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Design and self-assembly of PBLG-*b*-ELP hybrid diblock copolymers based on synthetic and elastin-like polypeptides

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Abstract

The precision synthesis and self-assembly of amphiphilic copolypeptides containing a recombinant elastin-like polypeptide (ELP) block used as macroinitiator for the ring opening polymerization (ROP) of γ -benzyl-*L*-glutamate (γ -BLG NCA) are herein presented. The molecular weight of the resulting PBLG-*b*-ELP block copolypeptides was precisely controlled without the use of complex initiators or demanding experimental setup. Diblock copolypeptides were obtained with an excellent control of the polymerization highlighted by the dispersity below 1.04. These amphiphilic hybrid synthetic/recombinant copolypeptides were self-assembled in water and nanoparticles obtained characterized by a combination of dynamic light scattering and electron microscopy. A variety of morphologies, namely polymersomes, inter-connected worm-like micelles and spherical micelles, were evidenced depending on the hydrophilic ratio of the diblocks as well as the self-assembly procedure.

Introduction

Over the past decades, several approaches have been developed for the elaboration of block copolymer systems based on proteins for various applications. For instance, therapeutic proteins can replace deficient or absent natural proteins, upregulate existing metabolic pathways, their conjugation with hydrophilic polymer being at the origin of important research area and biotechnology development. Upon them, poly(ethylene glycol) (PEG) is the most intensively used polymer for bioconjugation, allowing longer half-life in the bloodstream, and leading to less frequent dosing.¹ In addition, giant amphiphiles, copolymers resulting from the controlled conjugation of synthetic polymers to proteins² can be interestingly used for their self-assembly

behavior. In this context, the perfect control of the chemical structure and bioconjugation mechanism is a prerequisite. In pioneered work in this area, Nolte et al. studied the self-assembly of precisely defined giant amphiphiles consisting of a single hydrophobic polystyrene tail covalently connected to a lipase³ and *via* cofactor reconstitution to horseradish peroxidase (HRP) or myoglobin.^{4,5} Velonia and co-workers showed the potential of hybrids BSA-polystyrene to create bionanoreactors,⁶ and the self-assembly behavior of tri-block giant amphiphilic BSA-copolymer synthesized by a combination of Atom Transfer Radical Polymerization (ATRP) and “click chemistry”.⁷ Thereafter, Bulmus et al. developed thermoresponsive BSA-poly(N-isopropylacrylamide) *via* the growth of the polymer chains from BSA-macroRAFT (Reversible Addition Fragmentation Chain Transfer) agent.⁸ More recently, vesicles were obtained from the self-assembly of BSA-poly(caprolactone)⁹ or HSA-poly(N-(2-hydroxypropyl) methacrylamide)¹⁰ and demonstrated great potential as drug-nanocarriers, taking advantage of the stealth character of BSA as the hydrophilic segment. In most recent example, Mann et al.¹¹ used such conjugates to built stimuli-responsive biomimetic protocells using BSA-PNIPAM based copolymers.

In the past decade, the library of proteins was significantly extended, and recombinant protein-engineering techniques allowed the development of precision recombinant polymers with exquisite control over their primary structure. Among them, elastin-like polypeptides (ELPs) represent a unique class of recombinant proteins. Their main structure results in repeating sequences of [-Val-Pro-Gly-*Xaa*-Gly-] pentapeptides, the guest residue *Xaa* being any amino acid except proline. ELPs share structural characteristics with intrinsically disordered proteins naturally found in the body, such as tropoelastin, at the origin of their name.^{12,13} The repeat sequences found in the biopolymer, as well as the monomer sequence and chain length, give each ELP a distinct structure, and influence the lower critical solution temperature (LCST), also

referred to commonly as the T_t . Recent contributions highlighted the tunable thermoresponsive properties of ELPs in water by chemoselective oxidation¹⁴ or alkylation^{15,16} of all methionine residues, with the aim of providing polycationic derivatives in the later case or introducing new side-chain functionalities.

In this context, ELPs may find an original place as protein block in hybrid protein-polymer bioconjugates. Indeed, the elaboration of synthetic polymers-ELPs conjugates were reported, such as low molecular weight ELP-based side chain poly(methacrylate)s *via* ATRP^{17,18} and ELP-grafted poly(norbornene)s *via* ring-opening metathesis polymerization (ROMP)^{19,20} in presence of catalyst based on ruthenium. Moreover, ELPs obtained by chemical pathway (Boc-based liquid phase synthesis) were conjugated with polyamidoamine (PAMAM) dendrimers to gain potential stimuli-responsive drug-carrier.²¹ More recently, Kiick and co-worker²² reported the self-assembly behavior in vesicles of elastin-*b*-collagen-like peptide bioconjugates obtained by copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) “click” reaction.

Although peptide-polymer and protein-polymer conjugates were studied, conjugates of hybrid protein-synthetic polypeptide are surprisingly less explored whereas the polymerization of N-carboxyanhydrides (NCAs) allows obtaining well-defined macromolecular structures, even from a macro-initiator. Indeed, block copolymer are usually prepared by using the first block with an amino end-group as a macroinitiator for the ring opening polymerization (ROP) of NCAs, this block being often PEG,²³⁻²⁶ even if some other example referred to poly[N-(2-hydroxypropyl) methacrylamide] (PHPMA) of high molecular weight ($\overline{M}_n = 32,100 \text{ g.mol}^{-1}$).²⁷

The NCAs ROP can proceed *via* 2 mechanisms: the normal amine mechanism (NAM, induced by nucleophilic substitution) proposed by Waley and Watson²⁸ and the activated monomer

mechanism (AMM, induced by deprotonation of the NCAs).²⁹ AMM yields polypeptides with high molar mass and broad dispersity. As a result, the NAM mechanism must be favored to obtain well-defined polypeptides. Most commonly, nucleophiles are used to initiate the polymerization, with primary amines being the most intensively investigated species. In addition to the versatility of synthesis and the intrinsic biodegradability of peptide bonds, the supramolecular organization of polypeptides provides an opportunity to produce hierarchical structures and can also be used to promote specific “bioactivity”.³⁰⁻³² For instance, block copolymers combining polypeptide and oligosaccharide blocks have previously been employed to prepare glycoprotein biomimetic polymersomes.³³⁻³⁵

The goal of the present contribution is to combine the various advantages of ELPs and synthetic polypeptides to obtain a “hybrid” amphiphilic diblock recombinant/synthetic polypeptide capable of self-assembly in various biocompatible nano-structures. In this context, this article highlights the use of hydrophilic ELP as a macroinitiator for the ROP of γ -BLG-NCA. The diblock polypeptides were obtained with a high control of the polymerization mechanism, highlighted by the low dispersity of the resulting copolymers, below 1.04. The resulting amphiphilic hybrid diblocks were subsequently self-assembled, using a microfluidic chip system and more “classical” methods such as dialysis or direct dissolution. The goal of our study is to establish structure-property relationships between the weight fraction of each block and the size and morphology of the resulting nano-objects depending on the self-assembly process used.

Results and discussion

Synthesis of PBLG-b-ELP block copolypeptides

The most rapid and cost-effective process to prepare synthetic polypeptide chains with high degrees of polymerization and excellent control over the dispersity is the ROP of heterocycle α -amino acid *N*-carboxyanhydrides (NCAs).³⁶⁻³⁸ Poly(γ -benzyl-*L*-glutamate)-*block*-ELP (PBLG-*b*-ELP) copolypeptides studied in this work were therefore prepared in high efficacy and with high purity by ROP of γ -BLG NCA using the *N*-terminal group of the ELP as initiating group. A series of hybrid copolypeptides with different PBLG block lengths was synthesized in order to study the influence of this parameter, among others, on their self-assembly behavior.

The ELP used in this work was recombinantly produced in *Escherichia coli* using protein engineering techniques as described previously.¹⁴ This ELP contains a total of forty pentapeptide repeats according to the protein sequence MW[(VPGVG)(VPGMG)(VPGVG)₂]₁₀ corresponding to a molecular weight of 17,035 Da. From the ELP primary structure, the only functional group able to initiate the polymerization is indeed the primary amine present on the *N*-terminal group. The ¹H NMR spectrum in CDCl₃ with 15 % of trifluoroacetic acid (TFA, to compare with spectrum after polymerization) of the ELP (Figure S1) was recorded and all peaks fully assigned for subsequent comparison with PBLG-*b*-ELP ¹H NMR spectra. The peak at 0.94 ppm attributed to the resonance of the CH₃ of the 70 valine residues (420 ¹H total) was used for the calibration.

