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Poly(ϵ -L-lysine)-decorated particles with a tunable morphology by a combination of Ugi multicomponent polymerisation and RAFT-mediated PISA†

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We report herein the unprecedented combination of the Ugi multicomponent reaction (Ugi MCR) and Polymerisation-Induced Self-Assembly (PISA) to fabricate poly(ϵ -L-lysine) (P ϵ LL) decorated amphiphilic nanoparticles. First, using the one-pot Ugi MCR of Boc-protected L-lysine with the addition of a trithio-carbonate (TTC) compound, a macromolecular chain transfer agent (macroCTA) for Reversible Addition–Fragmentation chain-Transfer (RAFT) polymerisation was synthesised. The obtained P ϵ LL–TTC was then used for chain extension of the hydrophobic 2-hydroxypropyl methacrylate (HPMA) in water. The reaction proceeded *via* the PISA mechanism, leading to the formation of amphiphilic block copolymers that self-assembled into a variety of morphologies—including spheres, short worm-like structures, and vesicles—spanning sizes from below 100 nm to over 1 μ m. This work showcases a proof-of-concept to prepare water-dispersed nanoparticles decorated with polypeptoids, which are highly desired in biological applications (e.g. stealth-cargo in nanomedicine, bio-separation, antibacterial coating, etc.).

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Introduction

Polymerisation-Induced Self-Assembly (PISA) has emerged as a powerful technique for generating self-assembled nanoparticles, particularly in aqueous media, aligning with environmentally friendly practices. During PISA, the chain extension of a hydrophilic polymer block with hydrophobic monomers triggers *in situ* self-assembly, yielding nanostructures such as spherical micelles, fibers, worm-like particles, vesicles, and lamellas.¹ Unlike traditional methods requiring volatile organic solvents, PISA offers a sustainable alternative for biomedical applications.² For the purpose of chain-extension, PISA is dominantly coupled with controlled polymerisation techniques, including atom transfer radical polymerisation, nitroxide-mediated polymerisation, ring-opening metathesis

polymerisation, and ring-opening polymerisation, but predominantly with Reversible-Addition Fragmentation chain Transfer (RAFT) polymerisation.^{3–7} Typically, a hydrophilic macro-chain transfer agent (macroCTA) is synthesised in an organic solvent before initiating PISA.^{8–11} While polyethylene glycol, poly(meth)acrylamides and poly(meth)acrylates are common macroCTAs, only a few examples of alternatives are described in the literature.¹²

Polypeptoids, due to their structural similarity to natural polypeptides, are promising candidates for PISA-based biomaterials, offering biocompatibility, stability, and tunable functionality.^{13–15} Polypeptoids can be synthesised *via* solid-phase synthesis or through the ring-opening polymerisation of *N*-carboxyanhydrides.^{16–18} These methods have been combined with PISA to produce water-soluble coronas or hydrophobic cores. However, while solid-phase synthesis is effective for short oligomers, it requires labor-intensive protection–deprotection steps, whereas ring-opening polymerisation demands complex monomer synthesis and stringent reaction conditions.¹⁹ In contrast, the Ugi multicomponent reaction (Ugi MCR) provides an efficient route to obtain polypeptoids with atom economy, mild conditions, and broad structural diversity.^{20–24} Extensively applied in various fields,^{25–29} this approach has proven particularly useful for polymerisation of amino acids. For example, Zhang *et al.* synthesised γ -, δ -, and

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ϵ -polypeptoids from bifunctional amino acids for drug encapsulation,³⁰ while Debuigne's group developed alternating polypeptide-polypeptoid copolymers and explored their functionalisation.^{31,32} Tao *et al.* further confirmed the polycondensation nature of the Ugi MCR, leading to the formation of alternating polypeptoids and polyampholytes.³³ Among diverse polypeptoids that can be obtained by the Ugi MCR, PeLL is of great interest for further investigation. Resulting from the biosynthesis of various microorganisms,^{34,35} naturally existing PeLL is known for its antimicrobial activity, heat stability and low toxicity. The functionalisation of PeLL has, thus, attracted continued interest for various applications in the medical field.^{36–38} For this purpose, along with other chemosynthetic routes for the functionalisation of PeLL,^{30,39} the Ugi MCR has emerged as a promising technique for synthesising polymers while enabling the introduction of new functionalities.

The combination of the Ugi MCR with controlled radical polymerisation has been reported in the literature. For example, the group of Tao has described the use of the Ugi MCR to graft polymers on carbon nanotubes,⁴⁰ to conjugate polymers with proteins,⁴¹ or to prepare miktoarm polymers⁴² by RAFT polymerisation. Despite the efficiency of the Ugi MCR in polypeptoid synthesis, the use of the Ugi MCR to prepare hydrophilic blocks for PISA-RAFT remains unexplored.

In this work, we demonstrate the one-pot synthesis of a macroCTA trithiocarbonate (TTC) derived from PeLL using the Ugi MCR, hereinafter referred to as PeLL-TTC. The obtained PeLL-TTC is characterised by various techniques (NMR, SEC, UV-Vis, and MALDI-ToF) to confirm the chain-end fidelity and the molar mass. PeLL-TTC is then employed as a hydrophilic block for chain extension with the hydrophobic HPMA in water at 55 °C with systematic changes in mass content and degree of polymerisation (DP) of the PHPMA block. Formation of particles is observed during the polymerisation and characterised by various analyses including dynamic light scattering (DLS), transmission electron microscopy (TEM), and when adapted small angle X-ray scattering (SAXS).

Experimental

Materials

Unless otherwise stated, all chemicals are used as received. KOH (Sigma Aldrich), acetone (pure, Carlo Erba), ethanethiol (97% pure, Alfa Aesar), carbon disulfide (99.9%, Alfa Aesar), 4-toluenesulfonyl chloride (PTSC), dichloromethane (pure, stabilised with amylene, Carlo Erba), 4,4'-azobis(4-cyanovaleic acid) (AICV, 98% pure, Sigma Aldrich), *tert*-butyloxycarbonyl-L-lysine (Boc-Lys) (97% pure, Alfa Aesar), formaldehyde (37 wt% in water with 10–15% methanol as a stabiliser, Thermo Scientific), *tert*-butyl isocyanide (98% pure, Sigma Aldrich or 97%, Thermo Scientific), diethyl ether (pure, stabilised with BHT), trifluoroacetic acid (99% pure, Thermo Scientific), 2,2'-azobis(2-methylpropionamide) dihydrochloride (V-50, granular, 99% pure, Aldrich), 2-hydroxypropyl methacrylate (HPMA, 98% pure, mixture of isomers, stabilised by 0.02% 4-methoxy-

phenol, Alfa Aesar), and methanol (anhydrous, Carlo Erba). HPMA was filtered over silica gel to remove the stabiliser before use.

