Hydrophilic short single-stranded nucleic acid grafts. This composed of a hydrophobic poly(2-oxazoline) backbone and synthesis of an amphiphilic comb/graft DNA copolymer versatile self-assembling macromolecules, we report herein the molecular self-assembly of DNA copolymers and achieve highly materials with potential applications in various process. Copolymers organize into functional nanostructured macromolecular properties to the ensemble inherent to this biomedicine, biomaterials engineering, or catalysis. Keeping in mind future developments in biology and medicine, copolymers have thus been designed to assemble structures that might eventually enable the manipulation and comprehension of a biochemical mechanism to solve biological or medical issues in the future.

Along this line, the synthesis and self-assembly of copolymers containing a segment composed of a polypeptide, a polysaccharide, or a nucleotide sequence have been reported. The synthesis of linear self-assembling DNA copolymers is usually achieved according to either conventional organic routes or molecular biology techniques by solid-phase-supported synthesis. To advance the understanding of the molecular self-assembly of DNA copolymers and achieve highly versatile self-assembling macromolecules, we report herein the synthesis of an amphiphilic comb/graft DNA copolymer composed of a hydrophobic poly(2-oxazoline) backbone and hydrophilic short single-stranded nucleic acid grafts. This macromolecular architecture is of high interest because the formation of structures of higher order might be induced in a bottom-up approach from the molecular scale to the third dimension.

1. INTRODUCTION

Copolymer self-assembly is an elegant route to form structures with sizes in the submicrometer range in solution and on surfaces. Of high interest, though, is the propagation of the macromolecular properties to the ensemble inherent to this process. Copolymers organize into functional nanostructured materials with potential applications in various fields such as biomedicine, biomaterials engineering, or catalysis. Keeping in mind future developments in biology and medicine, copolymers have thus been designed to assemble structures that might eventually enable the manipulation and comprehension of a biochemical mechanism to solve biological or medical issues in the future.

Along this line, the synthesis and self-assembly of copolymers containing a segment composed of a polypeptide, a polysaccharide, or a nucleotide sequence have been reported. The synthesis of linear self-assembling DNA copolymers is usually achieved according to either conventional organic routes or molecular biology techniques by solid-phase-supported synthesis. To advance the understanding of the molecular self-assembly of DNA copolymers and achieve highly versatile self-assembling macromolecules, we report herein the synthesis of an amphiphilic comb/graft DNA copolymer composed of a hydrophobic poly(2-oxazoline) backbone and hydrophilic short single-stranded nucleic acid grafts. This macromolecular architecture is of high interest because the formation of structures of higher order might be induced in a bottom-up approach from the molecular scale to the third dimension.

Poly(2-oxazoline)s, in general, are considered for usage in biomedical applications. Poly[2-(3-butenyl)-2-oxazoline] (PBOX) is particularly interesting because of its low glass-transition temperature (~10 °C) and vinyl double bonds, which are susceptible to functionalization via thiol-ene photochemistry. Like poly(2-isobutyl-2-oxazoline) and poly(2-nonyl-2-oxazoline), PBOX exhibits an upper critical solution temperature (UCST) solution behavior in ethanol–water mixtures. The polymer is soluble in a mixture of ethanol–water 55:45 w/w above 44 °C, whereas below this temperature, phase separation and crystallization occur with the spontaneous formation of insoluble microspheres. (See Scheme 1.)

The crystallites are physically cross-linked polymer chains, which dissolve upon exposure to acid, for example, trifluoroacetic acid (TFA), without chain scission. Here we took advantage of this crystallization process in combination with the possibility to perform thiol-ene photochemistry to graft DNA.
chains onto the PBOX and synthesize a DNA molecular brush. Because the nucleic acid strands are modified at the 5′-end through a hexyl spacer by a thiol group, these are grafted onto the PBOX microspheres through a one-step reaction prior to dissolution in TFA. As demonstrated in the following, the grafting efficiency reached 67% in agreement with steric hindrance owing to molecular crowding of single-stranded nucleic acid strands grafted to the polymer backbone. The molecular brushes (released from the microspheres by treatment with TFA) organize in aqeous solution into spherical structures with sizes of ~300 nm. The nucleic acid strands remain functional subsequent to coupling, and stable structure formation enables speciation between complementary and non-complementary nucleotide sequences present in the surrounding.

