utilizing three Waters Ultrahydrogel Linear Columns (mixed beads, 7.8 × 300 mm). Elemental analysis (CHN testing) was performed by Schwarzkopf Microanalytical Laboratory (Woodside, NY). Light scattering studies were performed with an ALV light scattering apparatus equipped with an ALV-5000 board. A green laser (COHERENT) with a wavelength of 514.5 nm was used as the light source, and the temperature of the sample holder was held constant at 25 ± 0.1 °C by a circulating water bath.

Synthesis of 5-Pentalysine(Boc)-1-cyclooctene (1). Pentalysine-functionalized macromonomer **1** was synthesized by Fmoc-based SPPS with the coupling agent HBTU using 2-chlorotrityl chloride resin. First, 5.00 g of resin (with 1.40 mmol of functional groups per gram of resin, 7.00 mmol, 100−200 mesh) was weighed into an oven-dried, glass-fritted reaction tube and swollen with dry DCM (40 mL) for 5−10 min. The resin was filtered, and a solution of Fmoc−Lys(Boc)−OH (6.56 g, 14.0 mmol), dry DIPEA (6.10 mL, 35.0 mmol), and dry DCM (35 mL) was added to the reaction tube. The tube was capped, vented, and agitated with nitrogen. After 30 min, the resin was filtered and rinsed with dry DMF three times. The remaining reactive groups on the resin were capped with a 80/15/5 dry DCM/methanol/DIPEA solution (40 mL, 2 × 10 min) and then rinsed with reagent grade DMF. The Fmoc group was then removed with a 25/75 piperidine/DMF cleavage solution (2 × 40 mL). The solution was added to the resin, and the resin was agitated for 3 min. Following filtration, fresh cleavage solution was added and agitated for 20 min. The resin was filtered and rinsed with DMF six times, DCM three times, 2-propanol three times, and hexanes six times. The resin was dried by suction filtration for 15 min, transferred to a vial, and dried under vacuum for 24−36 h. The loading density was estimated by the change in mass from the starting resin to the final resin. Loading densities for these syntheses were generally in the range of 0.7−1.4 mmol/g with lower loading densities targeted for longer oligopeptide sequences. To tailor the degree of functionalization of the resin, the number of equivalents of the amino acid added to the coupling solution was changed. When 2.00 equiv of amino acid were used as described above, loading densities generally ranged from 1.20 to 1.40 mmol per gram of resin.

Following the addition of the first amino acid residue, the dried resin (6.61 g, 9.25 mmol) was transferred to a dry, glass-fritted reaction tube and swollen with 40 mL of DCM for 5 min. Fmoc−Lys(Boc)−OH (21.7 g, 46.3 mmol), HOBt (7.09 g, 46.3 mmol), HBTU (17.2 g, 45.3 mmol), and DMF (60 mL, reagent grade) were combined in an oven-dried round-bottom flask, and DIPEA (reagent grade, 16.1 mL, 92.5 mmol) was added to form a clear, yellow solution. The activated amino acid solution was then added to the resin, and the resin was agitated for 1 h, filtered, and washed with DMF three times. As previously described for the resin loading, the Fmoc protecting group was cleaved with 25/75 piperidine/DMF, after which the resin was washed with DMF six times. The same procedure was utilized to add lysine units until the desired oligopeptide sequence length was obtained.