Methods

Synthesis of 4-cyano-4-[[[(ethylthio)thioxomethyl]thio]penta-noic acid (CEPA). CEPA was synthesised as reported in the literature.⁴³ In brief, KOH (6.72 g, 0.07 mol) was dissolved in 25 mL of an acetone:water (20:5 v/v) mixture in a round bottom flask. Ethanethiol (7.4 mL, 0.10 mol) was added to the mixture under stirring. The flask was then cooled down in an acetone bath followed by the addition of carbon disulfide (6 mL, 7.61 g, 0.10 mol). After 45 minutes of stirring, PTSC (9.91 g, 0.052 mol) was added to the mixture and then stirred at room temperature for 1 hour. The mixture was then heated to 45 °C and stirred for 30 minutes. The remaining was then diluted in 150 mL of dichloromethane and washed with 50 mL of distilled water (twice). The volatile compounds were eliminated by evaporation under a fume hood, and an orange crude product was obtained. AICV (15.2 g, 0.053 mol) was added to the crude product followed by the addition of 300 mL of ethyl acetate. The whole mixture was then degassed with argon, stirred and then heated to 75 °C with reflux for 20 hours. The mixture was cooled down to room temperature and the solvent was then evaporated using a stream of argon. The final product was obtained by column chromatography on silica gel using a gradient solvent mixture of cyclohexane and dichloromethane with >99% dichloromethane at the end. The purified product was obtained as an orange solid after evaporation of the solvent. ¹H-NMR: DMSO-d₆, 400 Hz, δ : 1.36 ppm (triplet, 3H, CH₃-CH₂-), 1.88 ppm (singlet, 3H, CH₃-C(CN)-), 2.40–2.53 ppm (m, 2H, -C(CN)-CH₂-), 2.69 ppm (m, -CH₂-COOH), 2.35 ppm (quartet, 2H, CH₃-CH₂-S-C(=S)-S).

Synthesis of Boc-PeLL-TTC. In a 10 mL round bottom flask, CEPA (0.10 g, 0.38 mmol, 0.05 eq.) was weighed followed by Boc-Lys (1.78 g, 7.21 mmol, 0.95 eq.); then 8 mL of methanol:H₂O (1:1 v/v) was added and the mixture was stirred using a magnetic bar. Formaldehyde (9.87 mmol, 1.3 eq.) was added to the mixture using a micropipette, resulting in full dissolution of all solids. Finally, with precaution, *tert*-butyl isocyanide (9.87 mmol, 1.3 eq.) was added dropwise to the reaction mixture using a micropipette. The flask was secured and closed with a septum. The reaction proceeded at room temperature. After 24 hours, phase separation was observed. The polymers (Boc-PeLL-TTC) were isolated by discarding the transparent aqueous phase. The crude polymer was then diluted in methanol prior to precipitation in cold diethyl ether. After filtration through a Büchner funnel under reduced pressure, a light-yellow powder was obtained and subsequently dried under vacuum to yield purified Boc-PeLL-TTC (yield: 30%).

Deprotection of Boc-PeLL-TTC to obtain PeLL-TTC. 400 mg of Boc-PeLL-TTC was charged into a 10 mL round bottom flask with a stirring bar. The polymer was dissolved in 1 mL of molecular-sieve-dried dichloromethane. The flask was then immersed in an ice bath. 1 mL of trifluoroacetic acid was added dropwise to the reaction mixture. After 3–4 hours, the

reaction mixture was concentrated by rotary evaporation. The PeLL-TTC polymer was retrieved by precipitation in cold diethylether and dried under vacuum to obtain a light yellow powder (yield: 90%).

PISA-RAFT chain extension of PeLL-TTC with HPMA. A typical PISA-RAFT chain extension of PeLL-TTC with HPMA was carried out as follows: for a target degree of polymerisation (DP_0) of 200 and polymerisation at 5 wt% of the monomer: 10 mg of PeLL-TTC ($M_{n,UV} = 2900 \text{ g mol}^{-1}$, $5 \times 10^{-3} \text{ mmol}$) was weighed into a vial. Then 2.550 mL of Milli-Q water was added to dissolve the macroCTA followed by the addition of 0.250 mL of an aqueous solution containing 0.27 mg of V-50. Finally, 0.140 mL of HPMA was added into the reaction mixture. The vial was then closed with a silicon septum. The reaction mixture was stirred and degassed with a moderate flow of argon for 15 minutes. After degassing, the polymerisation commenced as soon as it was immersed in a preheated oil bath at 55 °C. The polymerisation was quenched by exposure to air after 21 hours of reaction, with >90% monomer conversion confirmed by ^1H NMR analysis.

Kinetics of PISA-RAFT chain extension of the PeLL macroCTA with HPMA. The kinetics of chain extension of the PeLL_{2,9k} macroCTA with HPMA in water was studied at $DP_0 = 100$ with 5 wt% in a 5 mL vial. First, 10.8 mg of V-50 was dissolved in 10 mL of Milli-Q water as a stock solution. 0.5 mL of the initiator stock solution (0.54 mg of V-50, $2.0 \times 10^{-6} \text{ mol}$, 0.2 eq.) was transferred into a vial containing 29 mg of PeLL_{2,9k} ($1.00 \times 10^{-5} \text{ mol}$, 1.0 eq.) and a magnetic stirring bar. 2.30 mL of Milli-Q water was added to the vial followed by the addition of 0.140 mL of HPMA (0.144 g, $1.00 \times 10^{-3} \text{ mol}$, 100 eq., 5 wt%). The vial was closed with a silicone septum and degassed with argon for 15 minutes at room temperature. Argon was removed at the end of degassing and the vial was sealed with parafilm and then immersed in a preheated oil bath at 55 °C to start the kinetics studies. An aliquot (200 μL) of the reaction mixture was taken at regular intervals using a degassed disposable syringe for NMR, SEC, and DLS analyses.

Characterisation

NMR spectroscopy. NMR was carried out using a Bruker 400 MHz spectrometer. The polymer concentration was 20 mg per 1 mL of deuterated solvent and the measurement was carried out in a 5 mm tube. The spectra were analysed using MestReNova 5.3.0 (Mestrelab Research S.L.). Chemical shifts are reported in *ppm*.

Size exclusion chromatography. The molar mass of the polymers was determined using size exclusion chromatography (SEC) in DMF (+ NH_4BF_4 5 mM) as the eluent at 50 °C. SEC analyses were performed using a Shimadzu instrument fitted with a mixed-C column and an RI detector. The molar mass in number (M_n), molar mass in weight (M_w) and molar mass dispersity (\mathcal{D}) were calculated using poly(ethylene glycol) (PEG) standards with the molecular weight in the range of 610 g mol^{-1} to 15 110 000 g mol^{-1} .

UV-Visible spectroscopy. Absorbance measurements of solutions were performed using an Agilent Cary 60 UV-Vis spectro-

photometer at room temperature. The calibration curve of CEPA was constructed from various solutions obtained by dilution of a stock solution of pure CEPA in methanol.

DLS/zeta potential. Dynamic Light Scattering (DLS) and zeta potential measurements were performed using a Malvern Zetasizer Nano S instrument at 25 °C. All measurements were performed using a diluted dispersion at 0.03 wt% in Milli-Q water. The Z-average hydrodynamic diameter (D_z) and polydispersity index (PDI) were calculated from 3 repeat measurements using the Stokes–Einstein equation, which assumes perfectly monodisperse, non-interacting spheres.