The ELP was subsequently used as macroinitiator for the ROP of γ -BLG NCA. Firstly, the polymerizations were initially performed in anhydrous DMF at 25 °C for 24 hours. Pure products were isolated by precipitation in diethyl ether. The degree of polymerization (DP) of each compound was determined by ¹H NMR in CDCl₃ containing 15% of TFA. The DP was

calculated by comparing the integral of the peak at 5.10 ppm corresponding to the resonance of the methylene group in α of the benzyl ring (2 ^1H per BLG unit) to the integral of the peak at 0.94 ppm used for calibration (*vide supra*, 420 ^1H). Four PBLG-*b*-ELP copolypeptides were obtained with DP ranging from 21 to 110. Because the experimental DP in each case was far below the targeted DP (Table S1, *e.g.* a DP 21 was obtained when DP 52 was targeted), we concluded that the polymerizations stopped before total consumption of the monomer. The increased polydispersity index \mathcal{D} of PBLG-*b*-ELP copolypeptides with DP above 61 (Table S1) attributed to the presence of a shoulder in the size exclusion chromatography (SEC) profiles (Figure 3a) also suggested the uncontrolled character of the polymerization under these conditions. Indeed, it was previously reported that side reactions, such as chain-end terminations and the formation of formyl end-groups can occur at 25 °C during the polymerization of NCAs.³⁹ More precisely, regarding the synthesis of PBLG, Hanby and co-workers⁴⁰ described in the 50's, the polypeptide chain termination, where the amine chain-end reacts with the carbonyl of the last unit side chain to form a stable 5-atoms heterocycle (*i.e.*, pyrrolidone). When occurring during polymerization, this reaction leads to the termination of the growing chain and is responsible for the poor control of the polymerization.^{36,39} To avoid this problem that is specific to the ROP of γ -BLG NCA, the polymerization reactions were performed in anhydrous DMF at low temperature (5 °C) (Scheme 1). Indeed, it was previously demonstrated that low reaction temperatures reduce the frequency of this premature termination since this reaction requires a higher activation energy.^{39,41-43}

Under these conditions, all *N*-terminal groups of ELP chains initiated the ROP as evidenced by the excellent yields. (Table 1) Complete conversions were achieved according to the excellent agreement between theoretical and experimental DPs as assessed by ^1H NMR and targeted DPs (Figure 1). Four PBLG-*b*-ELP copolypeptides with DP ranging from 25 to 180 were obtained,

corresponding to PBLG blocks between 5,500 and 39,500 g.mol⁻¹ molecular weights and diblock PBLG-*b*-ELP copolypeptides between 22,500 and 56,500 g.mol⁻¹ molecular weights, respectively. All hybrid copolypeptides were characterized by SEC in DMF (0.8 mL.min⁻¹) at 50 °C in the presence of LiBr (1 g.L⁻¹) with RI detector and polystyrene used as standard. Using this setup, the apparent molar mass of the ELP was 18,000 g.mol⁻¹, while diblock copolypeptides' apparent molar masses were comprised between 33,300 and 56,200 g.mol⁻¹. Values estimated by SEC were found in relatively good adequacy with the values obtained by ¹H NMR. (Table 1) The SEC chromatograms evidenced monomodal distributions of polypeptide chains with narrow dispersities (1.02 - 1.03) indicating the well-controlled character of the polymerization. (Table 1 and Figure 2b) Consequently the presence of the carboxylic group at the *C*-terminal end of the ELP did not appear to be disadvantageous in the polymerization. Aoi and co-workers actually showed that the presence of a nucleophilic carboxylate provides control over the polymerization of sarcosine-NCA.⁴⁴

To summarize the synthetic part of this work, the ROP of γ -NCA BLG initiated by a high molecular weight recombinant ELP was conducted at 5 °C to prevent chain termination reactions during the polymerization. A total of four, well-defined, hybrid diblock copolymers associating a synthetic hydrophobic polypeptide block and a biocompatible elastin-like polypeptide block were obtained. These differ by the length of the hydrophobic PBLG block and therefore feature different hydrophilic weight fractions (*f*) – PBLG₂₅-*b*-ELP (*f* = 76 %), PBLG₄₅-*b*-ELP (*f* = 63 %), PBLG₉₅-*b*-ELP (*f* = 45 %) and PBLG₁₈₀-*b*-ELP (*f* = 30 %) – a critical parameter that influences amphiphilic copolypeptide self-assembly.⁴⁵⁻⁴⁷

Self-assembly of PBLG-b-ELP block copolypeptides

The self-assembly in water of the different PBLG-*b*-ELP copolypeptides was performed using different methodologies, more precisely dialysis or microfluidics and direct solubilization in the case of PBLG₂₅-*b*-ELP with the highest hydrophilic weight fraction ($f = 76\%$). These methods were selected among others to obtain stable nanoparticles of controlled size and morphologies under kinetic or thermodynamic control. Temperature of the solutions during the self-assembly process and the dialysis was carefully maintained at 20°C to overcome the impact of this parameter. The self-assembly studies were performed at the concentration of 4 g.L⁻¹, namely the highest polypeptide concentration avoiding the formation of macroscopic aggregates in the dialysis tubing or in the microfluidic chip. Concentrations lower than 2 g.L⁻¹ resulted in the formation of similar nanostructures that were however difficult to characterize because of the high dilution after dialysis. The resulting self-assemblies were characterized by transmission electron microscopy (TEM) (Figures 3 and S2a) and dynamic light scattering at 90° (DLS) (Figures S2b and S3).

The block copolypeptide with the shortest hydrophobic PBLG block, and therefore the highest hydrophilic weight fraction, ($f = 76\%$), was first self-assembled by direct solubilization in ultrapure water at 1 mg.mL⁻¹ at room temperature. DLS measurements showed a monomodal size distribution, with a hydrodynamic radius (R_h) of 39 nm and relatively low polydispersity index (PDI) of 0.13 (Figure S2a) that is consistent with the formation of spherical nanoparticles. TEM micrographs revealed the presence of a highly homogeneous dispersion of spherical nanoparticles (Figure S2b) with a rather strong intrinsic contrast certainly due to the high electron density of the benzyl group of the hydrophobic PBLG block. It is worth mentioning that PBLG₂₅-*b*-ELP has the largest hydrophilic weight fraction of the whole series of copolypeptides; it is therefore supposed to have the highest critical aggregation concentration (CAC). The CAC of PBLG₂₅-*b*-

ELP was determined at $0.22 \text{ mg}\cdot\text{mL}^{-1}$. Self-assembly of all copolypeptides was therefore studied at concentrations above the CAC.

Self-assembly of PBLG₂₅-*b*-ELP ($f = 76 \%$) was triggered either by dialysis or microfluidics, leading to micellar morphology, irrespective of the technique used (Figure 3). Due to the use of DMSO as organic solvent to solubilize the diblock copolypeptides, the intrinsic α -helix conformation of the PBLG block was lost in favor of the coil conformation.⁴⁸ The α -helix conformation is however most likely reformed during the self-assembly process, as evidenced by circular dichroism (data not shown), ensuring the stability of the final structures.³¹ The suspension obtained by dialysis was heterogeneous as evidenced by the TEM micrograph (Figure 3a) as well as the DLS profile (Figure S3a) showing two distinct populations of particles: one most likely corresponding to individual nanoparticles with a R_h of 27 nm and one around 100 nm in diameter most likely corresponding to aggregates. Self-assembly from an organic solution in DMSO in which the copolypeptide is well solubilized thus leads to the formation of smaller spherical micelles than a direct solubilization of the copolypeptide in water. The microfluidic technique proved more efficient in producing nanoparticles of homogeneous size at a ratio of 70:30 DMSO/H₂O. The DLS intensity profile indeed showed a monomodal size distribution centered on $R_h = 15 \text{ nm}$ with a narrow polydispersity (PDI = 0.14) (Figure S3b).

The hybrid block copolypeptide with a 63 % hydrophilic weight fraction, namely PBLG₄₅-*b*-ELP, self-assembled similarly and formed spherical nanoparticles both by dialysis and microfluidics (Figure 3). The microfluidic technique allowed the formation of nanoparticle of homogeneous size at $R_h = 17 \text{ nm}$ (PDI = 0.08), while dialysis resulted in a more heterogeneous dispersion ($R_h = 30 \text{ nm}$, PDI = 0.25). (Figure S3, panels c and d, respectively).

The micellar morphology observed with PBLG₂₅-*b*-ELP and PBLG₄₅-*b*-ELP is consistent with previous studies performed on amphiphilic diblock copolymers based on PBLG. In particular, poly(ethylene oxide)-*b*-PBLG⁴⁹ (from 21 % to 85 % *f* values) as well as polysarcosine-*b*-PBLG⁵⁰ (*f* = 90 %) block copolymers self-assembled into spherical nanoparticles by dialysis of an organic solution against water or solvent displacement method. We also evidenced that, for these two copolypeptides with short hydrophobic PBLG chains and *f* above 60 %, microfluidics provided homogeneous solvents mixing resulting in monodisperse and smaller micelles as compared to the dialysis method.

The self-assembly technique showed however to have a greater impact in the case of larger hydrophobic PBLG block. Dialysis against water of PBLG₉₅-*b*-ELP (*f* = 45%) led to the formation of polydisperse inter-connected worm-like micelles as shown by the TEM micrographs. (Figure 3, panel e) This observation explained the large distribution obtained by DLS with R_h around 130 nm and high polydispersity (PDI = 0.32). (Figure S3, panel e).

Because the water content plays a very important role in the self-assembly, decreasing the solvent quality for the PBLG block firstly solubilized in DMSO and changing the chain mobility, in this part, we describe the formation of the copolymer self-assemblies depending on the DMSO/H₂O ratio precisely controlled by the microfluidic device.

