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Polypeptide- and Protein-Based Conjugate Nanoparticles via Aqueous Ring-Opening Polymerization-Induced Self-Assembly (ROPISA)

Hannah Beuseroy, Chloe Grazon, Segolene Antoine, Mostafa Badreldin, Pedro Salas-Ambrosio, Simon Harrisson, Elisabeth Garanger, Sebastien Lecommandoux,* and Colin Bonduelle*

Protein–polymer conjugates and polymeric nanomaterials hold great promise in many applications including biomaterials, medicine, or nanoelectronics. In this work, the first polymerization-induced self-assembly (PISA) approach performed in aqueous medium enabling protein–polymer conjugates and nanoparticles entirely composed of amino acids is presented by using ring-opening polymerization (ROP). It is indeed shown that aqueous ring-opening polymerization-induced self-assembly (ROPISA) can be used with protein or peptidic macroinitiators without prior chemical modification and afford the simple preparation of nanomaterials with protein-like property, for example, to implement biomimetic thermoresponsivity in drug delivery.

weight and compositions, and have been applied as drug delivery agents,^[1,2] biomaterials,^[3] and macromolecular therapeutics.^[4] Indeed, polypeptides are already used in pharmaceutical applications with products both on the market and in advanced clinical trials.^[5] Polypeptide-based materials combine advantageous features of synthetic polymers (solubility, processability, rubber elasticity, etc.) with those of natural proteins (secondary structure, functionality, biocompatibility, etc.).^[6,7] Polypeptides can be precisely designed to fit a particular function, while the combination of ring-opening polymerization

(ROP) and self-assembly approaches has been used to prepare nanomaterials with unprecedented properties.^[8,9]

ROP of amino acid *N*-carboxyanhydrides (NCA) is a widely used method for polypeptide synthesis.^[10] Until recently, NCA polymerization was limited by tedious monomer purification steps^[11] and significant sensitivity to moisture.^[12] Indeed, any water in the reaction medium can lead to hydrolysis of the NCA and uncontrolled polymerization.^[13,14] Recent research has focused on promoting the polymerization without the use of a catalyst, exploring more reactive initiators,^[15,16] developing new high-throughput approaches,^[17] and using aqueous-based processes including emulsion polymerization.^[18–20] We have recently reported the first examples of aqueous ring-opening polymerization-induced self-assembly (ROPISA) of NCA monomers in buffered aqueous solution with the hydrophilic macromolecular initiator α -amino-poly(ethylene glycol) (PEG–NH₂).^[21,22] Our work established that NCA hydrolysis and subsequent uncontrolled polymerization can be suppressed both by the formation of protective self-assembled structures and by reaction conditions (pH and solid contents) for which ring opening by the amine propagating species is faster than hydrolysis.

In this work, we present how aqueous ROPISA enables the direct use of polypeptide macroinitiators without prior chemical modification and under non-denaturing conditions, and provides efficient access to fully peptide-based block copolymers including protein–polymer conjugates. We demonstrate it with three different peptide-based macroinitiators of synthetic poly(sarcosine), (PSar), poly(L-proline) (PLP), or biological origin elastin-like polypeptide (ELP). Functional nanomaterials with polypeptide or

1. Introduction

Polypeptides are biomimetic polymers made of amino acid residues linked by native peptide bonds that are sequence-simplified protein analogs. They have well-defined molecular

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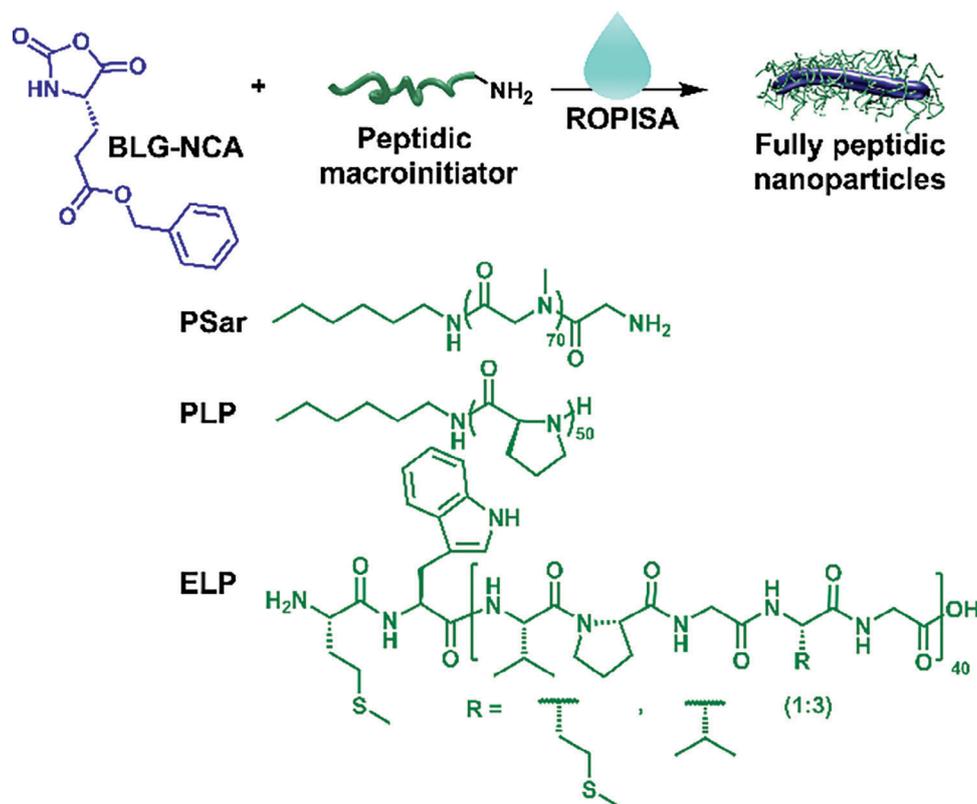
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Scheme 1. Nanomaterials made of amphiphilic diblock polypeptides synthesized in one-step by aqueous ROPISA. In green, chemical structure of employed peptidic macroinitiators using γ -benzyl-L-glutamate–NCA as monomer.