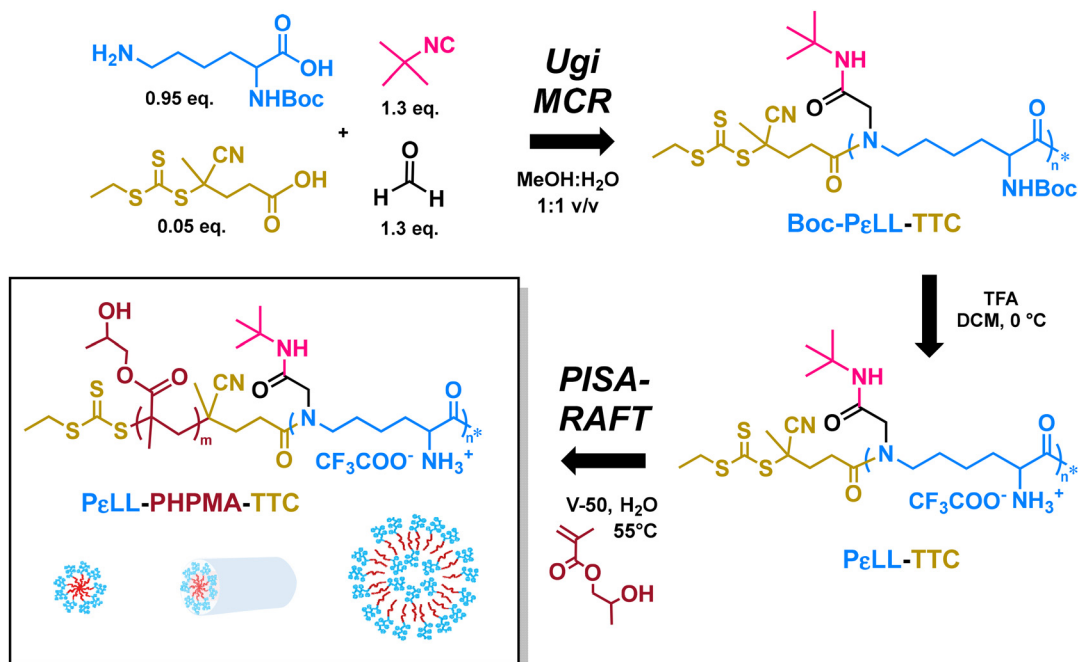
Transmission electron microscopy (TEM). Dry-state transmission electron microscopy (TEM) imaging was performed using an FEI TECNAI F20 microscope at an S3 acceleration voltage of 200 kV. All aqueous samples were diluted in Milli-Q water to 0.03–0.05 wt% and then deposited onto formvar/carbon film coated copper grids (400 mesh). After 90 s, the excess sample was blotted from the grid and the grid was stained with an aqueous 1 wt% uranyl acetate (UA) solution for 1 min prior to blotting, drying and microscopic analysis.

Results and discussion

In preliminary experiments, the synthesis of non-modified Boc-PeLL using the Ugi MCR of Boc-Lys (1.0 eq.) with *tert*-butyl isocyanide (1.3 eq.) and formaldehyde (1.3 eq.) in MeOH/water (1/1 v/v) was performed for 24 hours, 48 hours and 96 hours. The obtained results (Table S1†) show that higher molar masses can be obtained by extending the reaction duration. However, the molar mass of the obtained polymers remains limited ($M_n < 5.0 \text{ kg mol}^{-1}$). This is probably due to the presence of a side-reaction that gives rise to a six-membered ring, which is unable to propagate during polymerisation, as mentioned in the literature.³⁰

Inspired by our report on the chain-end functionalisation of polypeptoids,³² the synthesis of a macro-RAFT agent was achieved in a single step by the addition of a CTA during the Ugi polymerisation of Boc-lysine (Boc-Lys) (Scheme 1). The CEPA/Boc-Lys molar ratio was fixed at 0.05:0.95 throughout the present work. The resulting Boc-PeLL-TTC was purified by precipitation in cold diethylether. The removal of the Boc-protecting group was performed afterward in a mixture of dichloromethane/trifluoroacetic acid (1:1 v/v). Table 1 summarises the macromolecular characteristics of two Boc-PeLL-TTCs and their corresponding PeLL-TTCs used in the present work. Molar mass distributions of these polymers are shown in Fig. 2. The molar mass of the resulting Boc-PeLL-TTC is in the same range as that of Boc-PeLL obtained without the addition of CEPA under similar reaction conditions (24 or 48 hours).

Fig. 1 shows the ^1H -NMR spectra of Boc-PeLL, Boc-PeLL-TTC and CEPA. The addition of CEPA to the Ugi MCR of Boc-Lys successfully resulted in Boc-PeLL-TTC as confirmed by the presence of the methyl peak *g* that originated from CEPA at around 1.9 ppm, which is absent from pure Boc-PeLL. Furthermore, the characteristic signals of Boc-PeLL are present



Scheme 1 General strategy for the synthesis of poly(ϵ -L-lysine) (P ϵ LL)-decorated nanoparticles by sequential Ugi polymerisation and PISA-RAFT polymerisation in water.

Table 1 Macromolecular characteristics of Boc-P ϵ LL-TTC (entries a) and their corresponding P ϵ LL-TTC (entries b) obtained after Boc-deprotection

Entry	macroCTA	M_n , UV ^a (g mol ⁻¹)	SEC ^b		D
			M_n , SEC (g mol ⁻¹)	M_w , SEC (g mol ⁻¹)	
1a ^c	Boc-P ϵ LL _{1.5k} -TTC	1800	2300	4200	1.82
1b	P ϵ LL _{1.5k} -TTC	1500	6700	8900	1.36
2a ^d	Boc-P ϵ LL _{2.9k} -TTC	2100	4500	6000	1.35
2b	P ϵ LL _{2.9k} -TTC	2900	10 900	13 400	1.23

^a Determined by UV-Vis absorption of TTC. ^b Eluent: DMF + 5 mM NH₄BF₄ at 50 °C, PEG calibration. ^c 24 hour reaction. ^d 48 hour reaction.

with methylene protons of the P ϵ LL and methyl protons of the *tert*-butyl groups located from 1.0 ppm to 1.6 ppm. Protons adjacent to amide functions are found between 2.8 ppm and 4.4 ppm whereas protons of amide functions are present between 6.2 ppm and 7.8 ppm. In addition, the ¹³C-NMR spectrum of these three materials (ESI, Fig. S1†) also indicates a characteristic peak of methyl carbon at 12.8 ppm, which is observed in CEPA yet absent in Boc-P ϵ LL. Unfortunately, other characteristic peaks of CEPA are not observable in the ¹H-NMR spectrum for they are obscured by overlapping signals from Boc-P ϵ LL.