Using a total flow rate of 100 $\mu\text{L}\cdot\text{min}^{-1}$ and DMSO/H₂O ratio of 70:30, PBLG₉₅-*b*-ELP formed inter-connected worm-like micelles, similar to that obtained by dialysis, as shown on Figure 3f. Indeed, the DMSO content being rather large after microfluidic, the system is still dynamic and able to re-organize during the dialysis step, a situation similar to that resulting from the slow

solvent mixing process. This observation is consistent with previous results reported for different amphiphilic copolymer systems within a similar range of hydrophilic fraction.⁵¹⁻⁵³

When reducing the relative amount of DMSO in H₂O (30:70) and thus increasing the amount of selective solvent, slightly distorted micelles were observed on Figure 3g. We assumed these structures to be metastable morphologies kinetically trapped during the experimental time scale, due to the faster diffusion of water in DMSO and high hydrophobicity and rigidity of the PBLG segment. This observation confirmed previous findings that nanoparticle morphology can be controlled not solely by the thermodynamic rules, related to the macromolecular parameters (the degree of repulsion between the blocks, the lengths of the block and consequently the hydrophilic weight fraction) but also by inducing self-assembly with different techniques³² or by varying the ratio between the organic solvent and the selective solvent allowing the self-assembly *via* kinetic control.^{54,55} Indeed, if the water content is too high, reducing drastically the solubility and chain mobility, the kinetic process of polymer self-assembly and structural rearrangement of the aggregates can be too slow to establish a dynamic equilibrium.

We finally studied the last copolyptide of the series, namely PBLG₁₈₀-*b*-ELP, having the lowest hydrophilic weight fraction ($f = 30\%$). In this case, the process proved critical on the self-assembled morphologies observed. Dialysis against water of a solution of PBLG₁₈₀-*b*-ELP led to relatively polydisperse (PDI = 0.28) spherical particles of $R_h = 59$ nm (Figures 3h and S3g). Spherical micelles were also reported as stable self-assembled structures of PBLG-*b*-PEG diblock copolymers with similar hydrophilic fractions and *via* a similar dialysis method.⁴⁹ The use of microfluidics (DMSO:H₂O ratio of 70:30) however allowed the formation of vesicles as

evidenced by TEM and cryo-TEM (Figure 3i). DLS measurements provided an average $R_h = 76$ nm and PDI = 0.19 (Figure S3h). The size of vesicles as measured on TEM micrographs ($n = 150$) appeared slightly smaller with an average radius of 61 nm, this difference being attributed to the hydration sphere of the ELP segment in solution. These results mainly highlight the major advantage of the microfluidic device in controlling the formation of monodisperse nanoparticles in a metastable morphology and in highly reproducible manner. With microfluidics, the proportion of selective solvent (*i.e.*, water) in the organic phase containing the soluble copolypeptides is directly related to the solvent mixing rate and thus the flow rate of the streams directly controls the formation of spherical or worm-like micelles or polymersomes.⁵⁶

When the kinetic of self-assembly is slowed down by a highly selective solvent (here the water) and no free self-assembly is possible, the formation of polymersomes need significantly more time than the formation of spherical micelles. The vesicular structures obtained can be explained by the slower and homogenous mixing of DMSO and water in the micro-mixer, allowing the self-organization of the rigid and hydrophobic PBLG block to form the thin membrane.

Conclusion

A recombinant elastin-like polypeptides (ELPs) with a precise sequence MW[(VPGVG)(VPGMG)(VPGVG)₂]₁₀ and named ELP-M-40 was efficiently used as macroinitiator for the ROP of the γ -benzyl-*L*-glutamate N-carboxyanhydride. The controlled ammonium-mediated ROP mechanism allowed us to afford library of define hybrid recombinant/synthetic diblock polypeptides with varying hydrophilic weight fractions (f):

PBLG₂₅-*b*-ELP ($f = 76\%$), PBLG₄₅-*b*-ELP ($f = 63\%$), PBLG₉₅-*b*-ELP ($f = 45\%$) and PBLG₁₈₀-*b*-ELP ($f = 30\%$) with a dispersity below 1.04.

Thereafter, the self-assembly of this series of giant amphiphiles in water was studied using several methods: dialysis, microfluidics and direct solubilization only for PBLG₂₅-*b*-ELP ($f = 76\%$). This study allows to explore the thermodynamic and kinetic conditions the most suitable for the system to obtain stable nanoparticles of controlled size and shape.

Depending on the diblock copolypeptide composition and the self-assembly protocol, the morphology of the structures formed could be controlled, ranging from micelles, worm like micelles to polymersomes and the results highlighted the advantages of using a microfluidic device to obtain more monodisperse structures of nano-objects of interest in a reproducible manner.

Overall, this contribution provides the first example of nano-assemblies of hybrid diblock based on recombinant ELPs and synthetic polypeptides. Due to their intrinsic biocompatibility, these new nanomaterials can potentially serve as platforms for the development of original nano-vectors.

Experimental section

Materials

All chemicals were purchased from Sigma-Aldrich and used as received. γ -benzyl-*L*-glutamate *N*-carboxyanhydride (γ -BLG NCA) was purchased from Isochem.

NMR spectroscopy

¹H NMR experiments were performed in CDCl₃ (with 15 % of TFA) at 25 °C on a Bruker Avance I NMR spectrometer operating at 400 MHz, and equipped with a Bruker multinuclear z-gradient direct probe head capable of producing gradients in the z direction with 53.5 G.cm⁻¹ strength. ¹H NMR spectra were recorded with a D1 of 2 sec and 64 scans.

Size exclusion chromatography

The molar masses and the dispersities of poly(γ -benzyl-*L*-glutamate)-*block*-ELP and the ELP precursor were determined by size exclusion chromatography (SEC) on a PL GPC50 integrated system from Agilent equipped with two KD-804 Shodex gel columns (300 x 8 mm) (exclusion limits from 4000 Da to 200 000 Da). The detector used is a refractive index detector (Jasco 1530-RI), dimethylformamide (DMF) has been used as eluent (0.8 mL.min⁻¹) at 50 °C in the presence of LiBr (1 g.L⁻¹).

Dynamic Light Scattering

To determine the hydrodynamic radius (R_h) and the polydispersity index (PDI, a dimensionless measure of the broadness of the size distribution), single-angle light scattering analysis was achieved with an ALV laser goniometer, with a 22 mW linearly polarized laser (632.8 nm HeNe) and an ALV- 5000/EPP multiple tau digital correlator (125 ns initial sampling time). All the measurements were performed at a constant temperature of 25°C and with an angle of 90°. The solutions were placed in 10 mm diameter glass cells. Data were acquired with ALV correlator control software, and the counting time was fixed for each sample at 10 s. The concentration of the solutions was 1 mg.mL⁻¹. The autocorrelation functions ($g_1(t)$) were analyzed in terms of relaxation time distribution (τ) (equation 1).

$$g_1(t) = \int A_{(\tau)} \exp\left(-\frac{t}{\tau}\right) d\tau \quad \text{equation 1}$$

Hydrodynamic radius (R_h) was determined from the Stokes-Einstein relation (equation 2).

$$R_h = \frac{k_B T}{6\pi\eta_s D_0} \quad \text{equation 2}$$

Where D_0 is diffusion coefficient, η_s is the viscosity of the solvent, T is absolute temperature and k_B the Boltzmann constant.

For the suspension in a DMSO/water mixture (before dialysis) the hydrodynamic radius and the polydispersity index (PDI) were determined by DLS with a Zetasizer Nano ZS from Malvern Instruments operating with a He-Ne laser source (wavelength 633 nm, scattering angle 90°). The correlation functions were analyzed using the cumulants method.

Transmission Electron Microscopy (TEM) micrographs of polymer nanoparticles (NPs) were recorded on a Hitachi H7650 microscope working at 80 kV equipped with a GATAN Orius 10.5 Megapixel camera (Bordeaux Imaging Center, Bordeaux, France). A drop of NPs dispersion was deposited on an Agar Scientific Formvar/Carbon film grid (200 mesh) without staining and the excess was removed after 1 min.

Cryo-Transmission Electron Microscopy (cryo-TEM) micrographs were obtained as following. A drop of suspension was deposited on “quantifoil”® (Quantifoil Micro Tools GmbH, Germany) carbon membrane. The excess of liquid on the membrane was absorbed with a filter paper and the membrane was quench-frozen quickly in liquid ethane to form a thin vitreous ice film including NPs in the holes of the grid. Once placed in a Gatan 626 cryo-holder cooled with liquid nitrogen, the samples were transferred in the microscope and observed at low temperature (-180 °C). Cryo-TEM images were recorded on ultrascan 2k CCD camera (Gatan, USA), using a LaB6 JEOL 2100 (JEOL, Japan) cryo microscope operating at 200kV with a JEOL low dose system (Minimum Dose System, MDS) to protect the thin ice film from any irradiation before imaging and reduce the irradiation during the image capture.