protein surfaces are produced (**Scheme 1**), whose retention of function is illustrated by the thermoresponsive behavior of nanomaterials stabilized by ELP and PLP. Overall, this work proves the feasibility of synthesizing fully biodegradable nanoparticles made of amino acids, without organic solvents, using a simple polymerization-induced self-assembly (PISA) approach in aqueous media.^[23]

2. Aqueous ROPISA Initiated by Poly(sarcosine)

We first implemented aqueous ROPISA with a poly(sarcosine) macroinitiator that displays physicochemical similarities to PEG (similar critical aggregation concentration and hydrodynamic volume in various solvents).^[24,25] This polymer was prepared by conventional ROP of sarcosine–NCA in *N,N*-dimethylformamide (DMF) (PSar, $M_n = 5.3 \text{ kg mol}^{-1}$, $\mathcal{D} = 1.05$; Schemes S1 and S2, Supporting Information). To avoid water-initiated oligomers, PSar was functionalized with glycine (PSar–NH₂; Figures S1–S4, Supporting Information) to present a similar primary amine terminal group as in our previous work using PEG. It was then mixed with 20 equivalents of γ -benzyl-L-glutamate *N*-carboxyanhydride (BLG–NCA) monomer ($[M]_0 = 0.14 \text{ M}$, $[M]_0/[I]_0 = 20$) in aqueous sodium bicarbonate buffer (pH 8.5, 50 mM) at 4 °C while stirring at >900 rpm (Scheme S6, Supporting Information). As a comparison, another polymerization was performed using α -amino-PEG ($M_n = 5.0 \text{ kg mol}^{-1}$, $\mathcal{D} = 1.08$). In both cases, aqueous ROPISA afforded dispersions of well-

Table 1. Macromolecular characteristics of diblock copolymers obtained by ROPISA with different macroinitiators.

Macroinitiator	M_n^{th} [kg mol ⁻¹] ^{a)}	τ_s [%] ^{b)}	M_n^{RMN} [kg mol ⁻¹] ^{c)}	\mathcal{D} ^{d)}	D_z [nm] (σ) ^{e)}
PEG	9.1	7	10	1.09	83 (0.26)
PSar–NH ₂	9.7	7	10.3	1.17	170 (0.39)
PLP	9.9	7	10.8	– ^{f)}	402 (0.25)
ELP	21.4	0.5	21.9	1.03	83 (0.15)

^{a)} Theoretical number average molar mass, ^{b)} Solid contents, ^{c)} Number average molar mass (M_n) determined by ¹H nuclear magnetic resonance (NMR), ^{d)} Dispersity (\mathcal{D}) determined by size exclusion chromatography (SEC), ^{e)} Z-average hydrodynamic diameter (D_z) and dispersity (σ) determined by dynamic light scattering, ^{f)} SEC characterization was not possible due to polymer insolubility.

defined diblock copolymers with narrow molar mass dispersities ($\mathcal{D} < 1.2$; **Table 1**; Scheme S5 and Figures S5–S8, Supporting Information) in 79–90% yield (Table S1, Supporting Information).

After dialysis against water to remove salts, the nanomaterial suspensions were studied using microscopy and light scattering techniques. It should be noted that dynamic light scattering (DLS) analysis cannot accurately describe anisotropic nanoparticles and has been performed to approximate nanoparticle size. Homogeneous nanoparticles with elongated morphologies were obtained in all cases (Table 1; Figures S9–S13, Supporting Information). Unlike PEG-*b*-PBLG nanoparticles, which exhibit

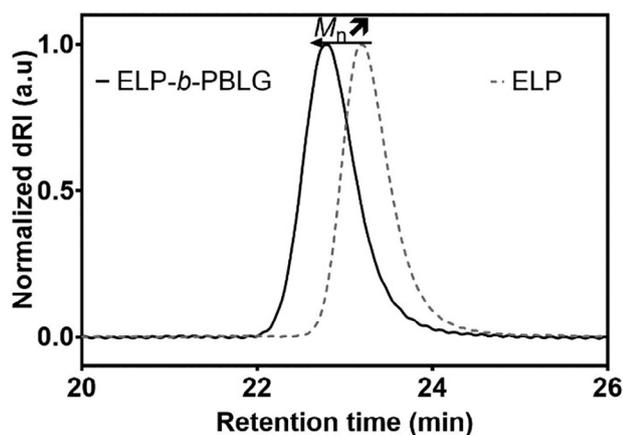


Figure 1. Size exclusion chromatogram (in DMF + 1% LiBr) of the pristine ELP and of the ELP-initiated peptide diblock copolymers obtained after ROPISA.

needle-like shapes by transmission electron microscopy (TEM) (rod-like morphology), PSar-*b*-PBLG gave apparently fewer rigid objects with a coiled ribbon morphology (Figures S12 and S13, Supporting Information).