Following the addition of the final lysine residue, the Fmoc protecting group was cleaved, and the resin was thoroughly washed with DMF. A solution of 5-carboxylic acid-1-cyclooctene (7.14 g, 46.3 mmol, prepared in three steps from 1,5-cyclooctadiene[26−28]), HOBt (7.09 g, 46.27 mmol), HBTU (17.20 g, 45.34 mmol), DIPEA (16.12 mL, 92.54 mmol), and 60 mL of DMF was then added to the resin and agitated for 1 h. The resin was filtered and washed with DMF and DCM three times. The macromonomer was then cleaved from the resin under mildly acidic conditions (4:1 DCM:TFE). After 45 min, the resin was filtered into a clean flask, and fresh solution was added. The resin was agitated for another 45 min, filtered, and washed with 4:1 DCM:TFE and then DCM three times. This resulting filtrate was concentrated on a rotary evaporator and then precipitated into diethyl ether. After standing at 4 °C for several hours, the product was isolated by filtration and then dried under vacuum. The white, powdery product was obtained in 80−85% yield based upon the estimated loading density and was characterized by ^{1}H and ^{13}C NMR, ATR−FTIR, low and high-resolution mass spectrometry, and elemental analysis. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (DMSO = 2.50 ppm) 12.49 (br, 1 H), 7.64−8.03 (br m, 5 H), 6.72 (br, 4 H), 6.40 (br, 1 H), 5.64 (br, 2 H), 4.04−4.30 (br m, 5 H), 2.86 (br, 10 H), 2.22−2.38 (br m, 2 H), 1.97−2.20 (br m, 3 H), 1.10−1.81 (m, 81 H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 177.12, 177.08, 173.42, 171.99, 171.48, 171.41, 171.25, 155.51 (5 C), 129.97, 129.88, 77.31(5 C), 52.70, 52.60, 52.39, 52.21, 51.81, 43.60, 40.35, 32.25, 31.98, 31.81, 31.67 (2 C), 31.22, 30.74, 29.89, 29.57, 29.25 (3 C), 29.14, 28.26 (15 C), 27.49, 27.35, 25.44, 25.37, 24.02, 22.53−22.86 (overlapping, 5 C) ppm. ATR−FTIR: 3282, 2931, 1683, 1630, 1521, 1452, 1392, 1365, 1249, 1169, 864 cm^{-1}. Low-resolution ESI (m/z): $[M + Na]^+$ calculated for $NaC_{64}H_{114}N_{10}O_{17}$, 1317.83; found, 1317.8. High-resolution FAB (m/z): $[M + Na]^+$ calculated for $NaC_{64}H_{114}N_{10}O_{17}$, 1317.8261; found, 1317.8959. Anal. (CHN) Calcd C, 59.33; H, 8.87; N, 10.81. Found: C, 58.28; H, 8.94; N, 10.44.

Synthesis of 5-Monolysine(Boc)-1-cyclooctene (2). 2-Chlorotrityl chloride resin was loaded with lysine as previously described. Following the loading of lysine on the resin, the resin was swollen, and a solution of 5-carboxylic acid-1-cyclooctene, HBTU, HOBt, DMF, and DIPEA was added. The resin was agitated for 1 h, filtered, washed with DMF 6 times, and washed with DCM 3−4 times. The macromonomer was cleaved from the resin as previously described for compound **1**, and the resulting white solid was characterized by ^{1}H and ^{13}C NMR, ATR−FTIR, low and high-resolution mass spectrometry, and elemental analysis. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (DMSO = 2.50 ppm) 12.39 (br, 1 H), 7.80−7.95 (br m, 1 H), 6.77 (br m, 1 H), 6.40 (br s), 5.64 (br m, 2 H), 4.05 (br m, 1 H), 2.86 (br m, 2 H), 2.21−2.38 (br m, 2 H), 1.93−2.21 (m, 3 H), 1.10−1.87 (br, 21 H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 176.99, 176.91, 174.03, 174.02, 155.63, 130.02, 129.98, 129.95, 77.37, 51.69, 43.58, 43.49, 40.17, 39.96, 39.75, 39.55, 39.34, 39.13, 38.92, 34.26, 32.24, 32.08, 30.67, 29.88, 29.61, 29.17, 28.97, 28.32, 27.61, 27.42, 25.52, 25.46, 24.10, 24.04, 22.97 ppm. ATR−FTIR: 3314, 2929, 2861, 1689, 1646, 1524, 1451, 1392, 1366, 1249, 1166, 988, 861, 778, 712 cm^{-1}. Low-resolution ESI (m/z): $[M + H]^+$ calculated for $C_{20}H_{35}N_2O_5$, 383.26; found, 383.3; $[M + Na]^+$ calculated for $NaC_{20}H_{34}N_2O_5$, 405.24; found, 405.2; $[M + K]^+$ calculated for $KC_{20}H_{34}N_2O_5$, 421.35; found, 421.3. High-resolution FAB (m/z): $[M + H]^+$ calculated for $C_{20}H_{35}N_2O_5$, 383.2546; found, 383.2558. Anal. (CHN) Calcd C, 62.80; H, 8.96; N, 7.32 Found: C, 62.30; H, 9.02; N, 7.21.