The critical aggregation concentration (CAC) was determined by fluorimetric method: Nile red (0.94 mg, 3.0 μmol) was dissolved in 9 mL of CH_2Cl_2 and 0.1 mL of this solution was added to a series of vials. The CH_2Cl_2 was removed under a stream of nitrogen. A series of concentrations of the nano-objects suspension ranging from to 2 $\text{mg}\cdot\text{L}^{-1}$ to 1000 $\text{mg}\cdot\text{L}^{-1}$ was prepared by serial two-fold dilutions. The suspensions were added to the vials containing Nile red and were allowed to equilibrate with stirring overnight. The fluorescence spectra were obtained on a Varian Cary Eclipse fluorescence spectrometer from Agilent Technologies. An excitation wavelength of 550 nm was used for Nile red and the emission spectra were recorded from 565 to 700 nm. The emission intensity at maximum of excitation was recorded for each concentration.

Design of encoding gene, bioproduction, purification and isolation of recombinant ELP-M-40

ELPs with high molecular weight have been efficiently produced in a large scale using *Escherichia Coli* as expression system. The ELP-M-40 was obtained as previously reported.¹⁴ The amino acid sequence is: MW[(VPGVG)(VPGMG)(VPGVG)₂]₁₀ so the molecular weight is precisely 17,035 $\text{g}\cdot\text{mol}^{-1}$. The ¹H NMR spectrum in CDCl_3 is shown in the supporting information (Figure S1).

Synthesis of block copolymers poly(γ -benzyl-L-glutamate)-block-ELP

Diblock copolypeptides poly(γ -benzyl-L-glutamate)-block-ELP were synthesized by ring opening polymerization (ROP) of γ -BLG NCA using ELP as macroinitiator. Various [monomers]/[initiator] molar ratios were used to obtain copolypeptides with different hydrophilic weight fractions *f*. For example, to prepare PBLG₂₅-*b*-ELP, γ -BLG NCA (9.42 $\times 10^{-2}$ g, 3.58 $\times 10^{-1}$ mmol) was weighed in a glovebox under pure argon, introduced in a flame-dried schlenk, and dissolved in 2 mL of anhydrous DMF. The solution was stirred for 10 min, and the ELP

(2.44×10^{-2} g, 1.43×10^{-3} mmol, $M = 17,035.4$ Da) was added with a nitrogen purged syringe from a solution in anhydrous DMF (1×10^{-2} g.mL⁻¹, injected volume: 24.4 mL). The mixture was stirred for one week at 5°C (or at 25°C) precipitated in diethyl ether, and dried under vacuum to afford a white powder. Yield: 3.12×10^{-1} g (97 %). A copolyptide with a molecular mass of 22.5×10^3 g.mol⁻¹ (determined by ¹H NMR) corresponding to the targeted degree of polymerization of 25 was obtained.

Study of self-assembly behavior of poly(γ -benzyl-L-glutamate)-block-ELP

The diblock amphiphilic copolyptides PBLG-*b*-ELP were self-assembled using various methods to compare the structure and the size of the nano-objects obtained depending on the process used.

Direct solubilization

The first method, the direct solubilization in water was used only for the copolyptide with the highest hydrophilic weight fraction f , PBLG₂₅-*b*-ELP ($f = 79$ %). Indeed, it was not possible to self-assemble under these conditions the others copolymers due to their lower f values without formation of macroscopic aggregates. Shortly, the copolyptide was added in ultrapure water at a concentration of 1 mg.mL⁻¹ and the mixture was stirred overnight at room temperature.

Dialysis

The second method used, the dialysis, consists in a solution of copolymers at 4 g.L⁻¹ in a common solvent of both blocks (here DMSO) dialyzed against a non-solvent of the hydrophobic block of PBLG (here deionized water). The membrane used has a cut off of 15 KDa and the water was renewed 4 times in 24 h. During this process, water diffused into the DMSO phase, leading to

aggregation of the hydrophobic chains and driving the self-assembly process of the amphiphilic block copolypeptides.

Microfluidics

The last method consists in using a microfluidics system from Dolomite[®]. A solution of copolypeptides at 4 g.L⁻¹ in DMSO and deionized water were mixed in a chip containing twelve steps of mixing. Two pumps under pressure of nitrogen brought the solutions with a flow rate controlled by the “Mitos Flow Control Center” software from Dolomite[®] (Scheme S.1). This technique allowed controlling perfectly the speed of mixing depending on the ratio DMSO/water and the total flow rate. For our study, the total flow rate is 100 $\mu\text{L}\cdot\text{min}^{-1}$, the flow rates of the solution of copolypeptides in DMSO and the water are 30 $\mu\text{L}\cdot\text{min}^{-1}$ and 70 $\mu\text{L}\cdot\text{min}^{-1}$ respectively (ratio DMSO:H₂O of 70:30), and also 70 $\mu\text{L}\cdot\text{min}^{-1}$ and 30 $\mu\text{L}\cdot\text{min}^{-1}$ for the PBLG₉₅-*b*-ELP ($f = 45\%$) (ratio DMSO:H₂O of 30:70). Thereafter, the DMSO was removed by dialysis (in a membrane bag with a cut off of 15 KDa). Except for the suspension of PBLG₁₈₀-*b*-ELP for which the characterizations were carried out before.

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References

- (1) Pelegri-O'Day, E. M.; Lin, E.-W.; Maynard, H. D. Therapeutic protein–polymer conjugates: advancing beyond PEGylation. *Journal of the American Chemical Society* **2014**, *136*, 14323-14332.
- (2) Dirks, A. T. J.; Nolte, R. J.; Cornelissen, J. J. Protein–polymer hybrid amphiphiles. *Advanced Materials* **2008**, *20*, 3953-3957.
- (3) Velonia, K.; Rowan, A. E.; Nolte, R. J. Lipase polystyrene giant amphiphiles. *Journal of the American Chemical Society* **2002**, *124*, 4224-4225.
- (4) Boerakker, M. J.; Hannink, J. M.; Bomans, P. H.; Frederik, P. M.; Nolte, R. J.; Meijer, E. M.; Sommerdijk, N. A. Giant amphiphiles by cofactor reconstitution. *Angewandte Chemie International Edition* **2002**, *41*, 4239-4241.
- (5) Boerakker, M. J.; Botterhuis, N. E.; Bomans, P. H.; Frederik, P. M.; Meijer, E. M.; Nolte, R. J.; Sommerdijk, N. A. Aggregation behavior of giant amphiphiles prepared by cofactor reconstitution. *Chemistry-A European Journal* **2006**, *12*, 6071-6080.
- (6) Le Droumaguet, B.; Velonia, K. In Situ ATRP-Mediated Hierarchical Formation of Giant Amphiphile Bionanoreactors. *Angewandte Chemie* **2008**, *120*, 6359-6362.
- (7) Le Droumaguet, B.; Mantovani, G.; Haddleton, D. M.; Velonia, K. Formation of giant amphiphiles by post-functionalization of hydrophilic protein–polymer conjugates. *Journal of Materials Chemistry* **2007**, *17*, 1916-1922.
- (8) Boyer, C.; Bulmus, V.; Liu, J.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. Well-defined protein–polymer conjugates via in situ RAFT polymerization. *Journal of the American Chemical Society* **2007**, *129*, 7145-7154.
- (9) Liu, Z.; Dong, C.; Wang, X.; Wang, H.; Li, W.; Tan, J.; Chang, J. Self-Assembled Biodegradable Protein–Polymer Vesicle as a Tumor-Targeted Nanocarrier. *ACS applied materials & interfaces* **2014**, *6*, 2393-2400.
- (10) Liu, X.; Gao, W. In Situ Growth of Self-Assembled Protein-Polymer Nanovesicles for Enhanced Intracellular Protein Delivery. *ACS applied materials & interfaces* **2017**.
- (11) Huang, X.; Li, M.; Green, D. C.; Williams, D. S.; Patil, A. J.; Mann, S. Interfacial assembly of protein-polymer nano-conjugates into stimulus-responsive biomimetic protocells. *Nature communications* **2013**, *4*, 2239.
- (12) Urry, D. W.; Trapane, T.; Prasad, K. Phase-structure transitions of the elastin polypentapeptide–water system within the framework of composition–temperature studies. *Biopolymers* **1985**, *24*, 2345-2356.
- (13) Urry, D. W. Physical chemistry of biological free energy transduction as demonstrated by elastic protein-based polymers. *The Journal of Physical Chemistry B* **1997**, *101*, 11007-11028.
- (14) Petitdemange, R.; Garanger, E.; Bataille, L.; Dieryck, W.; Bathany, K.; Garbay, B.; Deming, T. J.; Lecommandoux, S. Selective tuning of elastin-like polypeptide properties via methionine oxidation. *Biomacromolecules* **2017**, *18*, 544-550.
- (15) Kramer, J. R.; Petitdemange, R.; Bataille, L.; Bathany, K.; Wirotius, A.-L.; Garbay, B.; Deming, T. J.; Garanger, E.; Lecommandoux, S. Quantitative Side-Chain

Modifications of Methionine-Containing Elastin-Like Polypeptides as a Versatile Tool to Tune Their Properties. *ACS Macro Letters* **2015**, *4*, 1283-1286.

(16) Petitdemange, R.; Garanger, E.; Bataille, L.; Bathany, K.; Garbay, B.; Deming, T. J.; Lecommandoux, S. Tuning Thermo-responsive Properties of Cationic Elastin-like Polypeptides by Varying Counterions and Side-Chains. *Bioconjugate chemistry* **2017**, *28*, 1403-1412.