For further investigation, MALDI-ToF analysis was performed on two Boc-P ϵ LL-TTC macroCTAs (ESI, Fig. S2†). The obtained results confirm the integrity of the polymer structure given by a series of peaks with $\Delta(m/z) \approx 341$ corresponding to

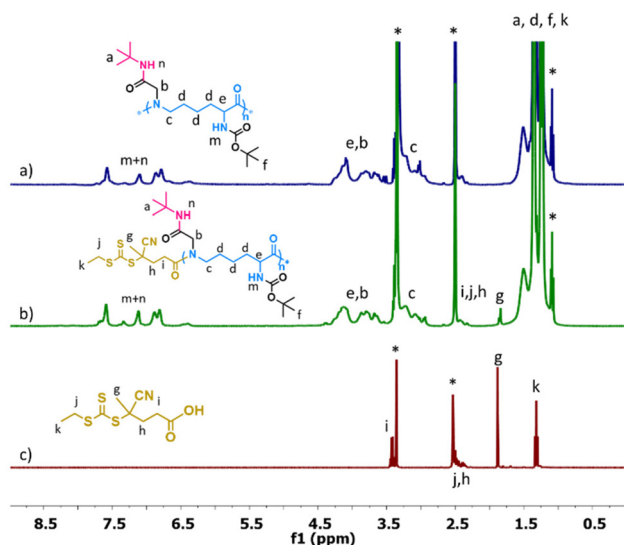


Fig. 1 ¹H-NMR spectrum of (a) Boc-P ϵ LL, (b) Boc-P ϵ LL-TTC, and (c) pure CEPA in DMSO-*d*₆ confirming the success in the synthesis of Boc-P ϵ LL-TTC using the Ugi multicomponent reaction, * indicates the residual solvent.

a repeating unit of Ugi derivatives Boc-P ϵ LL. More importantly, evidence for the presence of the trithiocarbonate function was also obtained from MALDI-ToF results (yellow triangle species, ESI, Fig. S2†). However, the signals from those species are less intense probably due to the sensibility of trithiocarbonate functions to MALDI measurement as reported elsewhere.⁴⁴

To obtain water-soluble PeLL-TTC, the Boc protecting group was removed in a mixture of dichloromethane/trifluoroacetic acid (1 : 1 v/v) at 0 °C for 3–4 hours.

The disappearance of signals at 1.3 ppm in the $^1\text{H-NMR}$ spectra of polymers after deprotection and purification (ESI, Fig. S3†) indicates successful quantitative elimination of the Boc protecting group. In accordance, $^{19}\text{F-NMR}$ shows the presence of trifluoroacetate counterions (ESI, Fig. S4†). $^{13}\text{C-NMR}$ further confirms the disappearance of peaks that originated from the *tert*-butyl group after Boc deprotection (ESI, Fig. S5†).

Molar masses of PeLL-TTC were then characterised by chain-end analysis *via* UV-Vis absorption and size exclusion chromatography (SEC). The results of these analyses are summarised in Table 1. First, quantification of TTC by UV-Vis absorption at $\lambda = 305$ nm in methanol was performed based on a calibration curve of pure CEPA (Fig. S6, ESI†). $M_{n,UV}$ can be deduced from the concentration of TTC as given by eqn (1) (ESI†) assuming that each polymer chain bears one TTC. As expected, polymers obtained from the Ugi MCR over a 48 hour reaction have higher molar masses, 2100 g mol^{-1} for Boc-PeLL-TTC (entry 2a, Table 1) and 2900 g mol^{-1} for PeLL-TTC (entry 2b, Table 1) in comparison with 1800 g mol^{-1} (entry 1a, Table 1) and 1500 g mol^{-1} (entry 1b, Table 1), respectively, for those obtained over 24 hours. SEC in DMF (+5 mM NH_4BF_4 with PEG calibration) was also performed to obtain relative $M_{n,SEC}$ and dispersity of Boc-PeLL-TTC as well as PeLL-TTC, as shown in Fig. 2. The results obtained from SEC and UV-Vis confirm the difference in molar mass at varying Ugi MCR reaction times, *i.e.* the longer the reaction, the greater the chain length. However, $M_{n,UV}$ is smaller than $M_{n,SEC}$ for all studied polymers, which is expected because the calculation of $M_{n,UV}$ was performed by assuming 100% chain-end functionalisation. Furthermore, $M_{n,SEC}$ was obtained as relative M_n with PEG calibration in DMF. As the chain-end functionalisation of PeLL was carried out *in situ* during the polycondensation reaction, there are possibly non-functionalised Boc-PeLL molecules mixed with functionalised Boc-PeLL-TTC. The difference between $M_{n,UV}$ and $M_{n,SEC}$ is particularly significant for the

cationic PeLL-TTC. This is because in SEC measurement, the charged nature of PeLL-TTC causes $M_{n,SEC}$ to shift to a higher molar mass. This shift can be attributed to the altered interaction of the polymers with the column material. Nevertheless, $M_{n,UV}$ before and after Boc-deprotection of the same polymer remains in the same range of molar mass, indicating that the TTC group remains intact after deprotection with trifluoroacetic acid. Due to the importance of quantifying TTC in the subsequent RAFT-PISA process, $M_{n,UV}$ was used as the reference value for further experiments. The macroCTAs are therefore denoted by their corresponding $M_{n,UV}$ values as PeLL $_{1.5k}$ -TTC and PeLL $_{2.9k}$ -TTC.

The resulting hydrophilic PeLL-TTC compounds then served as macroCTAs in the aqueous RAFT polymerisation of HPMA at 55 °C (see Scheme 1). Table 2 summarises the results of chain extension of PeLL $_{1.5k}$ -TTC with HPMA in water at 55 °C for 20 hours of reaction using V50 as an initiator. Under studied conditions, monomer conversion was >90% for all experiments with most achieving quantitative conversion. The molar mass after RAFT polymerisation of final formulations was analysed by SEC. These results are presented in Fig. S7, ESI.† Overall, except for $DP_0 = 70$, the increase in DP_0 , $_{PHPMA}$ gave rise to the molar mass of the obtained copolymers, featuring efficient chain extension. Though the inevitable presence of non-functionalised PeLL is noticed for all experiments, these signals are reduced in intensity when a higher DP is expected.

At the end of polymerisation, except for $DP_0 = 70$ at 15 wt%, final solutions were obtained as a stable dispersion given by their visual appearance (Fig. S8†). As is typical in the PISA process, nanoparticles are formed progressively as the polymerisation proceeds. Their formation causes light scattering, resulting in a transition to a turbid or milky-white appearance. Digital photos of polymer solutions, DLS results and TEM images of the final formulation can be found in ESI, Fig. S8–S10,† respectively. Fig. 3a illustrates the phase diagram of PeLL $_{1.5k}$ -*b*-PHPMA as a function of monomer content and DP_0 with representative TEM images of different morphologies obtained during the PISA process. Overall, various morphologies were obtained from PeLL $_{1.5k}$ -TTC by the PISA-RAFT process with HPMA at 5 wt%, 10 wt% or 15 wt% monomer content. First of all, at $DP_0 = 10$, based on the visual appearance of the final formulation, an evolution in morphology was expected with a turbid solution (5 wt%) giving spherical micelles, viscous solution at 10 wt% giving a mixture of spheres and short worm-like particles, and only gel at 15 wt% giving a fiber structure. Using TEM, a mixture of small spheres and short worm-like particles is observed from these three formulations. Unusual globular morphologies that appear intermediate or ill-defined may arise from the short HPMA chains used in these experiments, which may result in an unstable packing parameter, preventing the system from reaching well-defined equilibrium morphologies. Higher resolution TEM images of the short worm-like structures are presented in Fig. S10.† In agreement, DLS results show large polydispersity and significant deviation in particle size among number,

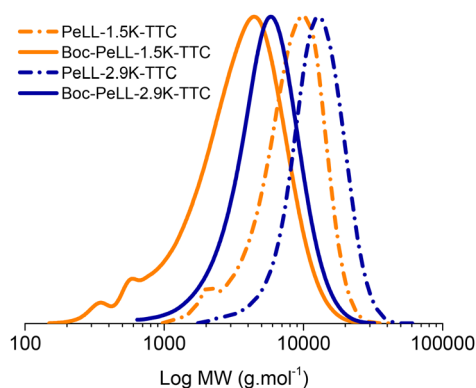


Fig. 2 Molar mass distribution of different Boc-PeLL-TTC and PeLL-TTC given in Table 1. SEC measurements were performed in DMF (+ NH_4BF_4 5 mM) at 50 °C with PEG calibration.