Synthesis of PEG1200-Functionalized Macromonomer 3. In a flame-dried air-free flask, 5-hydroxycyclooctene (6.8 mL of a 2.0 M solution in THF, 13.6 mmol) was added to dry THF (200 mL) while stirring under N_2. The solution was titrated with potassium napthalenide (0.2 M in THF) until a slight green endpoint was observed (~70.0 mL). The cyclooctene alkoxide solution was stirred for an additional 30 min at room temperature before

being cooled to 0 °C in an ice/water bath. Ethylene oxide (15.0 mL, 340.6 mmol) was condensed at −78 °C using a stainless steel gas transfer[46] manifold and then slowly warmed to room temperature while transferring to the cooled cyclooctene alkoxide solution under static vacuum. The reaction mixture was pressurized with argon, sealed, and stirred at room temperature for 16 h. The solution was concentrated, and the residue was purified by column chromatography on silica gel (93/7 → 90/10 $CHCl_3$/MeOH). The product recovered from the column was dissolved in a minimal amount of chloroform and precipitated into a hexane/diethyl ether mixture. A white powder was isolated by filtration and dried under vacuum to yield 12.5 g (83% yield) of PEG-functionalized macromonomer. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ($CHCl_3$ = 7.26 ppm) 5.62 (m, 2 H), 3.28−3.84 (complex, br m, 114 H), 2.54 (br s, 1 H), 1.28−2.36 (complex br m, 11 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ ($CHCl_3$ = 77.16 ppm) 130.2, 129.6, 81.1, 72.7, 71.0, 70.7, 70.4, 67.8, 61.8, 34.2, 33.5, 25.9, 25.8, 22.8 ppm. ATR−FTIR: 3491, 2882, 1467, 1359, 1341, 1280, 1242, 1100, 1060, 959, 841, 725 cm^{-1}. GPC (THF, PEG standards): M_n = 1200 g/mol, M_w = 1360 g/mol, PDI = 1.09.

Synthesis of Polycyclooctene-*graft*-pentalysine(Boc). Polycyclooctene-*graft*-pentalysine(Boc) was synthesized by ROMP of **1**. Macromonomer **1** (0.88 g, 0.68 mmol) was slowly added to a stirring solution of dry TFE (1.22 mL). The solution was vortexed until **1** completely dissolved. Grubbs Generation III catalyst ([(H_2-IMes)(3-Br-py)$_2$($Cl)_2$Ru = CHPh]) was prepared according to the literature.[29] 12.0 mg of the catalyst (0.014 mmol, 50:1 monomer:catalyst ratio) was dissolved in dry DCM (136 μL) in a separate vial and immediately added to the macromonomer solution. The reaction (final concentration 0.5 M 10/90 DCM/TFE) was vortexed for 5−10 min, stirred under nitrogen for a total of 40 min, and quenched with ethyl vinyl ether (∼1 mL). The reaction solution was precipitated into diethyl ether and isolated by filtration. After drying under vacuum, the light yellow polymer was obtained in 85% yield and characterized by ^{1}H and ^{13}C NMR. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (DMSO = 2.50 ppm) 12.52 (br s, 1 H), 7.60−8.20 (br m, 5 H), 6.68 (br s, 4 H), 6.38 (br s, 1 H), 5.29 (br s, 2 H), 3.92−4.40 (br m, 5 H), 2.87 (br s, 10 H), 1.09−2.32 (complex br m, 86 H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 173.60, 171.25, 171.00, 155.53, 77.32 (5 C), 51.90−52.10 (overlapping, 2 C), 31.90, 30.80, 29.20, 28.27 (15 C), 22.40−22.60 (overlapping, 5 C) ppm.

Synthesis of Polycyclooctene-*graft*-pentalysine (poly1). Boc-protected **poly1** (0.512 g, 0.526 mmol) was dissolved in 5 mL of dry methanol, and excess 4 M HCl in 1,4-dioxane (2 mL) was added. The vented reaction was allowed to stir at ambient temperature for several hours. The reaction solution was concentrated and precipitated from acetone. The product was isolated by filtration, rinsed with acetone, and dried under vacuum overnight, yielding 93% (0.479 g, 0.369 mmol) of the desired product. Residual macromonomer was removed by centrifugation with filter devices. The purified polymer was freeze-dried, yielding a white porous solid, and analyzed by ^{1}H and ^{13}C NMR and aqueous GPC. ^{1}H NMR (D_2O, 400 MHz): δ (H_2O = 4.79 ppm) 5.28−5.62 (br m, 2 H), 4.18−4.42 (m, 5 H), 2.88−3.10 (m, 10 H), 2.36 (br, 1 H), 1.23−1.94 (complex br m, 40 H) ppm. ^{13}C NMR (D_2O + 2 drops of DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 180.88, 175.58, 175.35 (2 C), 175.12, 132.48 (2 C), 55.10, 55.02, 54.85, 54.49, 54.35, 40.76, 33.66, 32.14 (5 C), 31.30 (5 C), 27.89 (4 C), 27.79, 23.84, 23.66 (3 C), 23.57 ppm. GPC (0.5 M acetic acid, 0.3 M Na_2SO_4 aqueous buffer): M_n = 87 000 g/mol, M_w = 144 000 g/mol, PDI = 1.7.