3. Aqueous ROPISA Initiated by Thermoresponsive Polypeptides and Proteins

The successful ROPISA from PSar-NH₂ and PEG encouraged us to extend this approach to functional polypeptide or protein macroinitiators^[26] presenting lower critical solution temperature (LCST) behavior: poly(L-proline) and elastin-like polypeptide.^[27,28] Water-enhanced ROP of L-proline NCA was used to prepare PLP ($M_n = 5.4 \text{ kg mol}^{-1}$, $\mathcal{D} = 1.03$; Scheme S3 and Figures S14 and S15, Supporting Information), a synthetic polymer that exhibits temperature-driven aggregation in water with a hysteresis around 50 °C.^[27] In a further step toward protein-polymer conjugates, sequence-controlled ELP presenting a protein-like thermoresponsive character that preferentially results in the formation of coacervates^[29] was also employed as macroinitiator. It was recombinantly prepared using *Escherichia coli* as an expression system^[30] (ELP, $M_n = 17\,035 \text{ g mol}^{-1}$; Scheme S4 and Figure S16, Supporting Information). In both cases, aqueous ROPISA afforded solutions of well-defined copolymers with narrow molar mass dispersity in $\approx 90\%$ yield (Figures 1 and 2 and Table 1; Schemes S7 and S8, and Figures S17 and S18, Supporting Information). In the case of ELP, a solid content of only 0.5% was used to avoid aggregation and a less controlled process. Due to lack of solubility, size exclusion

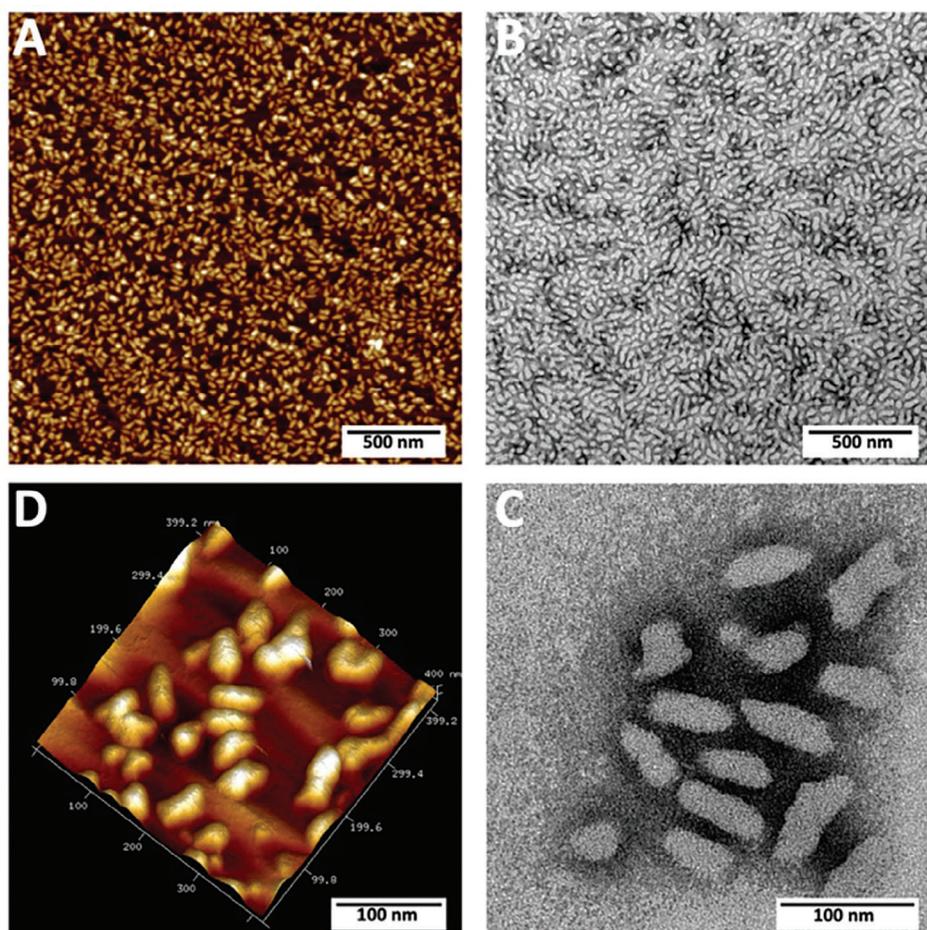


Figure 2. Fully peptidic nanoparticles obtained upon aqueous ROPISA initiated by ELP. A, D) Observed by atomic force microscopy (AFM). B, C) Observed by TEM, using uranyl acetate as a contrast agent.

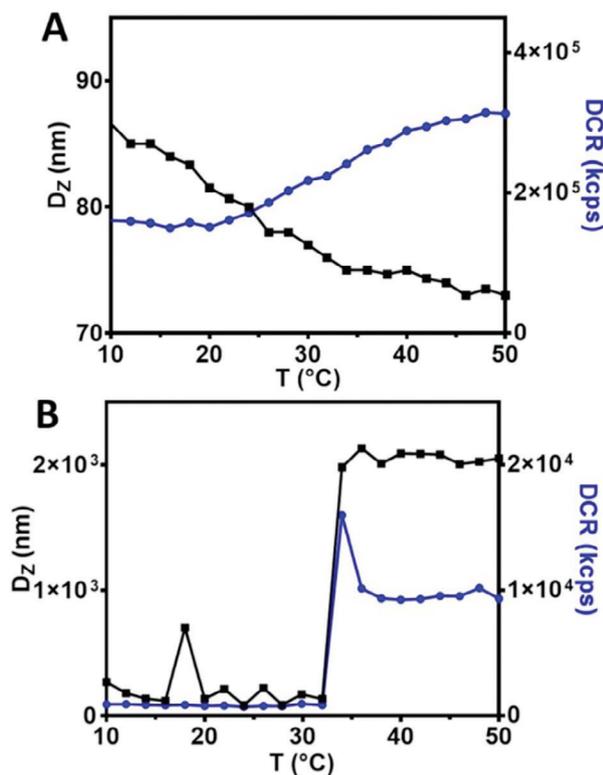


Figure 3. Evolution of the scattered light intensity with temperature of a solution of A) ELP-*b*-PBLG and B) ELP in pure water at 1 mg mL⁻¹.

chromatography (SEC) cannot be performed on PLP-*b*-PBLG copolymer, but Diffusion Ordered Spectroscopy (DOSY) analyses suggest that poly(L-proline) correctly initiates the ROP (Figure S19, Supporting Information). Overall, the ROPISA of BLG-NCA initiated by polypeptides such as PLP or ELP is an efficient way to obtain in aqueous solution, either well-defined amphiphilic protein-polymer conjugates or diblock copolypeptides.