Table 2 Summary of $\text{PeLL}_{1.5\text{k}}\text{-TTC}$ chain extension with HPMA by RAFT polymerisation in aqueous medium. Polymerisation conditions: $[\text{macroCTA}]_0:[\text{V-50}]_0 = 1/0.2$, $T = 55\text{ }^\circ\text{C}$, $t = 21\text{ h}$, wt%: mass content of HPMA in water, $\text{DP}_0 = [\text{PeLL-TTC}]_0/[\text{HPMA}]_0$

Entry	wt%	DP_0	Conv. (%)	DP_{NMR}	$M_{\text{n, theo}}^a$ (g mol^{-1})	SEC _{DMF} , PEG calibration			\mathcal{D}	TEM ^b	DLS	
						$M_{\text{n, SEC}}^a$ (g mol^{-1})	$M_{\text{w, SEC}}$ (g mol^{-1})	\mathcal{D}			Z-Ave (d , nm)	PDI
P1	5	10	>99	10	2900	12 700	25 700	2.02	sS, sW	235	0.29	
P2		20	>99	20	4380	18 800	65 800	3.49	S	310	0.02	
P3		30	>99	30	5820	17 300	99 500	5.74	S	376	0.01	
P4		50	91	46	8052	28 300	163 200	5.78	V	583	0.07	
P5		70	97	68	11 278	27 000	115 300	4.28	V	468	0.02	
P6	10	10	>99	10	2940	11 600	19 300	1.67	sS, sW	45	0.19	
P7		20	>99	20	4380	19 400	69 700	3.59	S	881	0.22	
P8		30	>99	30	5820	16 400	75 400	4.60	V	730	0.21	
P9		50	>99	50	8700	22 700	151 800	6.69	V	666	0.07	
P10		70	>99	70	11 580	27 700	140 900	5.08	V	769	0.26	
P11	15	10	>99	10	2940	11 400	18 000	1.58	sS, sW	73	0.12	
P12		20	>99	20	4380	18 400	49 200	2.67	V	1934	0.63	
P13		30	>99	30	5820	18 000	99 500	5.57	V	1383	0.68	
P14		50	>99	50	8700	29 100	160 300	5.52	V	1444	1.00	
P15		70	98	69	11 378	31 500	131 700	4.18	—	—	—	

^a $M_{\text{n, theo}}$ was calculated from conversion determined by ¹H-NMR and from $M_{\text{n, UV}}$. ^b Expected morphology: sS = small sphere, S = sphere, sW = short worm-like particle, and V = vesicle.

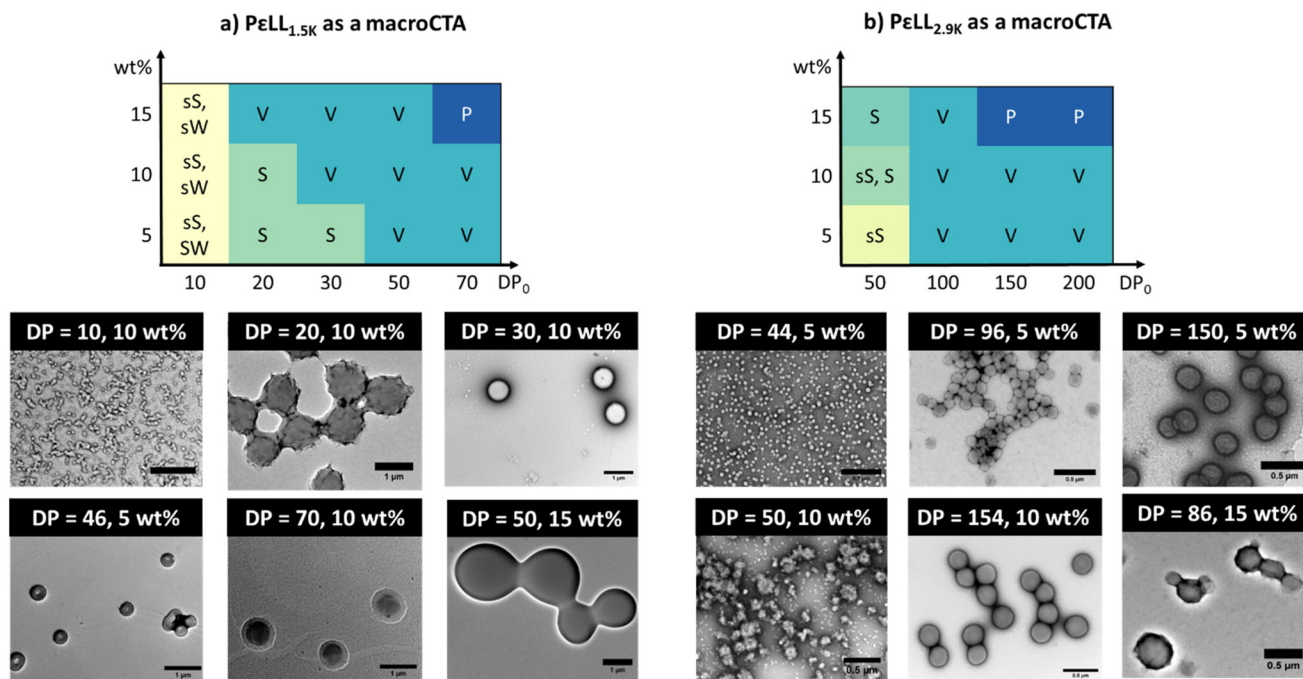


Fig. 3 Phase diagrams and representative TEM images of nanoparticles obtained by PISA-RAFT chain extension of (a) the $\text{PeLL}_{1.5\text{k}}$ macroCTA and (b) the $\text{PeLL}_{2.9\text{k}}$ macroCTA with HPMA in aqueous medium where DP_0 and the monomer content were varied. The scale bar is $1\text{ }\mu\text{m}$ for the $\text{PeLL}_{1.5\text{k}}$ macroCTA and $0.5\text{ }\mu\text{m}$ for the $\text{PeLL}_{2.9\text{k}}$ macroCTA. Abbreviation: sS = small sphere, sW = small worm-like particle, S = sphere, V = vesicle, and P = precipitation.