2. MATERIALS AND METHODS

2.1. Materials. Sodium chloride (>99.5%) and magnesium chloride (99.9%) were purchased from Acros Organics (Geel, Belgium), and sulfuric acid (96%) was purchased from Fisher Scientific SA (Wohlen, Switzerland). S′-CTCTCTCTCTTTT-3′ (S′- (CT)₂T₂-3′-DNA) modified at the 5′-end through a C₆ hexyl spacer with a thiol group, S′-AAAAAAGAGAGAG-3′ (S′-A₆(AG)₃-3′, “complementary DNA”), C₅S₅-(CT)₂T₂-3′, and FITC-C₅S₅-(AG)₃-3′ were purchased from Microsynth Laboratory (Balgrush, Switzerland). C stands for cytosine, T for thymine, G for guanine, and A for adenine. Poly(ethyleneimine) (PEI, nominally 40 000 g mol⁻¹) was purchased from Polysciences (Eppelheim, Germany). TCEP (tris(2-carboxyethyl)phosphine) was purchased from Roth AG (Arlsheim, Switzerland). Ethanol (98%), TFA (29%), tetrahydrofuran (≥99.9%), hydrogen peroxide (50%, diluted prior to usage), and dialysis tubing were ordered from Sigma-Aldrich (Buchs, Switzerland).

PBOX was prepared by cationic ring-opening isomerization polymerization of 2-(3-butenyl)-2-oxazoline, as described in the literature. The isolated PBOX chains had an average length of 43 repeat units, corresponding to a number-average molar mass (Mₙ) of 5380 g mol⁻¹, by ¹H NMR end group analysis, and a dispersity index (ratio of weight-over-number-average molar mass, Mₙ/Mₚ) of 1.05 by size exclusion chromatography (SEC). LCR 0.22 μm pore size syringe filters were purchased from Merck Millipore (Zug, Switzerland). Zeta cells were provided by Instrumat SA (Lausanne, Switzerland). Silicon wafers single side polished, orientation N/Phos <100>, (625 ± 25) μm thickness, 1–50 Ohm cm resistivity were purchased from Silchek (Freiburg, Germany). Mica was purchased from Plano (Wetzlar, Germany). The uranyl acetate staining solution and Mesh Copper Grids for transmission electron microscopy were purchased from Electron Microscopy Sciences (Hatfield, PA). Milli-Q water was used in all cases.

2.2. Synthesis of the DNA Molecular Brush. A 0.5 wt % polymer solution (0.12 mg) in an ethanol/water mixture (55:45 w/w) was dissolved at 60 °C prior to being cooled below the UCST and stirred at room temperature for 3 days. Subsequently ethanol is allowed to evaporate overnight. Thiol modification was then conducted in the same manner, as previously reported. In brief, 1 mg of thiol DNA is added to the solution of microspheres ([SH]/[C=C]₀ = 0.28) together with 75 μg of TCEP. TCEP was added to reduce DNA-S-S-DNA to 2 DNA-SH. Subsequent to degassing by bubbling argon for 5 min, the solution was exposed to UV light (λ = 305 nm, 24 h). The product was then purified by dialysis (MWCO 14000 Da) and SEC chromatography. Self-assembly was induced by direct dissolution of the product in water.