(17) Ayres, L.; Vos, M. R.; Adams, P. H. M.; Shklyarevskiy, I. O.; van Hest, J. C. Elastin-based side-chain polymers synthesized by ATRP. *Macromolecules* **2003**, *36*, 5967-5973.

(18) Ayres, L.; Koch, K.; Adams, P. H. H.; van Hest, J. C. Stimulus responsive behavior of elastin-based side chain polymers. *Macromolecules* **2005**, *38*, 1699-1704.

(19) Roberts, S. K.; Chilkoti, A.; Setton, L. A. Multifunctional thermally transitioning oligopeptides prepared by ring-opening metathesis polymerization. *Biomacromolecules* **2007**, *8*, 2618-2621.

(20) Conrad, R. M.; Grubbs, R. H. Tunable, Temperature-Responsive Polynorbornenes with Side Chains Based on an Elastin Peptide Sequence. *Angewandte Chemie International Edition* **2009**, *48*, 8328-8330.

(21) Kojima, C.; Irie, K. Synthesis of Temperature-Dependent Elastin-Like Peptide-Modified Dendrimer for Drug Delivery. *Biopolymers* **2013**, *100*, 714-721.

(22) Luo, T.; Kiick, K. L. Noncovalent Modulation of the Inverse Temperature Transition and Self-Assembly of Elastin-b-Collagen-like Peptide Bioconjugates. *Journal of the American Chemical Society* **2015**, *137*, 15362-15365.

(23) Pu, Y.; Zhang, L.; Zheng, H.; He, B.; Gu, Z. Synthesis and Drug Release of Star-Shaped Poly (benzyl L-aspartate)-block-poly (ethylene glycol) Copolymers with POSS Cores. *Macromolecular bioscience* **2014**, *14*, 289-297.

(24) Shen, J.; Chen, C.; Fu, W.; Shi, L.; Li, Z. Conformation-specific self-assembly of thermo-responsive poly (ethylene glycol)-b-polypeptide diblock copolymer. *Langmuir* **2013**, *29*, 6271-6278.

(25) Lee, H.; Park, J. B.; Chang, J. Y. Synthesis of poly (ethylene glycol)/polypeptide/poly (D, L-lactide) copolymers and their nanoparticles. *Journal of Polymer Science Part A: Polymer Chemistry* **2011**, *49*, 2859-2865.

(26) Gao, H.; Hu, Z.; Guan, Q.; Liu, Y.; Zhu, F.; Wu, Q. Synthesis and thermoreversible gelation of coil-helical polyethylene-block-poly (γ -benzyl-L-glutamate) diblock copolymer. *Polymer* **2013**, *54*, 4923-4929.

(27) Holley, A. C.; Ray, J. G.; Wan, W.; Savin, D. A.; McCormick, C. L. Endolytic, pH-responsive HEMA-b-(l-Glu) copolymers synthesized via sequential aqueous RAFT and ring-opening polymerizations. *Biomacromolecules* **2013**, *14*, 3793-3799.

(28) Waley, S.; Watson, J. In *Tilte*1949; The Royal Society.

(29) Ballard, D.; Bamford, C. 77. Reactions of N-carboxy- α -amino-acid anhydrides catalysed by tertiary bases. *Journal of the Chemical Society (Resumed)* **1956**, 381-387.

(30) Rodríguez-Hernández, J.; Lecommandoux, S. Reversible inside-out micellization of pH-responsive and water-soluble vesicles based on polypeptide diblock copolymers. *Journal of the American Chemical Society* **2005**, *127*, 2026-2027.

(31) Holowka, E. P.; Pochan, D. J.; Deming, T. J. Charged polypeptide vesicles with controllable diameter. *Journal of the American Chemical Society* **2005**, *127*, 12423-12428.

(32) Huang, J.; Bonduelle, C.; Thévenot, J.; Lecommandoux, S. b.; Heise, A. Biologically active polymersomes from amphiphilic glycopeptides. *Journal of the American Chemical Society* **2011**, *134*, 119-122.

(33) Upadhyay, K. K.; Meins, J.-F. L.; Misra, A.; Voisin, P.; Bouchaud, V.; Ibarboure, E.; Schatz, C.; Lecommandoux, S. Biomimetic doxorubicin loaded polymersomes from hyaluronan-block-poly (γ -benzyl glutamate) copolymers. *Biomacromolecules* **2009**, *10*, 2802-2808.

(34) Schatz, C.; Louguet, S.; Le Meins, J. F.; Lecommandoux, S. Polysaccharide-block-polypeptide Copolymer Vesicles: Towards Synthetic Viral Capsids. *Angewandte Chemie International Edition* **2009**, *48*, 2572-2575.

(35) Upadhyay, K. K.; Bhatt, A. N.; Mishra, A. K.; Dwarakanath, B. S.; Jain, S.; Schatz, C.; Le Meins, J.-F.; Farooque, A.; Chandraiah, G.; Jain, A. K. The intracellular drug delivery and anti tumor activity of doxorubicin loaded poly (γ -benzyl l-glutamate)-b-hyaluronan polymersomes. *Biomaterials* **2010**, *31*, 2882-2892.

(36) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. Synthesis of well-defined polypeptide-based materials via the ring-opening polymerization of α -amino acid N-carboxyanhydrides. *Chemical reviews* **2009**, *109*, 5528-5578.

(37) Cheng, J.; Deming, T. J.: Synthesis of polypeptides by ring-opening polymerization of α -amino acid N-carboxyanhydrides. In *Peptide-based materials*; Springer, 2011; pp 1-26.

(38) Huang, J.; Heise, A. Stimuli responsive synthetic polypeptides derived from N-carboxyanhydride (NCA) polymerisation. *Chemical Society Reviews* **2013**, *42*, 7373-7390.

(39) Habraken, G. J.; Peeters, M.; Dietz, C. H.; Koning, C. E.; Heise, A. How controlled and versatile is N-carboxy anhydride (NCA) polymerization at 0 C? Effect of temperature on homo-, block-and graft (co) polymerization. *Polymer Chemistry* **2010**, *1*, 514-524.

(40) Hanby, W.; Waley, S.; Watson, J. 632. Synthetic polypeptides. Part II. Polyglutamic acid. *Journal of the Chemical Society (Resumed)* **1950**, 3239-3249.

(41) Vayaboury, W.; Giani, O.; Cottet, H.; Deratani, A.; Schué, F. Living Polymerization of α -Amino Acid N-Carboxyanhydrides (NCA) upon Decreasing the Reaction Temperature. *Macromolecular rapid communications* **2004**, *25*, 1221-1224.

(42) Habraken, G. J.; Wilsens, K. H.; Koning, C. E.; Heise, A. Optimization of N-carboxyanhydride (NCA) polymerization by variation of reaction temperature and pressure. *Polymer Chemistry* **2011**, *2*, 1322-1330.

- (43) Lu, H.; Wang, J.; Song, Z.; Yin, L.; Zhang, Y.; Tang, H.; Tu, C.; Lin, Y.; Cheng, J. Recent advances in amino acid N-carboxyanhydrides and synthetic polypeptides: chemistry, self-assembly and biological applications. *Chemical Communications* **2014**, *50*, 139-155.
- (44) Nakamura, R.; Aoi, K.; Okada, M. Controlled Synthesis of a Chitosan-Based Graft Copolymer Having Polysarcosine Side Chains Using the NCA Method with a Carboxylic Acid Additive. *Macromolecular rapid communications* **2006**, *27*, 1725-1732.
- (45) Chécot, F.; Brûlet, A.; Oberdisse, J.; Gnanou, Y.; Mondain-Monval, O.; Lecommandoux, S. Structure of polypeptide-based diblock copolymers in solution: Stimuli-responsive vesicles and micelles. *Langmuir* **2005**, *21*, 4308-4315.
- (46) Peyret, A.; Trant, J. F.; Bonduelle, C. V.; Ferji, K.; Jain, N.; Lecommandoux, S.; Gillies, E. R. Synthetic glycopolypeptides: synthesis and self-assembly of poly (γ -benzyl-L-glutamate)-glycosylated dendron hybrids. *Polymer Chemistry* **2015**, *6*, 7902-7912.
- (47) Bonduelle, C.; Huang, J.; Ibarboure, E.; Heise, A.; Lecommandoux, S. Synthesis and self-assembly of “tree-like” amphiphilic glycopolypeptides. *Chemical communications* **2012**, *48*, 8353-8355.
- (48) Bradbury, E.; Crane-Robinson, C.; Paolillo, L.; Temussi, P. Nmr studies of the helix-coil transition of polypeptides in non-protonating solvent mixtures. *Polymer* **1973**, *14*, 303-308.
- (49) Jeong, Y.-I.; Seo, S.-J.; Park, I.-K.; Lee, H.-C.; Kang, I.-C.; Akaike, T.; Cho, C.-S. Cellular recognition of paclitaxel-loaded polymeric nanoparticles composed of poly (γ -benzyl L-glutamate) and poly (ethylene glycol) diblock copolymer endcapped with galactose moiety. *International journal of pharmaceutics* **2005**, *296*, 151-161.
- (50) Heller, P.; Mohr, N.; Birke, A.; Weber, B.; Reske-Kunz, A.; Bros, M.; Barz, M. Directed Interactions of Block Copolypept (o) ides with Mannose-binding Receptors: PeptoMicelles Targeted to Cells of the Innate Immune System. *Macromolecular bioscience* **2015**, *15*, 63-73.
- (51) Discher, D. E.; Eisenberg, A. Polymer vesicles. *Science* **2002**, *297*, 967-973.
- (52) Lee, C.-U.; Lu, L.; Chen, J.; Garno, J. C.; Zhang, D. Crystallization-driven thermoreversible gelation of coil-crystalline cyclic and linear diblock copolypeptoids. *ACS Macro Letters* **2013**, *2*, 436-440.
- (53) Kakkar, D.; Mazzaferro, S.; Thevenot, J.; Schatz, C.; Bhatt, A.; Dwarakanath, B. S.; Singh, H.; Mishra, A. K.; Lecommandoux, S. Amphiphilic PEO-b-PBLG Diblock and PBLG-b-PEO-b-PBLG Triblock Copolymer Based Nanoparticles: Doxorubicin Loading and In Vitro Evaluation. *Macromolecular bioscience* **2015**, *15*, 124-137.
- (54) Zhang, L.; Eisenberg, A. Thermodynamic vs kinetic aspects in the formation and morphological transitions of crew-cut aggregates produced by self-assembly of polystyrene-b-poly (acrylic acid) block copolymers in dilute solution. *Macromolecules* **1999**, *32*, 2239-2249.
- (55) Cui, H.; Chen, Z.; Zhong, S.; Wooley, K. L.; Pochan, D. J. Block copolymer assembly via kinetic control. *Science* **2007**, *317*, 647-650.