Synthesis of Polycyclooctene-*graft*-monolysine(Boc). Similar procedures were utilized to generate the monolysine derivative as used for the pentalysine analog. The polymerization was performed in 1.2 M 50/50 DCM/MeOH at room temperature for 3 h and was catalyzed by Grubbs Generation III catalyst (50:1 monomer:catalyst). The resulting polymer was precipitated from diethyl ether, isolated in 89% yield, and characterized by ^{1}H and ^{13}C NMR and THF GPC. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (DMSO = 2.50 ppm) 12.39 (br s, 1 H), 8.72 (br s, 1 H), 7.29 (br m, 1 H), 6.37 (br s), 5.32 (br s, 2 H), 4.15 (br s, 1 H), 2.88 (br s, 2 H), 2.21 (br s, 1 H), 0.9−1.89 (complex br m, 25 H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 174.89, 173.87, 155.56, 129.91. 129.63, 77.33, 51.53, 45.06, 32.99, 32.68, 32.33, 32.15, 30.53, 30.06, 28.99, 28.46, 28.27, 26.81, 24.78, 22.85 ppm. GPC (THF, PEG standards): M_n = 7500 g/mol, M_w = 11 000, PDI = 1.5. GPC (THF, PMMA standards): M_n = 13 000 g/mol, M_w = 18 000, PDI = 1.4.

Synthesis of Polycyclooctene-*graft*-monolysine (Poly2). **Poly2** was deprotected according to the same procedure used for **poly1** and isolated as an off-white solid in 83% yield. ^{1}H NMR (D_2O, 400 MHz): δ (H_2O = 4.79 ppm) 5.44 (br s, 2 H), 4.39 (br s, 1 H), 3.75 (br m, 3 H), 3.00 (br m, 2 H), 2.40 (br s, 1 H), 1.20−2.15 (complex br m, 16 H) ppm. ^{13}C NMR (D_2O + 2 drops of DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 175.72, 131.82, 54.35, 54.02, 47.28, 40.74, 33.63, 31.45, 27.88, 23.99 ppm.

Synthesis of Polycyclooctene-*graft*-pentalysine(Boc)-*co*-polycyclooctene-*graft*-PEG. In a representative polymerization, **1** (0.52 g, 0.40 mmol) and **3** (0.49 g, 0.40 mmol) were copolymerized at room temperature with Grubbs Generation III catalyst (50:1 monomer:catalyst) in 0.6 M 50/50 DCM/MeOH. The reaction proceeded for 3 h under nitrogen and was quenched with ethyl vinyl ether. The polymer was precipitated from diethyl ether, isolated by filtration, and freeze-dried. The Boc-protected polymer was isolated in 71% yield as an off-white solid. The resulting material was analyzed by ^{1}H and ^{13}C NMR and was determined to be composed of 70/30 mol % pentalysine(Boc)/PEG1200. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (DMSO = 2.50 ppm) 12.49 (br s, 1 H), 7.65−8.14 (br m, 5 H), 6.69 (br s, 5 H), 6.38 (br s), 5.33 (br m, 4 H), 4.55 (br s, 2 H), 4.05−4.38 (br m, 5 H), 3.10−3.79 (complex br m, 114 H), 2.85 (br m, 10 H), 0.98−2.32 (complex br m, 98 H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 173.42, 171.50, 171.20, 155.51, 129.94, 77.30 (5 C), 72.35, 70.15, 69.80, 67.51, 60.22, 52.22, 51.76, 31.83, 30.73, 29.14, 28.26 (15 C), 22.66 (5 C), 22.50 ppm. GPC (THF, PEG standards): M_n = 36 000 g/mol, M_w = 61,000, PDI = 1.7.