intensity and volume signals. The difference in the expected morphology and TEM image could be attributed again to the very short hydrophilic or HPMA block, which may cause instability upon dilution for TEM preparation, as reported in the literature for short poly(α -lysine) used as a macroCTA.⁴⁵ Specifically, as reported in the literature, nanostructures derived from short poly(α -lysine) ($\sim 780\text{ g mol}^{-1}$) have been

shown to lose stability upon dilution, leading to poorly defined structures observed in post-experiment TEM and DLS analyses. On the other hand, at a fixed monomer content of 5 wt%, as the proportion of DP_{HPMA} increases, the morphology evolution of the diblock system was observed. In comparison with $\text{DP} = 10$, when the PHPMA block has $\text{DP} = 20$ or $\text{DP} = 30$, well-defined spherical structures were seen under TEM with

DLS giving a Z-average of 300–370 nm and PDI = 0.01–0.02; at a DP of ~50, a clear donut-type vesical structure was observed in TEM. At a higher monomer content, 10 wt% for example, the same phenomenon is observed with changes between $DP_{\text{PHPMA}} = 10$ and a higher DP_{PHPMA} . At DP = 70, a clear vesicular structure was seen in TEM images. Interestingly, when $DP_{\text{PHPMA}} \geq 20$, the change in the nanoparticle size appears to be mostly dependent on the monomer content rather than the DP of the PHPMA block. As expected, as the hydrophobic core gets bigger, the stability of the system is lost given by the formation of unstable aggregates and eventually precipitates are observed visually in the final solution.

To explore the effect of the PeLL chain length on the PISA process, another series of experiments was performed with PeLL_{2.9k}. A longer stabilising block is expected to form more stable nanoparticles with certain changes in particle sizes. Table S3 (ESI†) shows the results of chain extension of PeLL_{2.9k}-TTC with HPMA under aqueous conditions. Digital photos of the final appearances of these formulations are presented in Fig. S12 (ESI†). Fig. 3b shows the phase diagram and TEM images of acquired formulations. Overall, compared to PeLL_{1.5k}-TTC, the conversion of HPMA was lower, especially when a high DP₀ or high monomer concentration was targeted. In SEC profiles presented in Fig. S11,† molar masses achieved with the use of PeLL_{2.9k}-TTC also increased with an increase in target DP_{HPMA}. However, at 15 wt% and DP ≥ 150 (P26, P27, Table 3), precipitation was observed at 50–60% monomer conversion and SEC analysis was not performed as the polymer solution cannot be filtered over a 0.45 μm filter. Similar to PeLL_{1.5k}-TTC, the residual of non-functionalised PeLL was present in all formulations.

In terms of morphology, unlike PeLL_{1.5k}-PHPMA where some small worm-like particles can be found at a low DP, only spherical structures were found for the PeLL_{2.9k}-b-PHPMA system, which was also described in the literature for other ionic diblocks obtained by PISA-RAFT in aqueous medium.^{46–48} As seen in Fig. 3b and Fig. S14,† at DP ~ 50,

small spheres and a mixture of spheres with varying sizes were found at 5 wt% and 10 wt%. At a higher DP_{HPMA}, the particle sizes increase significantly. As expected, the size of the nanoparticles based on PeLL_{2.9k}-PHPMA is about twice as small as that obtained by PeLL_{1.5k}-PHPMA due to the longer stabilizer block.

To assess the stability of the nanoparticles, zeta-potential measurement was performed for formulations at 10 wt% monomer content. As seen in Fig. S15,† as expected, all nanoparticles show positive zeta potentials due to the cationic nature of PeLL as a stabilising block. Furthermore, the zeta potential of the obtained nanoparticles increases gradually with an increase of the PHPMA block length. When DP_{HPMA} < 140, all nanoparticles have zeta-potentials between 45 and 55 mV, indicating the good colloidal stability of ionic particles. However, at DP_{HPMA} = 154, the zeta potential of nanoparticles drops to ~22 mV, indicating the loss of ionic stability. This result is consistent with the visual observation where aggregation was found on the formulation of PeLL_{2.9k}-PHPMA₁₅₄.

SAXS analysis was performed for samples whose characteristic size observed in TEM was smaller than 50 nm including PeLL_{1.5k}-PHPMA₁₀ and PeLL_{2.9k}-PHPMA₅₀ prepared at 5 wt% and 10% monomer contents. For each of these samples, the scattering intensity as a function of wave vector is shown in Fig. S16, ESI.† The data were analysed in agreement with the TEM data and images using polydisperse hard spheres (eqn (2), ESI†). The fitting results are in good agreement with TEM and DLS analyses as given in Table S4, ESI.†

Finally, the kinetics of chain extension were studied on PeLL_{2.9k} with a target degree of polymerisation DP₀ = 100 and 5 wt% solid content, to investigate the process kinetics during PISA-RAFT, as summarised in Fig. 4. As seen in Fig. 4a, under studied conditions, the chain extension of PeLL_{2.9k}-TTC with HPMA took place quite rapidly and reached >90% monomer conversion after ~70 minutes. An onset of polymerisation was observed at around 35 minutes, which is consistent with the visual observation where a rapid change in solution appear-

Table 3 Summary of PeLL_{2.9k}-TTC chain extension with HPMA by RAFT polymerisation in aqueous medium. Polymerisation conditions: [macroCTA]₀:[V-50]₀ = 1/0.2, T = 55 °C, t = 21 h, wt%: mass content of HPMA in water, DP₀ = [PeLL-TTC]₀/[HPMA]₀

Entry	wt%	DP ₀	Conv. (%)	DP _{NMR}	M _{n, theo} ^a (g mol ⁻¹)	SEC _{DMF} , PEG calibration			TEM ^b	DLS	
						M _{n, SEC} (g mol ⁻¹)	M _{w, SEC} (g mol ⁻¹)	D		Z-Ave (d, nm)	PDI
P16	5	50	>99	44	9240	18 800	42 500	2.26	sS	92	0.25
P17		100	87	96	16 740	31 500	124 700	4.00	sS, S	188	0.12
P18		150	>99	150	24 530	33 000	136 500	4.14	S	283	0.02
P19		200	>99	200	31 740	29 700	84 400	2.84	V	123	0.06
P20	10	50	>99	50	10 110	72 300	344 000	4.76	V	254	0.02
P21		100	80	80	14 430	57 100	187 000	3.28	V	396	0.03
P22		150	88	132	21 930	17 500	85 100	4.88	V	463	0.07
P23		200	77	154	25 100	84 700	417 200	4.93	V	319	0.04
P24	15	50	96	48	9820	21 700	53 400	2.46	V	374	0.20
P25		100	85	85	15 160	30 100	142 100	4.72	V	377	0.06
P26		150	57	86	15 300	NA	NA	NA	—	—	—
P27		200	51	102	17 610	NA	NA	NA	—	—	—

^a M_{n, theo} was calculated from conversion determined by ¹H-NMR and from M_{n, UV}. ^b Expected morphology, sS = small sphere, S = sphere, sW = short worm-like particle, and V = vesicle; NA: measurement was not performed due to impossibility of filtration.