(56) Bleul, R.; Thiermann, R.; Maskos, M. Techniques to control polymersome size. *Macromolecules* **2015**, *48*, 7396-7409.

Table 1. Molecular characteristics of ELP and of the different hybrid diblock copolypeptides PBLG-*b*-ELP obtained by ROP of γ -BLG NCA at 5 °C.

Copolypeptide	Expected DP (PBLG)	DP PBLG (¹ H NMR)	M_n ¹ H NMR (g.mol ⁻¹)	f (%) [*]	\overline{M}_n SEC ^{**} (g.mol ⁻¹)	\overline{D} SEC ^{**}	Yield (%)
ELP	-	-	-	-	18,000	1.03	-
PBLG ₂₅ - <i>b</i> -ELP	25	25	22,500	76	33,300	1.02	97
PBLG ₄₅ - <i>b</i> -ELP	52	45	26,900	63	40,100	1.02	85
PBLG ₉₅ - <i>b</i> -ELP	117	95	37,800	45	49,800	1.03	88
PBLG ₁₈₀ - <i>b</i> -ELP	181	180	56,500	30	56,200	1.03	93

* f : hydrophilic weight fraction determined from the \overline{M}_n calculated by ¹H NMR

**SEC in DMF (0.8 mL.min⁻¹) at 50 °C in the presence of LiBr (1 g.L⁻¹) with RI detector and polystyrene used as standard.

Table 2. Characteristics of nanometer-sized particles obtained from the self-assembly of amphiphilic PBLG-*b*-ELP hybrid copolypeptides.

Copolypeptide	f (%) ¹ H NMR	Self-assembly process	R_h^* PDI	Structure of nano- objects**
PBLG ₂₅ - <i>b</i> -ELP	76	Dialysis	27 nm 0.32	Micelles + aggregates
		Microfluidics	15 nm 0.14	Micelles
		Direct solubilization	39 nm 0.13	Spheres
PBLG ₄₅ - <i>b</i> -ELP	63	Dialysis	30 nm 0.25	Micelles
		Microfluidics	17 nm 0.08	Micelles
PBLG ₉₅ - <i>b</i> -ELP	45	Dialysis	133 nm 0.32	Worm-like micelles
		Microfluidics	128 nm 0.29	Worm-like micelles
		Microfluidics***	42 nm 0.47	Spheres
PBLG ₁₈₀ - <i>b</i> -ELP	30	Dialysis	63 nm 0.28	Spheres
		Microfluidics	76 nm 0.19	Vesicles

*Determined by DLS at 90° at 1 mg.mL⁻¹

**Observed by TEM

***Ratio DMSO:H₂O of 30:70

Scheme 1. Synthesis of hybrid diblock copolymers poly(γ -benzyl-L-glutamate)-*block*-ELP by ROP of γ -BLG NCA initiated by the primary ammonium terminal group of the ELP.

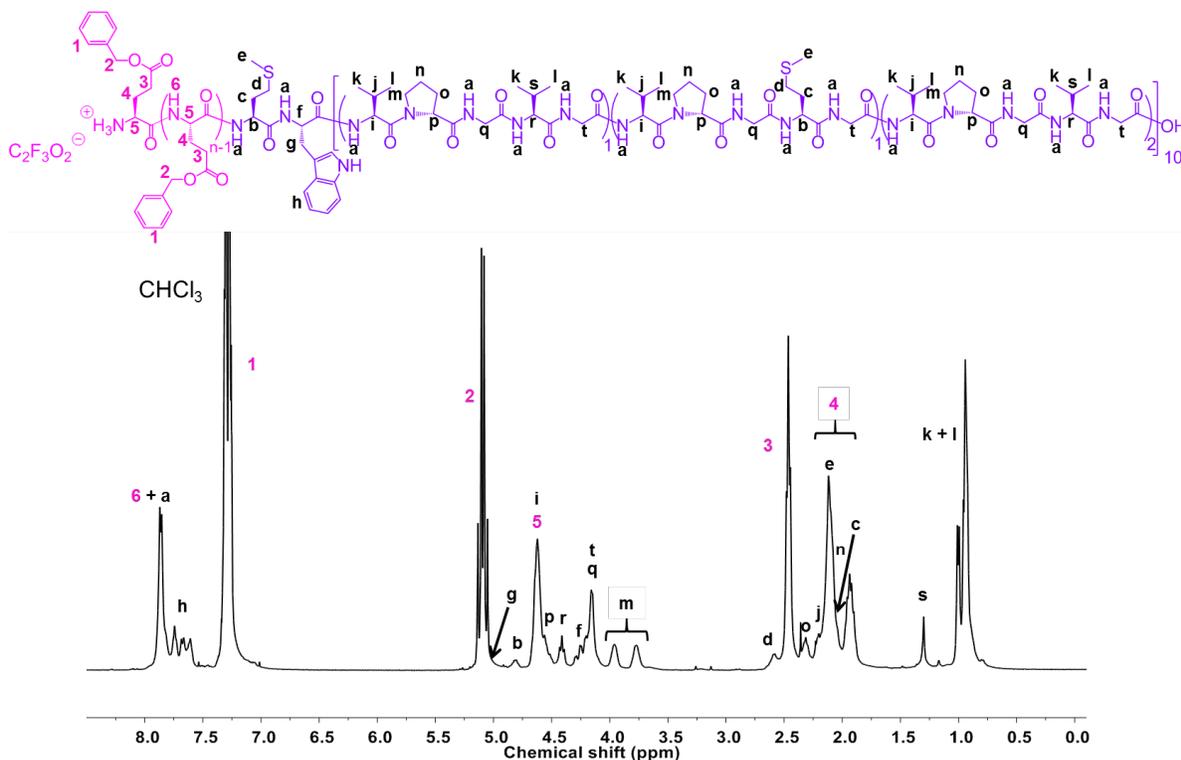


Figure 1. ^1H NMR spectrum of poly(γ -benzyl-L-glutamate)₁₈₀-*block*-ELP in CDCl_3 (15 % TFA).

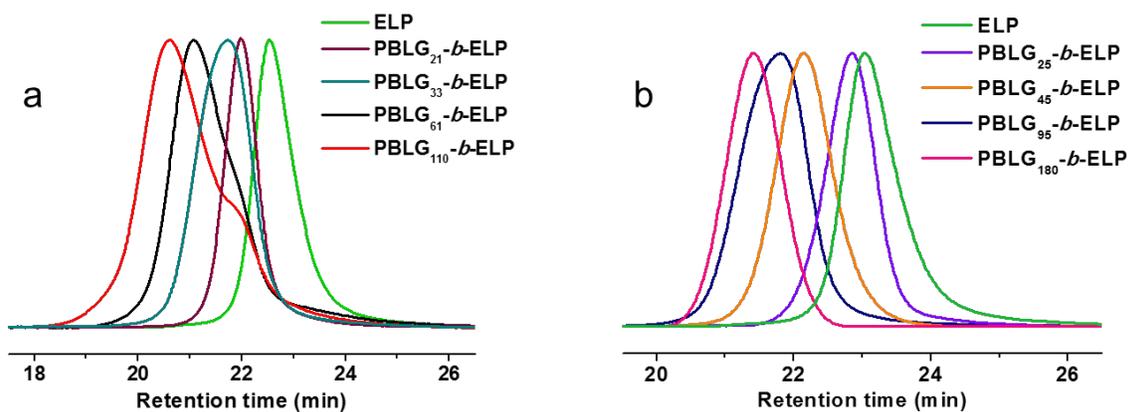


Figure 2. Size exclusion chromatography traces of ELP and hybrid diblock copolypeptides PBLG-*b*-ELP obtained by ROP of γ -BLG NCA at 25 °C (a) and at 5 °C (b).