Synthesis of Polycyclooctene-*graft*-pentalysine-*co*-polycyclooctene-*graft*-PEG (Poly3). The Boc-protected copolymer was deprotected and purified using the same conditions as **poly1**. The final product was an off-white solid and obtained in 93% yield. ^{1}H NMR (D_2O, 400 MHz): δ (H_2O = 4.79 ppm) 5.44 (br s, 4 H), 4.38 (br s, 5 H), 3.08−3.20 (m, 2 H), 2.85−3.08 (m, 10 H), 1.18−2.52 (complex br m, 53 H) ppm. ^{13}C NMR (D_2O + 2 drops DMSO-d_6, 100 MHz): δ (DMSO = 39.52 ppm) 175.67, 175.37 (2 C), 175.15, 73.32, 71.77, 71.15, 61.97, 55.16, 54.51, 40.80, 32.25, 31.37, 27.97, 27.87, 23.72, 23.61 ppm. GPC (0.5 M acetic acid + 0.3 M Na_2SO_4 aqueous buffer): M_n = 45 000 g/mol, M_w = 91,000, PDI = 2.0.

Light Scattering Studies: Solution Preparation. NaCl solutions with concentration of 0.1 and 0.5 M were prepared by dissolving corresponding amounts of NaCl pellets in water purified by a Milli-Q UF system (Millipore, Billerica, MA) with a resistance of 18.2 Ω. To adjust the pH of a solution, HCl (diluted from concentrated solution) was used. Stock solutions were prepared by dissolving the polymer in the NaCl solutions and were then allowed to equilibrate at room temperature for at least 24 h prior to further dilution. Light scattering samples for each polymer were prepared at 3−4 concentrations, ranging from 0.78 to 5.85 g/L. **Poly1** was analyzed at concentrations of 1.46, 2.93, 5.85 g/L (0.1 M NaCl aqueous solution) and 1.41, 2.83, and 5.65 g/L (0.5 M NaCl aqueous solution). At lower salt concentrations, **poly2** was characterized at solution concentrations of 0.78, 1.77, and 3.53 g/L and 1.45, 2.93, and 5.65 g/L for 0.5 M NaCl. Because of its high scattering abilities at low concentrations, **poly3** was studied by light scattering at four concentrations: 0.56, 1.12, 2.23, and 4.46 g/L (0.1 M NaCl) and 0.35, 0.80, 1.60, and 3.20 g/L (0.5 M NaCl).

Light Scattering Studies: Static and Dynamic Light Scattering. For static light scattering experiments, the scattering intensity of a toluene solution was first used as the standard. Sample solutions were directly filtered into precleaned cuvettes by a syringe equipped

with a 0.22 μm diameter membrane. Static light scattering was carried out in the angle range of 35−135° with three readings at each angle. d*n*/d*c* measurements were made on a refractometer (RM-102 differential refractometer, Ostuka Electronics) and used to calculate absolute M_w for **poly1** and **poly2**. In 0.1 M NaCl, the d*n*/d*c* values of **poly1** and **poly2** were measured as 0.18 and 0.22. **Poly3** aggregated in solution at the concentration range used to determine d*n*/d*c*, and therefore, its d*n*/d*c* in 0.1 M NaCl was estimated as 0.20.

In dynamic light scattering studies, the scattering intensity autocorrelation, $g^2(t)$, was recorded and analyzed by CONTIN algorithm[46] to generate a relaxation spectrum where a single dominant peak was identified. Its corresponding relaxation time, $1/\tau$, was then plotted against scattering vector square, q^2. The slope of a linear fit of $1/\tau$ vs q^2 yielded the diffusion coefficient of the polymer at that specific concentration, $D(c)$. The diffusion coefficient of the polymer at zero concentration, D_0, was obtained by extrapolating the $D(c)$ vs c curve to $c = 0$ with the relationship of $D(c) = D_0(1 + kc)$ where k is a constant. To determine the hydrodynamic radius R_h, the Stoke-Einstein equation, $D_0 = k_BT/6\pi\eta R_h$ was applied where k_B is the Boltzmann constant, T represents temperature, and η is the solvent viscosity at T.

For DNA complexation experiments, a dilute solution of **poly1** (1 mL of 0.5 mg/mL) was mixed with dsDNA (50 μL of a 0.3 mg/mL calf thymus DNA from Sigma-Aldrich, catalog number D1501). Concentrated aqueous NaOH was then added to this mixture to give pH ~11. Subsequently, aqueous HCl was added stepwise in 1−5 μL integrals. Dynamic light scattering data was collected following equilibration at room temperature for at least 10 min. The scattering angle was fixed at 90° and the temperature was 25 °C. For imaging with atomic force microscopy (Digital Instrument, Nanoscope IIIa), the mixture solution was spin-cast (rpm 2000) onto a freshly cleaved mica surface, then transferred to the microscope stage. AFM micrographs were obtained in tapping mode under ambient conditions.

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Supporting Information Available: Figure S.1, ^{1}H NMR spectrum of boc-protected **poly3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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