Next, we studied the resulting nanomaterial suspensions by light scattering and microscopy (Figure 2; Figures S11–S13, Supporting Information). While poly(L-proline) formed branched elongated aggregates (Figures S12B and S13B, Supporting Information), initiating ROPISA with the recombinantly synthesized ELP yielded homogeneous cocoon-like nano-objects displaying much less anisotropy (Figure 2; Figures S12C and S13C, Supporting Information).

4. Fully Peptidic and Thermo-responsive Nanomaterials

As poly(L-proline) and ELP are thermo-responsive polymers, we studied the thermally induced phase transition behavior of these nanomaterials. First, we monitored the nanoparticle suspension of ELP-*b*-PBLG by light scattering analysis while increasing the temperature at a rate of 2 °C min⁻¹. Interestingly, the scattered light intensity (measured as the derived count rate (DCR); Figure 3A) was found to increase below the cloud point temperature of the pure ELP (i.e., 32 °C at 1 mg mL⁻¹; Figure 3B) as expected by the covalent fusion of a hydrophobic peptide (PBLG) to the ELP.

The decrease in DCR is correlated to an increase in nanoparticle density, resulting from the dehydration of the ELP on the

surface of the nano-objects.^[31–33] It is accompanied by a decrease in the objects' diameter of about 20% which is due to this dehydration, leading to a shrinking of the ELP shell. No aggregation of the nanomaterial was, however, observed in contrast to the analysis performed with the ELP alone in solution (Figure 3B), showing that the nanoparticles stayed colloidal stable. We then monitored the absorbance (at 550 nm) of the PLP-*b*-PBLG dispersion while increasing the temperature at a rate of 1 °C min⁻¹ (Figure S20, Supporting Information). A clear cloud point was observed at 76 °C confirming the LCST aggregation properties of the PLP^[27] at the surface of the nanomaterial. After a 10 min isotherm at 85 °C, the solution was cooled down by 1 °C min⁻¹ and a clearing point temperature was found at a much lower temperature of 4 °C. This revealed a hysteresis width of over 50 °C provided by the polypeptide shell, in agreement with the thermo-responsiveness of the PLP alone (Figures S20 and S21, Supporting Information).

5. Conclusion

In conclusion, using aqueous ROP of NCA monomers, we present an efficient process to obtain protein-polymer conjugates and polymeric nanomaterials that are entirely composed of amino acid residues, in high yield and with high purity. This ROPISA method is easy to implement, can involve the native amines of any polypeptide structures, and is time efficient. To the best of our knowledge, this work proves also for the first time the feasibility of synthesizing fully biodegradable nanoparticles in aqueous media using a PISA approach.^[23] Moreover, as proved with thermo-responsiveness in this work, the use of polypeptides or proteins opens the field to many possible functional nanomaterials that take advantage of the great chemical diversity of amino acids.

6. Experimental Section

Materials: Solvents for size-exclusion chromatography like DMF, acetonitrile (ACN), and hexafluoropropan-2-ol (HFIP) were bought from Sigma. DMF and ACN for macroinitiators synthesis were obtained from a solvent system purifier (PureSolv, Innovative Technology), kept under argon atmosphere, and freshly used. Dichloromethane was bought from Sigma and trifluoroacetic acid from TCI. BLG-NCA, sarcosine *N*-carboxyanhydride (Sar-NCA), and L-proline *N*-carboxyanhydride (L-Pro-NCA) were supplied by PMC Isochem (Vert-le-Petit, France), and stored at -20 °C under argon atmosphere and weighed in the glove box (Jacomex GP13 No. 2675). *N*-(*Tert*-butoxycarbonyl)glycine (Boc-Gly-OH), 1-ethyl-3-carbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole hydrate (HOBT), and hexylamine were bought from Sigma, and were used without any purification, except for hexylamine which was freeze-thawed and cryo-distilled on the Schlenk line prior to use. PEG_{5k}-NH₂ (*M*_p = 5516 g mol⁻¹, *D* = 1.02) was bought from RAPP Polymer GmbH (Tübingen, Germany). Ultrapure water was obtained from a Milli-Q system (Merck Millipore, France) with a resistivity of 18.2 MΩ. Deuterated solvent (CDCl₃, Dimethyl Sulfoxide (DMSO-*d*₆), Trifluoroacetic acid (TFA-*d*)) were purchased from Eurisotop.

Instrumentation: ¹H nuclear magnetic resonance (NMR) spectra were recorded at room temperature or at 323 K with a Bruker Avance 400 (400 MHz). CDCl₃ with TFA, TFA-*d*, and DMSO-*d*₆ were used as solvent and signals were referred to the signal of residual protonated solvent signals. DOSY NMR was performed at 296 K on a Bruker Avance NEO 400 MHz NMR spectrometer, equipped with a 5 mm BBO Bruker Cryoprobe Prodigy, capable of producing gradients in the *z*-direction with a strength of 50 G cm⁻¹. The DOSY spectra were acquired with the