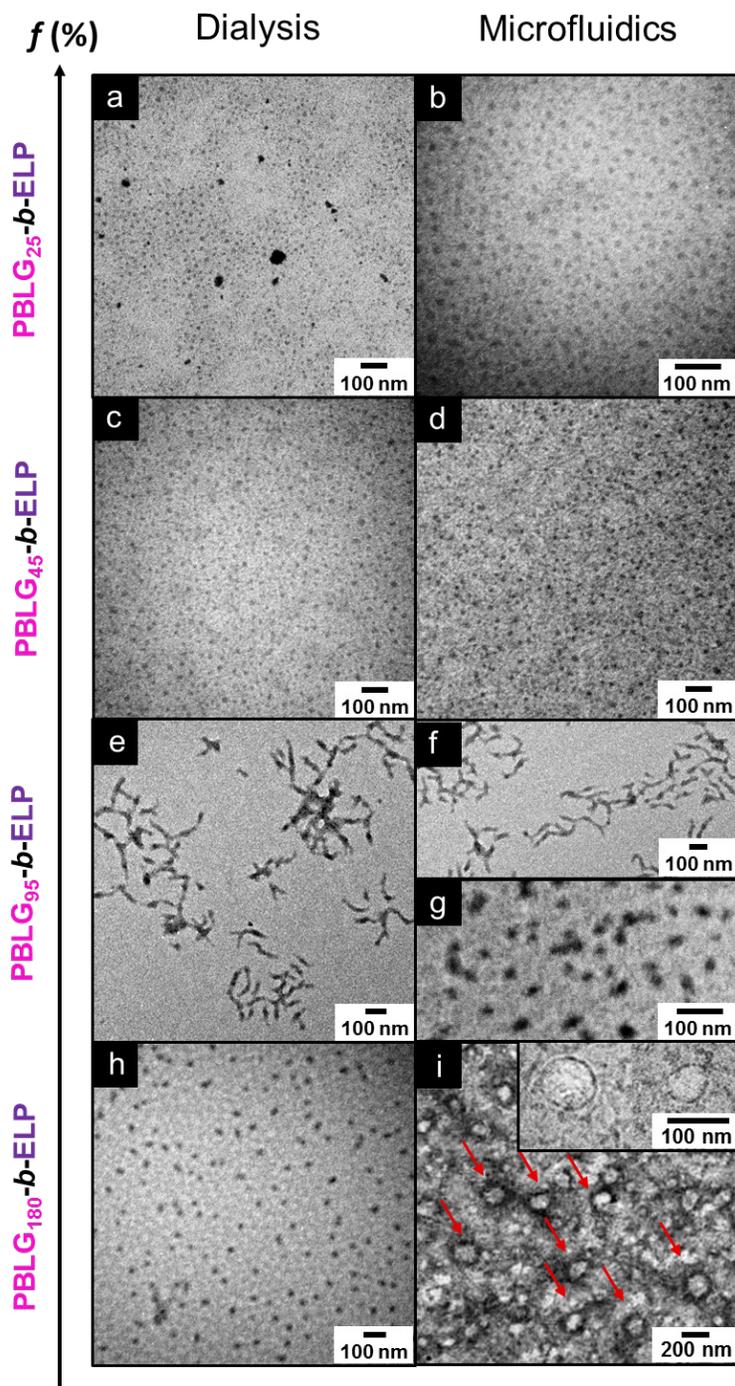
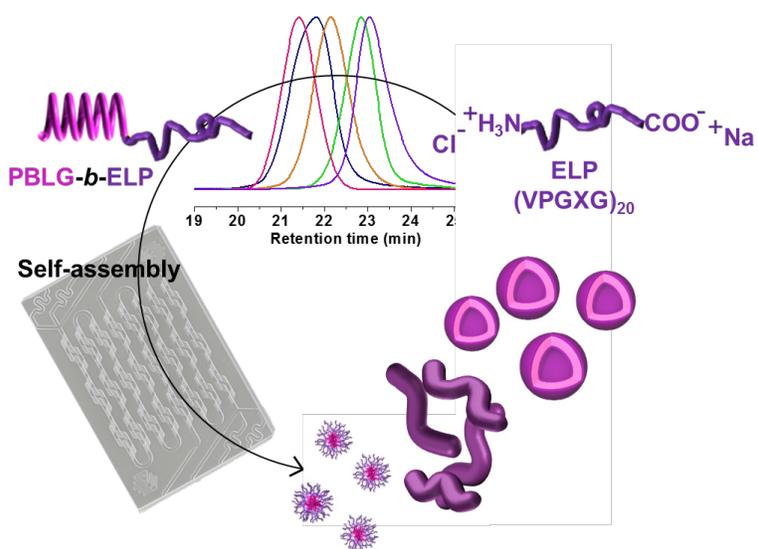


Figure 3. TEM micrographs of nano-objects obtained from PBLG_n-b-ELP depending on the self-assembly process. (f) Ratio DMSO:H₂O by microfluidics 70:30, (g) Ratio DMSO:H₂O by

microfluidics 30:70, (i) observed directly after the formation of nano-objects (cryo-TEM in the insert).

TOC



SUPPORTING INFORMATION:

Design and self-assembly of PBLG-*b*-ELP hybrid diblock copolymers based on synthetic and elastin-like polypeptides

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Synthesis mechanism hypothesis

Because the purification of ELP involves the use of sodium chloride, we can reasonably assume the polymerization to be initiated by the *N*-terminal ammonium group of the ELP with the chloride Cl⁻ as counter-anion. Consequently, the block copolypeptides poly(γ -benzyl-*L*-glutamate)-*block*-ELP were synthesized by ROP of the γ -BLG NCA initiated by the primary ammonium end group of the ELP.

It was previously postulated by Schlaad et *al.* that the ammonium-mediated ROP mechanism may lead to a controlled propagation comparable to nitroxyde-mediated radical or living cationic polymerizations involving an equilibrium between dormant (ammonium) and active (amine) chain ends¹ provided that the counter-anion, here the nucleophilic chloride anion, is quite mobile in the medium.^{1,2} The ammonium salt in addition is suspected to suppress the activated monomer mechanism (AMM) due to protonation of the NCA anions.¹

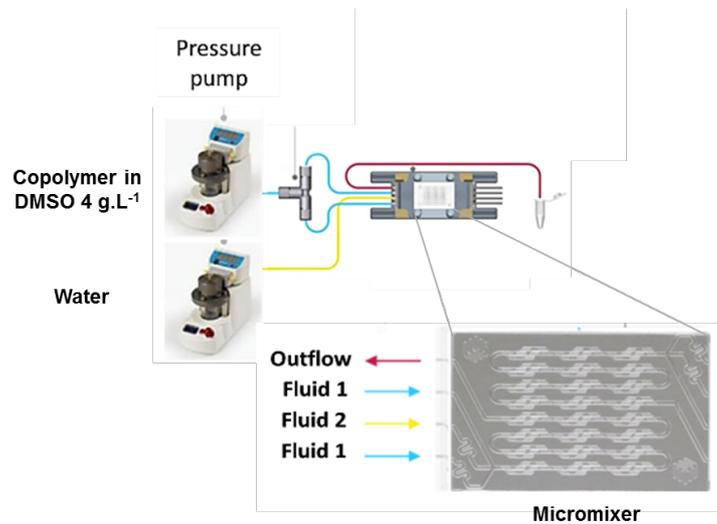
Interestingly, in the present case of the polymerization of γ -BLG NCA, we suspect the ELP macroinitiator to afford sufficient polarity to the medium to allow an excellent control of ammonium-mediated ROP with the chloride counterion. We additionally presume the formation of hydrogen bonds between the polypeptide chain amide groups and the γ -BLG NCA, stabilizing the latter and limiting the AMM mechanism, and consequently favoring the normal NAM amine mechanism.

Table S1. Molecular characteristics of ELP and hybrid diblock copolypeptides PBLG-*b*-ELP obtained by ROP of γ -BLG NCA at 25 °C.

#	Copolypeptide	Expected DP (PBLG)	DP PBLG (¹ H NMR)	\overline{M}_n ¹ H NMR (g.mol ⁻¹)	<i>f</i> (%) ¹ H NMR	\overline{M}_n SEC* (g.mol ⁻¹)	<i>D</i> SEC*	Yield (%)
-	ELP	-	-	17,000	-	18,000	1.03	-
1	PBLG₂₁-<i>b</i>-ELP	52	21	21,600	79	24,900	1.03	73
2	PBLG₃₃-<i>b</i>-ELP	78	33	24,300	70	30,200	1.06	80
3	PBLG₆₁-<i>b</i>-ELP	117	61	30,400	56	37,100	1.15	77
4	PBLG₁₁₀-<i>b</i>-ELP	181	110	41,200	41	44,800	1.20	70

**f*: hydrophilic weight fraction determined from the \overline{M}_n calculated by ¹H NMR

**SEC in DMF (0.8 mL.min⁻¹) at 50 °C in the presence of LiBr (1 g.L⁻¹) with RI detector and polystyrene used as standard.