2.3. Analytical Instrumentation and Methods. ¹H NMR measurements were carried out at room temperature using a Bruker DPX-400 spectrometer operating at 400.1 MHz. ATR-IR spectra were recorded on a Bruker VERTEX 80v spectrometer with a liquid-nitrogen-cooled narrow-band mercury-cadmium telluride (MCT) detector; 1 mg mL⁻¹ sample solutions were spread on a Germainium plate and left for drying for 30 min. The germanium background was recorded prior to sample analysis. X-ray diffraction measurements were performed on a SuperNova setup (Oxford Diffraction, U.K.) using the Cu Kα radiation source (λ = 1.5418 Å). An Abbemat reflectometer WR MW (Anton Paar, Germany) was used to determine the refractive index increment (dn/dc = 0.0082 cm g⁻¹) of the PBOX-g-DNA copolymer. UV–vis spectra were registered on a Varian Cary-50 in a quartz cuvette of 1 cm path length for all measurements in the same manner as previously reported. SEC of PBOX was performed on a system with simultaneous UV (λ = 270 nm) and RI detection using N-methyl-2-pyrrolidone (NMP + 0.5 wt % LiBr) as the eluent at +70 °C, flow rate: 0.8 mL min⁻¹, and a column set of two 300 × 8 mm² PSS-GRAM 7 μm spherical polyester particles with porosities of 10² and 10³ Å. Calibration was done with polystyrene standards. Aqueous SEC with PBOX-g-DNA was performed on a Viscotec GPCmax system (Viscotek, Houston, TX) with differential refractive index, right-angle light scattering (RALS), and low-angle light scattering (LALS) detectors. A 0.3% aqueous solution of sodium azide was used as the eluent at 35 °C, flow rate: 1.0 mL min⁻¹. Separation was achieved using two Viscotec columns (ViscoGEL column, TSK-GEL GMRW XL products 300 × 7.8 mm²). Calibration was done with polystyrene sulfonate standards (Polymer Standards Service, Mainz, Germany). Concentration- and angle-dependent light-scattering studies were conducted on the multilayer ALV-CGS-8 goniometer (Langen, Germany). This device is equipped with eight detectors and a solid-state laser operating at λ = 532 nm (VerdiV2, Coherent). For all measurements, low-volume borosilicate quartz cuvettes were used, which were cleaned in a mixture of H₂SO₄:30% aqueous H₂O₂:3:1 v/v prior to any measurement. Dilution series were prepared by sequential dilution of the initial 1 mg mL⁻¹ aqueous solutions of the copolymer. All experiments were carried out at a temperature of 25 °C. The apparent hydrodynamic radius (cumulant analysis) and apparent radius of gyration (Berry analysis) were evaluated according to conventional routes. For atomic force microscopy (AFM), 20 μL of sample was dropcasted on a mica surface, gently washed with ethanol, and dried under nitrogen. For single-molecule imaging of the copolymer, mica surfaces were dipped overnight in MgCl₂ (30 mM) and afterward extensively washed to allow attachment of DNA molecules to the surface by double-charged ions subsequent to spin coating. The self-assembled structures were dissolved in a small volume of TFA and subsequently diluted in THF/EtOH (1:1) mixture to obtain 5 × 10⁻⁸ g L⁻¹ final concentration of copolymer. Twenty μL
Subsequently, 0.1 nmol of the complementary sequence (0.75 μM of DNA (in a 50 mM NaCl solution)) was added to a solution of the self-assembled molecular brush (containing 3 g L⁻¹) because the outer layer of the self-assembled structures is composed of the negatively charged DNA sequences. In brief, the solution of PEI was spread on the microscopy glass slide and left for adsorption for 5 min. Afterward, the glass surface was extensively washed with Milli-Q water, and 10 μL of the solution containing the mixture of stained nucleotide sequences was spread on the glass slide, covered by the cover glass and observed simultaneously by CLSM (Zeiss LSM 700 confocal microscope; lens 100× 1.4 NA) at two different excitation wavelengths with suitable filters (FITC ex. 488 nm, em. 520 nm; Cy5 ex. 639 nm, em. 679 nm). Subsequently, the remaining solution was centrifuged (15 min, 14,000 rpm) and the supernatant was removed to get rid of the sequences that did not hybridize. The self-assembled structures were subsequently suspended again and washed with water three times (by centrifugation). Ten μL of this solution was dropped on a glass slide and covered with a cover glass for imaging by CLSM. To quantify the speciation, we conducted fluorescence spectroscopy in parallel to CLSM. Fluorescence intensities of FITC (ex. 485 nm) and Cy5 (ex. 639 nm) were recorded, keeping the same slit aperture for all measurements. For all solutions, a 50 mM NaCl aqueous solution was used. Encapsulation and release of a fluorescent dye was monitored by fluorescence spectroscopy. The water-soluble fluorescein dye was selected to assess the hollow sphere morphology of the self-assembled PBOX-g-DNA. Imaging was performed by CLSM before and subsequent to the dye release, which was triggered by the addition of a 2% aqueous solution of sodium azide. The release time course was recorded by fluorescence spectroscopy. In brief, 1 mg of PBOX-g-DNA copolymer was dissolved in 50 μg mL⁻¹ fluorescein aqueous solution to encapsulate the fluorescent dye during the spheres formation. The free dye was removed by 4 days dialysis against water (MWCO 14000). The time course of dye release was recorded with a Horiba Jobin Yvon Fluoromax 2 (New York). Toward this end, 90 μL of sample was placed in a quartz cell (QS 3.0 mm, Hellma Analytics), to which 10 μL of 2% sodium azide was added during the measurement to induce disassembly of the spheres and monitor the release time course (485/512 with an integration time of 0.5 s, standard error of 1%, for a duration of 900 s). For the control measurement, 1 mg of PBOX-g-DNA was added to 1 mL of 0.5 mg mL⁻¹ Nile Red solution dissolved in methanol. The resulting solution was dialyzed against methanol/water mixture and water for 5 days to induce self-assembly as well as to remove the free dye. Samples were as well imaged by CLSM prior and subsequent to disassembly by the addition of sodium azide subsequent to dye encapsulation at a concentration of 2 μg mL⁻¹.