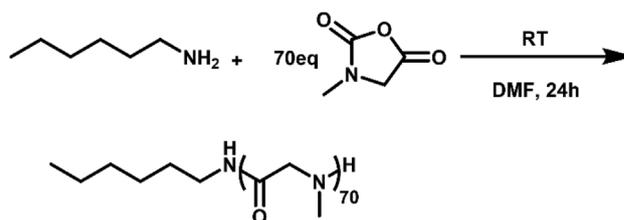
dsteppp3s pulse program. All spectra were recorded with 16k time domain data points in the t_2 dimension and 16 t_1 increments. The gradient strength was linearly incremented in 16 steps from 5% up to 95% of the maximum gradient strength. All measurements were performed with a compromise diffusion Δ of 100 ms. The gradient pulse length δ was 2.3 ms. Polymer molar masses were determined by SEC) using DMF + LiBr 1 g L⁻¹, HFIP + 0.05% KTFA (Potassium trifluoroacetate), or aqueous solvent as eluent. Measurements in DMF were performed on an Ultimate 3000 system from Thermo Fischer Scientific (Illkirch, France) equipped with a diode array detector (DAD). The system also includes a multiangle light scattering detector (MALS) and differential refractive index detector (dRI) from Wyatt Technology (Santa Barbara CA, USA). Polymers were separated on three Shodex Asahipack gel columns (GF 310 (7.5 × 300 mm), GF510 (7.5 × 300), exclusion limits from 500 to 300 000 Da) at a flow rate of 0.5 mL min⁻¹. Column temperature was held at 50 °C. Easivial kit of polystyrene from Agilent (Santa Clara, CA, USA) was used as calibration standard (M_n from 162 to 364 000 Da). Measurements in HFIP were performed on an Ultimate 3000 system from Thermoscientific equipped with a UV detector. The system also included a differential refractive index detector from Wyatt Technology. Polymers were separated on PSS PFG 100 Å and PFG 1000 Å columns (300 × 8 mm, 2 μm) (exclusion limits from 100 to 12 000 000 Da) at a flow rate of 1 mL min⁻¹. Column temperature was held at 30 °C. Easivial kit of PMMA (poly(methyl methacrylate) from Agilent was used as the standard. Poly(L-proline) samples were analyzed using two TOSOH TSK Gel G4000PWL and G3000PWL columns (300 × 7.8 mm) (exclusion limits from 200 to 300 000 Da) at a flow rate of 0.6 mL min⁻¹. Aqueous solvent composed of acetic acid (AcOH) 0.3 M, ammonium acetate 0.2 M, and ACN (H₂O/ACN: 6.5/3.5, v/v) was used as the eluent. Column temperature was held at 25 °C. dn/dc measurements were performed on the differential refractive index detector (dRI) from Wyatt Technology by injecting 500 μL of each sample dissolved in the aqueous phosphate buffer at 0.5–5 mg mL⁻¹. The chromatograms were recorded with Chromeleon 7.2 software and Astra 7.1.0 software and analyzed using the latter. dn/dc values calculated were of 0.1616 mg mL⁻¹ for L-Proline homopolymers. Atomic force microscopy (AFM) measurements were performed at room temperature in a dry state using a Multimode 8 microscope (Veeco Instruments, Inc., Bruker, Santa Barbara CA, USA). Both topographic and phase images of needle-like nanoparticles were obtained in tapping mode using a rectangular silicon cantilever (AC 160-TS, Atomic Force Microscopy probes Asylum, Wiesbaden, Germany) with a spring constant of 26 N m⁻¹, a resonance frequency lying in the 270–320 kHz range, and a radius of curvature of less than 10 nm. Samples were prepared by solvent casting at ambient temperature from a stock solution (70 mg mL⁻¹). A 5 μL drop of suspension was deposited onto freshly cleaved mica, and after 10 min the excess of solution was removed with blotting paper. Subsequently, the substrate was dried under nitrogen flow for several minutes. Cryo-TEM (cryo-TEM) images were obtained by depositing 4 μL of samples on an EM grid coated with a perforated carbon film. After draining the excess liquid with a filter paper, grids were plunge-frozen into liquid ethane cooled by liquid nitrogen using a Leica EMCPD cryo-chamber (Leica Microsystems, Wetzlar, Germany). For cryo-TEM observation, grids were mounted onto a Gatan 626 cryoholder and transferred to a Tecnai F20 microscope (Thermo Fisher Scientific) operated at 200 kV. Images were recorded with an Eagle 2k CCD camera (FEI, USA). Z-average hydrodynamic diameters (D_z) and the polydispersity index (σ) of the nanoparticles were determined by DLS with a Zetasizer Nano ZS from Malvern Instruments (Malvern, UK) operating with a He–Ne laser source (wavelength = 633 nm, scattering angle = 90° or 173°). The correlation functions were analyzed using the cumulant method. The dispersions were analyzed at 0.1 wt% in water. Cloud point temperatures (T_{CP}) were determined by DLS between 10 and 55 °C at a 2 °C min⁻¹ scan rate in water for ELP40 and ELP-*b*-PBLG nanoparticles (1 mg mL⁻¹). Z-average hydrodynamic diameters (D_z) and derived count rates (kcps) were plotted as a function of temperature. TEM images were recorded on a Hitachi H7650 microscope working at 80 kV. Samples were prepared by spraying a 0.1 g L⁻¹ solution of the block copolymer onto a copper grid (200 mesh coated with carbon) using a homemade spray tool or by depositing a drop of nanomaterial dispersion grid and removing excess after 1 min. Both types of grids were negatively stained with 1.2%

uranyl acetate or Uranyless. UV–vis spectroscopy, the cloud point temperature (T_{CP}), and the clearing point temperature (T_{CL}) of PLP and PLP-*b*-PBLG solutions in pure water were determined by measuring the turbidity at 550 nm between 10 and 85 °C at a 1 °C min⁻¹ scan rate according to Badreldin et al.^[27] Data were collected on a Cary 100 UV–Vis spectrophotometer equipped with a multicell thermoelectric temperature controller from Agilent Technologies. T_{CP} is defined as the temperature at the inflection point of the transmittance–temperature curves during heating ramps and concordantly for T_{CL} during the cooling ramps. A typical experiment followed the following heating programs:

- 1) 10 min isotherm at 10 °C
- 2) 1 °C min⁻¹ ramp to 85 °C
- 3) 10 min isotherm at 85 °C
- 4) 1 °C min⁻¹ ramp to 10 °C