Scheme S1. Schematic representation of the microfluidics device used.

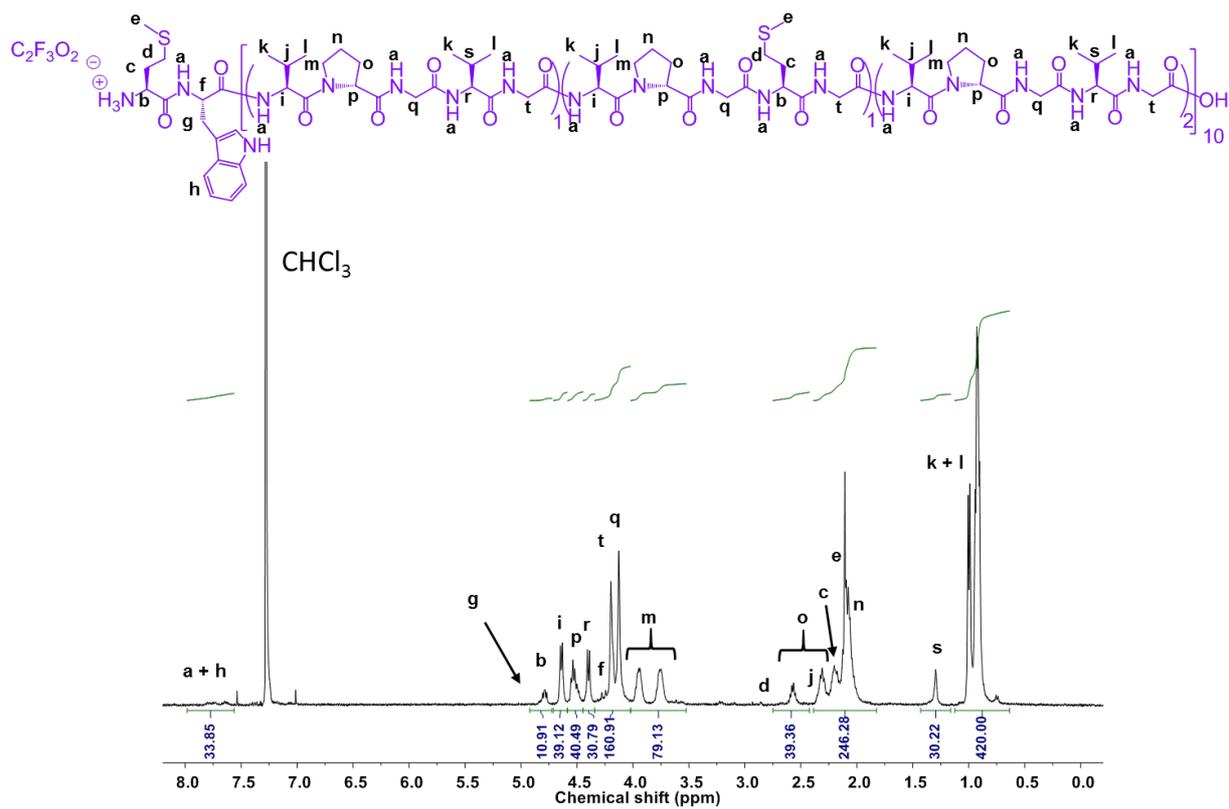


Figure S1. ^1H NMR spectrum of ELP in CDCl_3 containing 15 % trifluoroacetic acid (TFA).

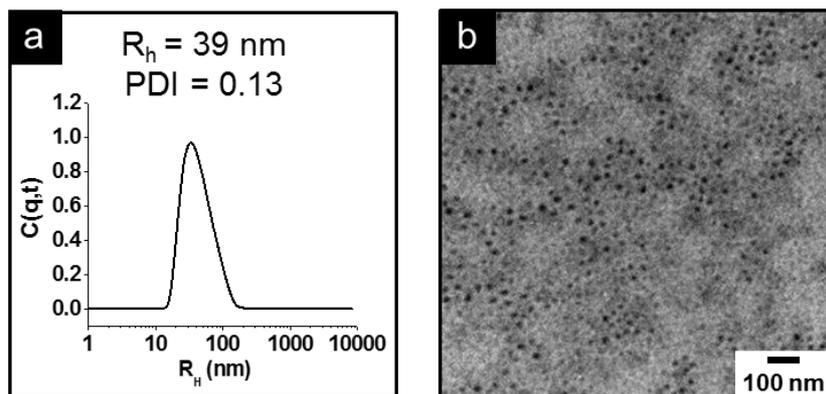


Figure S2. (a) Size distribution of nano-particles by DLS.(b) TEM micrographs of nano-particles obtained from PBLG₂₅-*b*-ELP by direct solubilization.

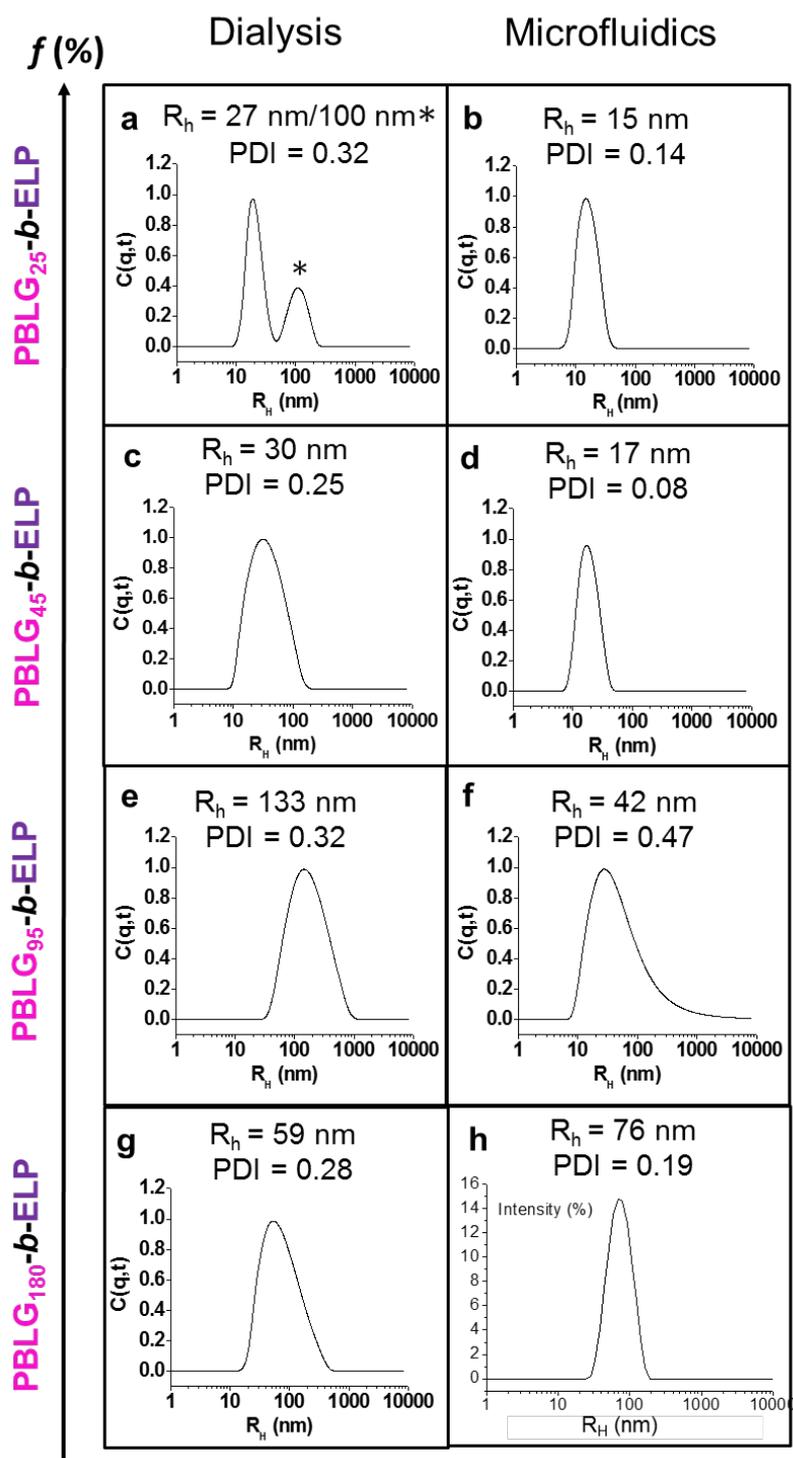


Figure S3. Size distribution of nano-particles by DLS depending on the technique of self-assembly process. (i) Characterized directly after the formation of nano-objects. Ratio DMSO:H₂O by microfluidics is 70:30.

- (1) Dimitrov, I.; Schlaad, H. *Chemical Communications* **2003**, 2944-2945.
- (2) Lutz, J. F.; Schütt, D.; Kubowicz, S. *Macromolecular rapid communications* **2005**, 26, 23-28.