For isindol synthesis, 20 nmol of S′-A2(AG)5-T2(CS)3-NH2 (200 μL, 100 μM) and 20 nmol of S′-A2(AG)5-T2(CS)3-NH2 (200 μL, 100 μM) were added to a solution of the self-assembled molecular brush (containing 40 nmol of S′-T2(CT)3-NH2) mixed and incubated at 4 °C for 30 min (total volume 1 mL, 50 mM NaCl solution). Afterward, 40 nmol (1.3 mg) of o-phthalaldehyde was added to the solution and left in a light-
protected place under stirring for 24 h. Fluorescence spectra were recorded after 30 min and 24 h. The control consists of a comparable experiment in the absence of the self-assembled brush: no fluorescence signal increase could be detected. Hellma Ultra micro cuvettes 3 x 3 mm² were used for all fluorescence spectroscopy measurements. All spectra were recorded on a Horiba Jobin Yvon Fluoromax 2 (New York).

3. RESULTS

The nucleotide sequences are purchased desalted of HPLC purity grade. The nucleic acid strand is a model linear sequence of melting temperature (34 °C in 50 mM NaCl) higher than the working temperature (20 °C), which does not self-hybridize and assemble a thermodynamically stable double helix with its complementary sequence. Like poly(2-isobutyl-2-oxazoline) and poly(2-nonyl-2-oxazoline),7 PBOX (average number of repeat units, 43) is soluble in a mixture of ethanol−water 55:45 w/w above 44 °C, whereas below this temperature phase separation and crystallization occur with the spontaneous formation of insoluble microspheres (insert in Scheme 1). The crystallinity of the freeze-dried sample was verified by X-ray diffraction (Figure S1 in the Supporting Information), which shows two predominant sharp peaks at 2θ ≈ 12° (d spacing ≈ 7.4 Å) and 2θ ≈ 23° (d spacing ≈ 3.8 Å), peaks of lower intensity at 2θ ≈ 14° (d spacing ≈ 6.3 Å), 2θ ≈ 28° (d spacing ≈ 3.1 Å), and 2θ ≈ 31° (d spacing ≈ 2.8 Å), and a broader peak at 2θ ≈ 19° (d spacing ≈ 4.6 Å).

Thiol-terminated nucleotide sequences were added to a solution of the crystalline PBOX microspheres ([SH]₀/[C=O]₀ = 0.28) in degassed water and irradiated with UV light (λ = 305 nm) over 24 h. Chemical characterization was performed subsequent to dialysis and SEC to remove any nonreacted product, in particular, DNA fragments.

The success of the coupling reaction and the purity of the PBOX-g-DNA brush copolymer product (absence of PBOX or DNA fragments) could be confirmed by FT-IR and SEC analyses (Figure 1, Table S1 in the Supporting Information). As compared with the pristine PBOX, the intensity of the band at 912 cm⁻¹, attributable to the stretching of vinyl groups, is much lower in the PBOX-g-DNA copolymer. In addition, a peak at 1060 cm⁻¹ appears in the spectrum of the copolymer, which corresponds to the C−N stretching of the amines present along the nucleotide sequence. Quantitative analysis of the PBOX-g-DNA copolymer by UV−vis spectroscopy (brief description in Figure S2 in the Supporting Information) shows that the grafting density is ∼20%, or, in other words, on average, every sixth unit along the PBOX backbone carries a nucleotide sequence. SEC further reveals a copolymer of low polydispersity (23 000 Mᵢ, 24 800 M₉₀, 1.08 PDI).

The structure formation of the resulting PBOX-g-DNA molecular brushes into spherical structures takes place in aqueous solution. Both scanning and transmission electron microscopy reveal the formation of structures between 100 and 400 nm (Figure 2). Structure formation is induced by direct dissolution in aqueous solution under stirring. This method usually leads to the formation of structures of wide size distribution, which is reduced by sequential extrusion through membrane filters to perform light-scattering investigations.¹²

To achieve both sizing and refine the morphological determination, we combined multiance dynamic and static light scattering (Figures S3−S5 in the Supporting Information)
ciency was monitored between the pristine complementary sequences in solution (Figure S7D in the Supporting Information). With the nucleic acid strands under investigation, no self-hybridization could be observed (data not shown).