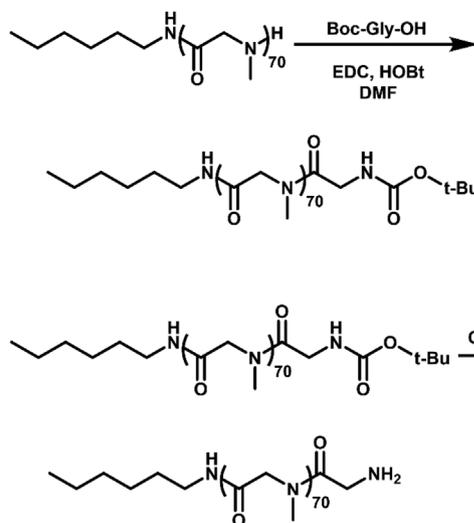
Transmittance was plotted as a function of temperature, with isotherm data not being collected. The y-axis in the results was displayed as 1-transmittance (%) to help the reader relate the soluble and the aggregated forms to low and high values respectively.

Synthesis of Poly(sarcosine) Macroinitiator:



For ring-opening polymerization, Sar-NCA (3 g, 2.6 × 10⁻¹ mol, 70 eq.) was introduced to a flame-dried Schlenk equipped with a stirrer and dissolved in 44 mL of dry DMF. While stirring, hexylamine (50 μL, 1.4 × 10⁻² mmol, 1 eq.) was added to the solution to initiate the ROP. The consumption of Sar-NCA was monitored by Fourier transform infrared (FT-IR) spectroscopy by checking the disappearance of the bands at 1859 and 1787 cm⁻¹, characteristics of the C=O stretching of NCAs. Upon completion of the polymerization reaction, the final PSar homopolymer was precipitated in diethyl ether and dried under vacuum overnight. The powder was then solubilized in water and dialyzed against deionized water in a 1 kDa dialysis membrane before being lyophilized. Yield = 50%, white powder.

¹H NMR (400 MHz, DMSO-*d*₆, 50 °C, δ): 0.86 (*t*, 3H; –CH₃, hexylamine), 1.24 (6H; –CH₂–CH₂–CH₂, hexylamine), 1.38 (*m*, 4H; –CH₂–CH₂–NH–, hexylamine), 2.73–2.94 (br *m* 3H; CH₃ of PSar side chain), 3.93–4.34 (br *m*, CH₂ of PSar backbone). M_n^{NMR} = 5300 g mol⁻¹ (D_p^{NMR} = 73). SEC (1% KTFA in HFIP) M_n^{SEC} = 33 600 g mol⁻¹, and D = 1.06.



For coupling with Boc-glycine-OH, PSar (500 mg, 1×10^{-1} mmol, 1 eq.) and EDC (90 μ L, 5×10^{-1} mmol, 5 eq.) were solubilized in 10 mL DMF in a round-bottom flask. Boc-Gly-OH (88 mg, 5×10^{-1} mmol, 5 eq.) and HOBt (68 mg, 5×10^{-1} mmol, 5 eq.) were solubilized in a second flask in 10 mL DMF. After 1 h, the solution containing Boc-Gly-OH was added to the PSar solution. The mixture was left to stir for 16 h. The product was then precipitated in diethyl ether and dried under vacuum overnight. The powder was then solubilized in water and dialyzed against deionized water in a 1 kDa dialysis membrane before being lyophilized. Yield = 50%, white powder.

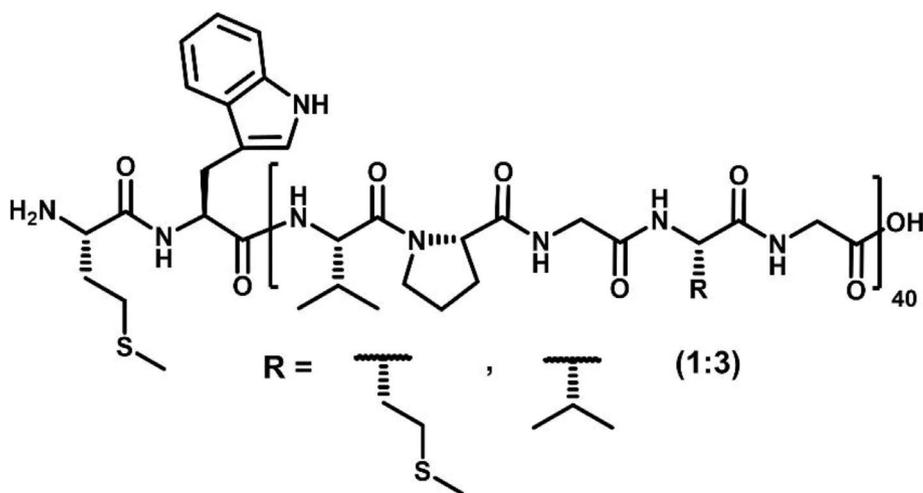
$^1\text{H NMR}$ (400 MHz, DMSO- d_6 , 50 $^\circ\text{C}$): δ = 0.86 (t, 3H; $-\text{CH}_3$, hexylamine), 1.24 (6H; $-\text{CH}_2-\text{CH}_2-\text{CH}_2$, hexylamine), 1.38 (m, 13H; $-\text{CH}_2-\text{CH}_2-\text{NH}-$, hexylamine and $(\text{CH}_3)_3-$, Boc-Gly-), 2.73–2.94 (br m 3H; CH_3 of PSar side chain), 3.93–4.34 (br m, $-\text{CH}_2$ of PSar backbone).