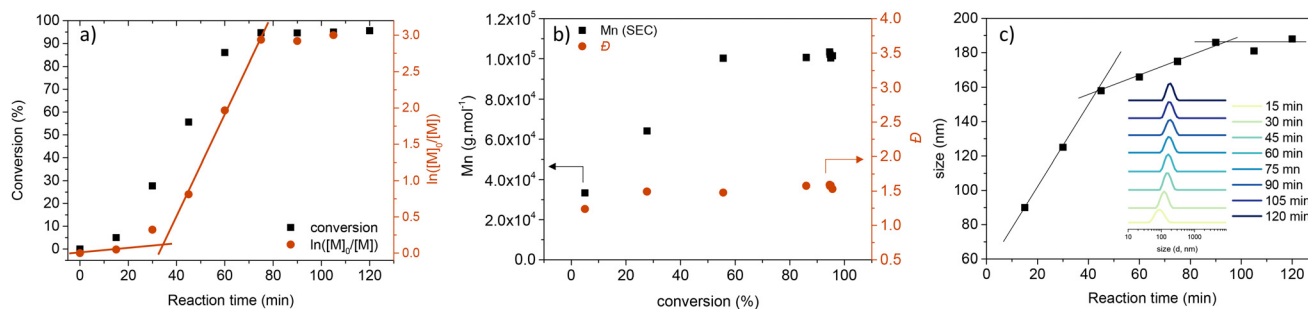


Fig. 4 Kinetics of chain extension of the P ϵ LL_{2.9k} macroCTA with HPMA ($DP_0 = 100$) at 5 wt% in terms of (a) conversion over the reaction time and pseudo-first linear plot, (b) molar mass and polydispersity evolution from SEC measurements with the exclusion of non-functionalised P ϵ LL residuals, and (c) size evolution of the formed nanoparticles over time by DLS measurement.

ance from transparent to turbid was observed between 30 minutes and 45 minutes. Fig. S17a (ESI[†]) presents global molar mass evolution during the kinetics. It is seen that after 15 minutes (5% monomer conversion determined by ¹H-NMR), a second population appears on SEC profiles, which takes ~15% of the whole molar mass distribution. As the monomer conversion increases with the reaction time, a linear increase in molar mass was obtained until ~50% monomer conversion was given by the shift to a higher molar mass of the second population in the SEC profiles. Nonetheless, above 50% monomer conversion, no clear shift was observed. As discussed previously, as P ϵ LL is positively charged, certain interactions with column materials could occur, leading to difficulty in molar mass separation.⁴⁷ Even though a bimodal molar mass distribution was observed due to the presence of non-functionalised P ϵ LL, by excluding non-functionalised P ϵ LL from the molar mass determination of the diblock, the molar mass distribution appears monomodal, and the weight fraction of this population (Fig. S17b, ESI[†]) increases with monomer conversion. The molar mass dispersity of the obtained diblock copolymers also remains around 1.5, featuring a relatively controlled polymerisation.

In terms of size evolution as presented in Fig. 4c, the diameter of the nanoparticles increases with the reaction time and monomer conversion. Three regimes can be observed in the evolution of the nanoparticle size. The first regime corresponds to the onset of polymerisation, with the initial formation of nanoparticles observed when monomer conversion is below 30%. Even at a slow monomer conversion of ~5% at 15 minutes, small particles of ~90 nm were already formed, which increased to ~125 nm at ~25% monomer conversion. After 35 minutes, as shown in Fig. 4a, micelle structures were formed and gradually increased in size due to an increase in monomer conversion. Once the monomer conversion reached >90%, almost no change in particle size was observed by DLS. TEM images taken for solution at 5%, 55.6% and 95.6% monomer conversion (Fig. S18, ESI[†]) also confirm the evolution in particle sizes obtained by DLS.

Lastly, the presence of non-functionalised P ϵ LL is consistently observed throughout this work. This is inevitable, as during the Ugi MCR polymerisation, CEPA competes with the

carboxylic acid group of Boc-Lys rather than being selectively incorporated. Technically, due to the similar molar masses of non-functionalised P ϵ LL and P ϵ LL-TTC, separating them using conventional purification methods such as dialysis, precipitation, or centrifugation poses a major challenge. Nonetheless, for the particle formation during the PISA process, these non-functionalised P ϵ LL molecules appear to act as a co-stabiliser for the P ϵ LL-HPMA system with prospective ionic interaction between the charges, proven by a low to very low PDI (0.02–0.25) obtained by DLS measurements. Further study to synthesise P ϵ LL-TTC with better chain-end fidelity may provide deeper insights into the role of non-functionalised P ϵ LL in the self-assembly of P ϵ LL-HPMA during the PISA process, which is essential regarding the application of this system in biological fields.

Conclusions

In conclusion, this work provides a proof of concept for the successful one-pot synthesis of hydrophilic polypeptoids *via* the Ugi multicomponent reaction (MCR), using P ϵ LL-TTC as a macroCTA for the PISA-RAFT process. The chain extension of P ϵ LL-TTC with HPMA in aqueous medium proceeds as a controlled polymerisation, despite the presence of non-functionalised P ϵ LL in the formulation, which shows minimal interference with the formation of P ϵ LL-HPMA nanoparticles of various morphologies (small spheres, small worm-like particles and small vesicles). Furthermore, the size of these nanoparticles was demonstrated to depend on target $DP_{HPMA, 0}$ and the monomer content as demonstrated by DLS results. In addition, zeta potential measurements showed good stability of the formed particles and confirmed the ionic colloidal stabilisation nature of P ϵ LL featuring zeta-potential values between 45 and 55 mV.

Given the combinatorial versatility of the Ugi MCR to prepare polypeptoids, with the robustness of PISA-RAFT in producing nanoparticles with diverse morphologies and tuneable sizes, this novel combination of the Ugi MCR and PISA is expected to advance the synthesis and self-assembly of novel functional macromolecules.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Data availability

All raw data generated and analyzed during the course of this study are securely stored at the Institut de Chimie et des Matériaux Paris-Est (ICMPE), CNRS. These include all experimental procedures, nuclear magnetic resonance (NMR) spectroscopy data, dynamic light scattering (DLS) measurements, transmission electron microscopy (TEM) images, size exclusion chromatography (SEC) data, and other relevant characterization files supporting the results and conclusions presented in the manuscript.

These datasets are not publicly accessible. However, they are available from the corresponding author upon request.

Conflicts of interest

There are no conflicts to declare.