Further evidence that the nucleic acid strands involved in structure formation are functional is gathered by the fact that the PBOX-g-DNA structures could be used as 3D scaffolds to carry out organic reactions. We resorted to an approach previously reported.13 In brief, hybridization between the nucleic acid strands involved in the PBOX-g-DNA self-assembly and complementary sequences modified either with an amine or a thiol group is induced to produce isoinodiol by the addition of free o-phthalaldehyde (Figure S8A in the Supporting Information). As can be seen in Figure S8B in the Supporting Information, the fluorescence of the reaction product could be monitored by spectroscopy. No fluorescence signal could be detected in the absence of the self-assembled structures. The intensity increases with reaction time, which corresponds to the formation of the compound with higher fluorescence quantum yield. This outcome further confirms that the nucleic acid sequences, subsequent to grafting and structure formation, remain functional at the interface between the aqueous surrounding and the self-assembled structures.

Because the hydrophilic weight fraction, the charge, as well as the rigidity of the macromolecule are increased, a morphological transition could have been observed upon hybridization. However, structures of larger size (949 ± 70 nm) are observed upon the formation of the double helix between the complementary sequences (Figure 4), which is indicative of the stability of the self-assembled spherical structures. The structures swell, as evidenced by the release of an encapsulated dye (data not shown) but retain their morphology.

This stability of the self-assembled structures against hybridization further enabled the assessment of the potential of these sub micrometer size spheres as sensing tools for nucleic acid strands speciation, which was carried out through the combination of CLSM and fluorescence spectroscopy. A solution containing two nucleotide sequences varying in their composition stained with fluorescent dyes emitting at different wavelengths have been incubated with the PBOX-g-DNA self-assembled structures. The complementary sequence is labeled with FITC, whereas the non complementary strand is labeled with Cy5. After sample incubation at 4 °C, the mixture was imaged under CLSM and the fluorescence intensity was monitored (Figure S9 in the Supporting Information). As can be seen in Figure S5A,C, the complementary sequences engaged in the PBOX-g-DNA structure formation do hybridize (green glow), whereas the non complementary sequences remain in the background, which shines in red. These could be simply separated by centrifugation and washing steps (Figure S5B, Scheme S1 in the Supporting Information). The green labeled spherical structures subsequent to hybridization solely remain.

4. DISCUSSION

Amphiphilic DNA block copolymers are usually achieved by grafting to nucleic acids synthesized by solid-phase-supported synthesis (SPS) on controlled pore glass (CpG).30 To prevent tedious synthesis and purification steps, coupling is performed prior to cleavage of the amphiphilic block copolymer from the porous phosphoramidite CpG resin. However, limited diffusion of the macromolecules through the pores of the phosphoramidite solid phase limits both the efficiency of the reaction up to ∼30% and the choice of the polymer to molecular weights up to ∼5000 Da.14 The polymer crystallization-assisted thiol-ene synthesis of a polymer-graft-DNA molecular brush described herein enables achieving reaction efficiencies above 60% without tedious synthesis routes or purification steps. The polymer crystallites are of smaller size than the conventional beads used in automated SPS synthesis. The interfacial area available for reaction is therefore higher in comparison with structures of larger size. In addition to the inherent efficiency of thiol-ene photosynthesis, the reactive PBOX vinyl groups might be located at the outer surface of the crystallites and the diffusion of the short single-stranded synthetic nucleotide sequences through the physically cross-linked polymer structures might be less limited than through the pores of a phosphoramidite solid support.15 The latter hypothesis is strongly supported by the degree of grafting of nucleic acid strands to the PBOX backbone. According to steric hindrance, eight nucleotide sequences of ∼3 nm Flory radius16 at best could be grafted along a 24 nm long PBOX polymer backbone, which is in agreement with the grafting density we quantified through UV spectroscopy.