Then, PSar-Gly(NBoc) (260 mg, 5×10^{-2} mmol, 1 eq.) was solubilized in a 6 mL mixture of $\text{CH}_2\text{Cl}_2/\text{TFA}$ (50:50) and left to stir for 2 h. The final product was precipitated in diethyl ether, neutralized in saturated

at 10 $^\circ\text{C}$ was added to the first solution to start the ROP under vigorous stirring. The solution quickly became clear and bubbling was observed. FT-IR confirmed the reaction completion after 10 min by monitoring the disappearance of the bands at 1859 and 1787 cm^{-1} characteristic of the $\text{C}=\text{O}$ stretching of NCAs. The reaction mixture was dialyzed against deionized water in a 100–500 Da dialysis membrane to remove all unreacted monomers and solvent before being lyophilized. Yield: 90%, white powder.

$^1\text{H NMR}$ (400 MHz, D_2O , δ): 0.85 (t, 3H; $-\text{CH}_3$, hexylamine), 1.26 (6H; $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$, hexylamine), 1.45–1.48 (m, 4H; $-\text{CH}_2-\text{CH}_2-\text{NH}-$, hexylamine), 2.03 (t, 2H; $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$, polymer backbone), 1.80–2.40 (m, 2H; $-\text{CH}_2\text{CH}_2-\text{CH}_2-$, polymer backbone), 3.55–3.90 (m, 2H; $-\text{CH}_2-\text{CH}_2-\text{N}-$, polymer backbone), 4.71 ppm (t, 1H; $\text{OC}-\text{CH}-\text{N}$, polymer backbone). $M_n^{\text{NMR}} = 5400 \text{ g mol}^{-1}$ ($\text{DP}^{\text{NMR}} = 55$). SEC (acetate/ACN) $M_n^{\text{SEC LS}} = 5790 \text{ g mol}^{-1}$, $\text{D} = 1.03$.

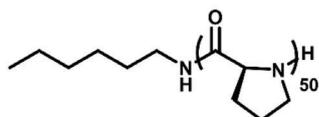
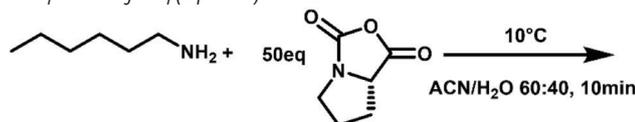
Recombinant Biosynthesis of Elastin-like Polypeptide Macroinitiator:



NaHCO_3 solution, and then dialyzed against deionized water in a 1 kDa dialysis membrane and lyophilized. Yield = 33%, white powder.

$^1\text{H NMR}$ (400 MHz, DMSO- d_6 , δ): 0.86 (t, 3H; $-\text{CH}_3$, hexylamine), 1.24 (6H; $-\text{CH}_2-\text{CH}_2-\text{CH}_2$, hexylamine), 1.38 (m, 4H; $-\text{CH}_2-\text{CH}_2-\text{NH}-$, hexylamine), 2.73–2.94 (br m 3H; $-\text{CH}_3$ of PSar side chain), 3.93–4.34 (br m, $-\text{CH}_2$ of PSar backbone and of Gly chain end). $M_n^{\text{NMR}} = 5360 \text{ g mol}^{-1}$ ($\text{DP}^{\text{NMR}} = 73$). SEC (1% KTFA in HFIP) $M_n^{\text{SEC RI}} = 35\,600 \text{ g mol}^{-1}$, $\text{D} = 1.06$.

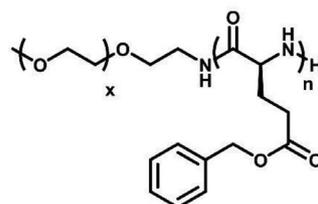
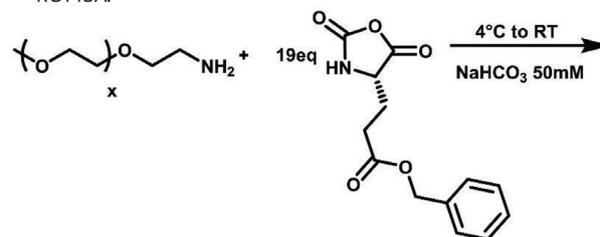
Synthesis of Poly(L-proline) Macroinitiator:



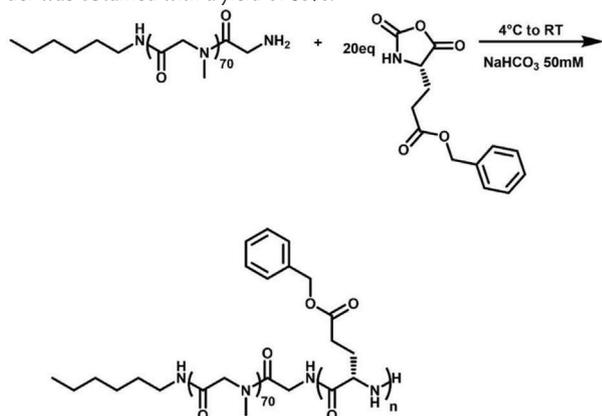
Poly(L-proline) was synthesized as reported previously.^[27] L-Pro-NCA (1 g, 71×10^{-1} mmol, 50 eq.) was introduced in a flame-dried Schlenk equipped with a stirrer and suspended in 0.5 mL of dry ACN. Hexylamine (10.43 mg, 1.4×10^{-1} mmol, 1 eq.) dissolved in 10.5 mL $\text{H}_2\text{O}:\text{ACN}$ (1:1)

ELP[M_1V_3 -40]—ELP—used for this study was produced using *E. coli* as an expression system, purified by inverse transition cycling and characterized as previously described.^[30] The amino acid sequence is $\text{MW}[(\text{VPGVG})(\text{VPGMG})(\text{VPGVG})_2]_{10}$, the molecular weight was precisely $17.035 \text{ g mol}^{-1}$. $^1\text{H NMR}$ spectrum in DMSO- d_6 is provided in Figure S16 (Supporting Information).