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References

- J. Wan, B. Fan and S. H. Thang, *Chem. Sci.*, 2022, **13**, 4192–4224.
- H. Phan, V. Taresco, J. Penelle and B. Couturaud, *Biomater. Sci.*, 2021, **9**, 38–50.
- C. Liu, C.-Y. Hong and C.-Y. Pan, *Polym. Chem.*, 2020, **11**, 3673–3689.
- C. Grazon, P. Salas-Ambrosio, E. Ibarboure, A. Buol, E. Garanger, M. W. Grinstaff, S. Lecommandoux and C. Bonduelle, *Angew. Chem., Int. Ed.*, 2020, **59**, 622.
- D. B. Wright, M. A. Touve, L. Adamiak and N. C. Gianneschi, *ACS Macro Lett.*, 2017, **6**, 925–929.
- S. Varlas, J. C. Foster and R. K. O'Reilly, *Chem. Commun.*, 2019, **55**, 9066–9071.
- M. J. Derry, L. A. Fielding and S. P. Armes, *Prog. Polym. Sci.*, 2016, **52**, 1–18.
- L. Lei, N. Patil, A. Arnoux, C. Le Cœur, Y. de Rancourt de Mimérand, D. Grande, B. Le Droumaguet, X. Feng, Y. Gnanou and B. Couturaud, *Macromolecules*, 2024, **57**, 10513–10521.
- H. Phan, R. Cavanagh, D. Destouches, F. Vacherot, B. Brissault, V. Taresco, J. Penelle and B. Couturaud, *ACS Appl. Polym. Mater.*, 2022, **4**, 7778–7789.
- H. Phan, R. Cavanagh, P. Jacob, D. Destouches, F. Vacherot, B. Brugnoli, S. Howdle, V. Taresco and B. Couturaud, *Polymers*, 2023, **15**, 3070.
- N. Audureau, F. Coumes, J.-M. Guigner, T. P. T. Nguyen, C. Ménager, F. Stoffelbach and J. Rieger, *Polym. Chem.*, 2020, **11**, 5998–6008.
- D. Ikkene, J.-L. Six and K. Ferji, *Eur. Polym. J.*, 2023, **188**, 111848.
- J. Sun and R. N. Zuckermann, *ACS Nano*, 2013, **7**, 4715–4732.
- N. Gangloff, J. Ulbricht, T. Lorson, H. Schlaad and R. Luxenhofer, *Chem. Rev.*, 2016, **116**, 1753–1802.
- K. Klinker and M. Barz, *Macromol. Rapid Commun.*, 2015, **36**, 1943–1957.
- A. M. Clapperton, J. Babi and H. Tran, *ACS Polym. Au*, 2022, **2**, 417–429.
- D. Zhang, S. H. Lahasky, L. Guo, C.-U. Lee and M. Lavan, *Macromolecules*, 2012, **45**, 5833–5841.
- M. Hartweg, C. J. C. Edwards-Gayle, E. Radvar, D. Collis, M. Reza, M. Kaupp, J. Steinkoenig, J. Ruokolainen, R. Rambo, C. Barner-Kowollik, I. W. Hamley, H. S. Azevedo and C. R. Becer, *Polym. Chem.*, 2018, **9**, 482–489.
- A. Ma, X. Yu, M. Liao, W. Liu, S. Xuan and Z. Zhang, *Macromol. Rapid Commun.*, 2023, **44**, 2200301.
- R. Kakuchi, *Angew. Chem., Int. Ed.*, 2014, **53**, 46–48.
- B. Couturaud, Z. H. Houston, G. J. Cowin, I. Prokeš, J. C. Foster, K. J. Thurecht and R. K. O'Reilly, *ACS Macro Lett.*, 2019, **8**, 1479–1483.
- A. K. Pearce, A. Travanut, B. Couturaud, V. Taresco, S. M. Howdle, M. R. Alexander and C. Alexander, *ACS Macro Lett.*, 2017, **6**, 781–785.
- E. Ruijter and R. V. A. Orru, *Drug Discovery Today: Technol.*, 2013, **10**, e15–e20.
- E. Ruijter, R. Scheffelaar and R. V. A. Orru, *Angew. Chem., Int. Ed.*, 2011, **50**, 6234–6246.
- O. Kreye, O. Türünç, A. Sehlinger, J. Rackwitz and M. A. R. Meier, *Chem. – Eur. J.*, 2012, **18**, 5767–5776.
- A. C. Boukis, A. Llevot and M. A. R. Meier, *Macromol. Rapid Commun.*, 2016, **37**, 643–649.
- A. C. Boukis and M. A. R. Meier, *Eur. Polym. J.*, 2018, **104**, 32–38.
- W. Konrad, F. R. Bloesser, K. S. Wetzels, A. C. Boukis, M. A. R. Meier and C. Barner-Kowollik, *Chem. – Eur. J.*, 2018, **24**, 3413–3419.
- P.-K. Dannecker, A. Sehlinger and M. A. R. Meier, *Macromol. Rapid Commun.*, 2019, **40**, 1800748.
- X. Zhang, S. Wang, J. Liu, Z. Xie, S. Luan, C. Xiao, Y. Tao and X. Wang, *ACS Macro Lett.*, 2016, **5**, 1049–1054.
- P. Stiernet, B. Couturaud, V. Bertrand, G. Eppe, J. De Winter and A. Debuigne, *Polym. Chem.*, 2021, **12**, 2141–2151.
- A. A. Samad, J. D. Winter, P. Gerbaux, C. Jérôme and A. Debuigne, *Chem. Commun.*, 2017, **53**, 12240–12243.

- 33 Y. Tao, S. Wang, X. Zhang, Z. Wang, Y. Tao and X. Wang, *Biomacromolecules*, 2018, **19**, 936–942.
- 34 M. Saimura, M. Takehara, S. Mizukami, K. Kataoka and H. Hirohara, *Biotechnol. Lett.*, 2008, **30**, 377–385.
- 35 I.-L. Shih, M.-H. Shen and Y.-T. Van, *Bioresour. Technol.*, 2006, **97**, 1148–1159.
- 36 J.-N. Liu, S.-L. Chang, P.-W. Xu, M.-H. Tan, B. Zhao, X.-D. Wang and Q.-S. Zhao, *J. Agric. Food Chem.*, 2020, **68**, 1101–1109.
- 37 Y. Wang, L. Wang, Y. Hu, J. Qin and B. Yu, *Int. J. Biol. Macromol.*, 2024, **262**, 129513.
- 38 A. Traeger and M. N. Leiske, *Biomacromolecules*, 2025, **26**, 5–32.
- 39 M. Li and Y. Tao, *Polym. Chem.*, 2021, **12**, 1415–1424.
- 40 B. Yang, Y. Zhao, X. Ren, X. Zhang, C. Fu, Y. Zhang, Y. Wei and L. Tao, *Polym. Chem.*, 2014, **6**, 509–513.
- 41 H. Wu, B. Yang, Y. Zhao, Y. Wei, Z. Wang, X. Wang and L. Tao, *Polym. Chem.*, 2016, **7**, 4867–4872.
- 42 B. Yang, Y. Zhao, C. Fu, C. Zhu, Y. Zhang, S. Wang, Y. Wei and L. Tao, *Polym. Chem.*, 2014, **5**, 2704–2708.
- 43 X. Pan, F. Zhang, B. Choi, Y. Luo, X. Guo, A. Feng and S. H. Thang, *Eur. Polym. J.*, 2019, **115**, 166–172.
- 44 A. Kerr, G. Moriceau, M. A. Przybyla, T. Smith and S. Perrier, *Macromolecules*, 2021, **54**, 6649–6661.
- 45 L. Luppi, T. Babut, E. Petit, M. Rolland, D. Quemener, L. Soussan, M. A. Moradi and M. Semsarilar, *Polym. Chem.*, 2019, **10**, 336–344.
- 46 M. Semsarilar, V. Ladmiraal, A. Blanazs and S. P. Armes, *Langmuir*, 2013, **29**, 7416–7424.
- 47 V. Baddam, L. Välinen, L. Kuckling and H. Tenhu, *Polym. Chem.*, 2022, **13**, 3790–3799.
- 48 G. N. Smith, L. L. E. Mears, S. E. Rogers and S. P. Armes, *Chem. Sci.*, 2018, **9**, 922–934.