Subsequent to coupling of the nucleic acid grafts to the PBOX backbone, crystallization could no longer be induced. The resulting macromolecule is a nonlinear comb/grafted amphiphilic macromolecule (Scheme 2). Unfortunately, high-resolution AFM did not enable imaging single molecular brushes, which have the tendency to collapse on the surface (Figure S10B in the Supporting Information). This macromolecule self-assembles into ∼150 nm radius spherical structures in aqueous solution. Although the hydrophilic weight fraction (∼90%) is high, the factor $R_C/R_H = (1.25 ± 0.07)$ is above one, which is indicative of the formation of hollow spheres or elongated structures.10a The fact that the PBOX-g-DNA self-assembled structures might undergo interaction owing to the negative charges of the nucleic acid sequences should also be taken into consideration to interpret the light-scattering data (Berry analysis of the SLS data) and the resulting $\rho$-factor value. The formation of compound micelles cannot be ruled out. However, some indirect evidence points toward the formation of vesicular structures. Imaging shows the formation of spherical particles. Light-scattering investigations enable sizing of particles of low size distribution and assessing an aggregation number of ∼20 000 PBOX-g-DNA molecules within each spheres, which points toward the formation of vesicular structures. Indeed, the molecular area occupied by a DNA molecule within a bilayer, as estimated experimentally, 8 nm², is in close agreement with the theoretical value.16 These
results indicate that about half of the nucleotide sequences are pointing toward the aqueous surrounding, and the other half is hindered within the structure. This hypothesis is further supported by the hybridization efficiency, estimated to 30%. Solely, the nucleotide sequences that are pointing toward the surrounding are available for hybridization, ~50% statistically. It may be that some nucleotide sequences are not accessible for hybridization because of steric hindrance upon structure formation. A molecular area above 7 nm² is indeed needed for optimal hybridization efficiency of nucleic acid sequences tethered on a surface. Nevertheless, the vesicular morphology is supported by TEM, which shows the presence of spheres characterized by a lighter inner core than the background and successful encapsulation of a water-soluble fluorescent dye, not possible with a hydrophobic probe.

Because the structures are composed of a PBOX membrane-like layer surrounded by a corona of the nucleic acid strands that point toward the inner aqueous pool and the surrounding medium of the vesicular structure, upon hybridization, the size increases and release of an encapsulated water-soluble dye takes place. Self-assembly does not take place when the process is induced in a solution of the complementary sequence. No morphological transition or disassembly could be observed up to the addition of 130% of complementary sequences in the surrounding (Figure 4). The large molecular weight of the copolymer (30 000 Da) infers mechanical stability to the self-assembled structures owing to the low chain mobility of the copolymer. The stability might be further supported by intramolecular hydrogen bonds between the nucleic acid grafts engaged in structure formation (Scheme 2). Upon hybridization, only the nucleotide sequences pointing toward
the aqueous surrounding are solicited. The ones pointing toward the inner aqueous pool might maintain their reinforcing interaction to preserve the structures.

5. CONCLUSIONS

Overall, these experimental results demonstrate that the ability of the polymer to crystallize into microspheres could be used to carry out the straight synthesis of a PBOX-g-DNA molecular brush and induce structure formation into submicrometer size spherical structures that are stable against hydrogen bonding, as evidenced by demonstrating the stability of the structures in the presence of nucleic acid strands in the surrounding. These could therefore be used for the speciation of complementary and non complementary strands both present in the surrounding. Although the formation of compound micelles cannot be ruled out, some indirect evidence points toward the formation of vesicular structures.

■ ASSOCIATED CONTENT

* Supporting Information
Atomic force microscopy imaging, UV−vis, FTIR and fluorescence spectroscopy, light scattering supporting data, and illustrative schemes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
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■ ABBREVIATIONS

A, adenine; AFM, atomic force microscopy; ATR-IR, attenuated total reflectance infrared spectroscopy; AUC, analytical ultracentrifugation; C, cytosine; CLSM, confocal laser scanning microscopy; DNA, deoxyribonucleic acid; FITC, fluorescein isothiocyanate; FOA, free oscillation amplitude; G, guanine; 1H NMR, proton nuclear magnetic resonance; LCR, low compound retention; \( M_n \), number-average molar mass; \( M_w \), weight-average molar mass; MWCO, molecular weight cutoff; NMP, N-methyl-2-pyrrolidon; PBOX, poly(2-(3-butenyl)oxazoline); PEI, poly(ethylenimine); RI, refractive index; SEC, size-exclusion chromatography; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TCEP, \((\text{tris(2-carboxyethyl)}\text{phosphine)}\); T, thymine; TFA, trifluoroacetic acid; UCST, upper critical solution temperature; UV, ultraviolet; UV−vis, ultraviolet−visible spectroscopy; WAXS, wide-angle X-ray scattering

■ REFERENCES