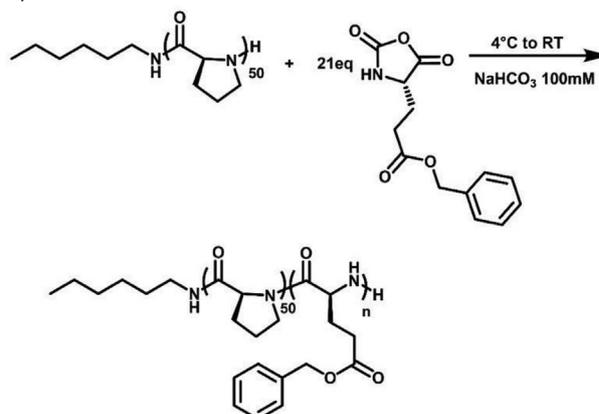
Synthesis of Poly(ethylene glycol)-block-Poly(γ -benzyl-L-glutamate) by ROPISA:



In a glove box, the NCA monomer of γ -benzyl-L-glutamate (150 mg, 0.57 mmol) was weighted in a Schlenk tube containing a magnetic stirring bar. The Schlenk was removed from the glove box and cooled on ice. 4 mL of an ice-cooled solution of NaHCO_3 0.05 M containing the initiator $\text{PEG}_{5k}\text{-NH}_2$ (150 mg, 0.03 mmol, $[\text{M}]/[\text{I}] = 19$) was added to the BLG-NCA powder under a strong agitation ($\tau_s = 7\%$). The reaction was left to stir 1) first in an ice-cold water bath; 2) then at room temperature overnight. The opalescent dispersion obtained was then transferred to a 3.5 kDa dialysis membrane and dialyzed against deionized water for 2 days. An aliquot was kept for further microscopy imaging and dynamic light scattering and the remaining dispersion was lyophilized. A white powder was obtained with a yield of 85%.^[21]

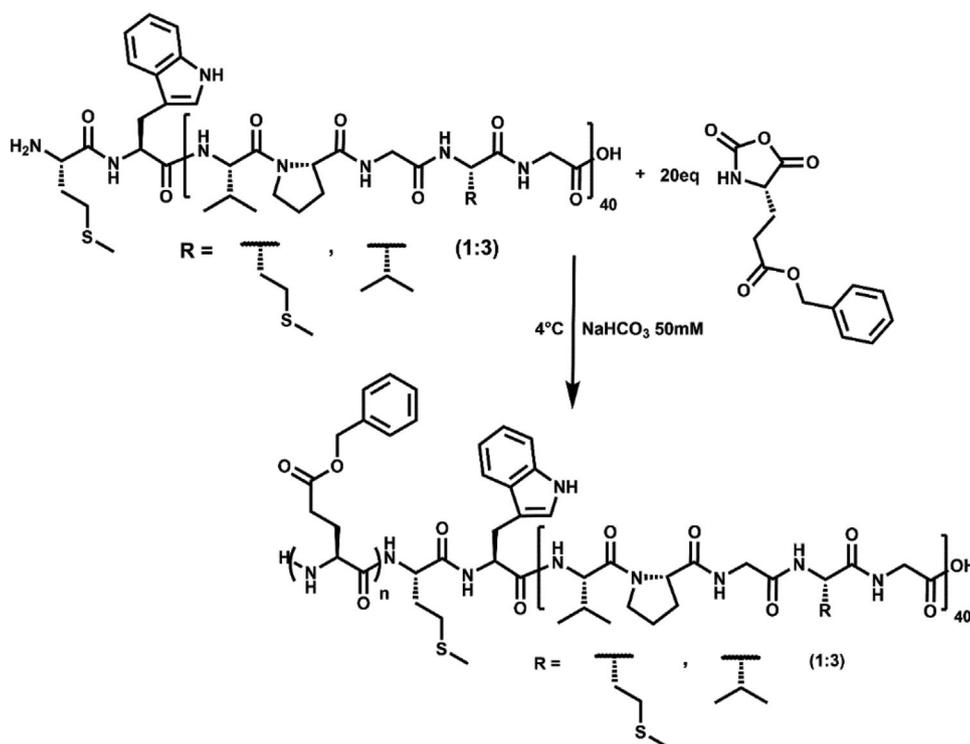


Synthesis of Poly(sarcosine)-block-Poly(γ -benzyl-L-glutamate) by ROPISA:
In a glove box, the NCA monomer of γ -benzyl-L-glutamate (40 mg, 0.142 mmol) was weighted in a Schlenk tube containing a magnetic stirring bar. The Schlenk was removed from the glove box and cooled on ice. 1 mL of an ice-cooled solution of NaHCO_3 0.05 M containing the initiator PSar-NH_2 (40 mg, 0.0071 mmol, $[\text{M}]/[\text{I}] = 20$) was added to the BLG-NCA powder under a strong agitation ($\tau_s = 7\%$). The reaction was left to stir 1) first in an ice-cold water bath; 2) then at room temperature overnight. The opalescent dispersion obtained was then transferred to a 3.5 kDa dialysis membrane and dialyzed against deionized water for 2 days. An aliquot was kept for further microscopy imaging and dynamic light scattering and the remaining dispersion was lyophilized. A white powder was obtained with a yield of 79%.



Synthesis of Poly(L-proline)-block-Poly(γ -benzyl-L-glutamate) by ROPISA:
PLP was also used as an initiator using the same protocol. In this case, the buffer used was NaHCO_3 0.1 M. A white powder was obtained with a yield of 90%.

Synthesis of ELP-block-Poly(γ -benzyl-L-glutamate) by ROPISA:



A similar protocol was employed with ELP as an initiator. The protocol as described for PEG-*b*-PBLG was slightly modified to decrease the solid content of the reaction to 0.5 wt% and temperature was maintained at 4 °C for the whole duration of the reaction. A white powder was obtained with a yield of 89%.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

N-carboxyanhydrides, PISA, polypeptide, protein–polymer conjugate, ring-opening polymerization

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