



HAL
open science

Amphiphilic diblock and triblock copolymers based on poly(2-methyl-2-oxazoline) and poly(D,L-lactide): Synthesis, physicochemical characterizations and self-assembly properties

Gaëlle Le Fer, Clémence Le Cœur, Jean-Michel Guigner, Catherine Amiel, Gisèle Volet

► To cite this version:

Gaëlle Le Fer, Clémence Le Cœur, Jean-Michel Guigner, Catherine Amiel, Gisèle Volet. Amphiphilic diblock and triblock copolymers based on poly(2-methyl-2-oxazoline) and poly(D,L-lactide): Synthesis, physicochemical characterizations and self-assembly properties. *Polymer*, 2019, 171, pp.149-160. 10.1016/j.polymer.2019.03.037 . hal-02952302

HAL Id: hal-02952302

<https://hal.science/hal-02952302>

Submitted on 22 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Amphiphilic diblock and triblock copolymers based on poly(2-
2 methyl-2-oxazoline) and poly(D,L-lactide): synthesis,
3 physicochemical characterizations and self-assembly properties.

4 Gaëlle Le Fer[†], Clémence Le Cœur^{†§}, Jean-Michel Guigner^{||}, Catherine Amiel[†], and Gisèle
5 Volet^{*†‡}

6 [†] Université Paris-Est, ICMPE (UMR7182), CNRS, UPEC, 94320 Thiais, France

7 [‡] Université d'Evry Val d'Essonne, Rue du Père Jarlan, 91025 Evry Cedex, France

8 [§] Laboratoire Léon Brillouin, UMR 12 CEA–CNRS, CEA Saclay, 91191 Gif-sur-Yvette
9 Cedex, France

10 ^{||} Institut de Minéralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), Sorbonne
11 Universités, UPMC Paris 6, IRD, CNRS UMR7590, MNHN, 4 place Jussieu, 75252 Paris
12 Cedex 05, France

13 Key-words: poly(2-methyl-2-oxazoline), block copolymers, micelles, Small-Angle Neutron
14 Scattering.

15

16 **Abstract**

17 A range of amphiphilic diblock and triblock copolymers based on poly(2-methyl-2-
18 oxazoline) (PMeOx) and poly(D,L-lactide) (PLA) with various compositions and molecular
19 masses were prepared combining cationic ring-opening polymerization and click chemistry.
20 Their hydrophilic weight ratios (f) covered a wide range of composition (21 to 77 %). In
21 water, these amphiphilic copolymers self-assembled to form, in the case of diblock
22 copolymers, core-shell micellar structures with a dense PLA core and a hydrated PMeOx
23 shell in the whole range of f values. The triblock copolymers formed micellar aggregates of
24 finite sizes made of bridged micelles, the micelles being connected by some of the PLA end-
25 blocks. Moreover, the micelles displayed original core-multishell nanostructures in a specific

1 range of f values (48 %), never reported for this kind of triblock copolymers. The amphiphilic
2 copolymers of this study displayed a rich variety of controlled self-assembled morphologies
3 which could be promising candidates for drug delivery applications.

4

5 **Introduction**

6 Amphiphilic block copolymers capable of self-assembling into micellar structures draw a
7 large interest over the last decades to improve hydrophobic drug encapsulation and delivery
8 because of the mesoscopic size range and the high drug-loading capacity of the inner core.¹⁻⁴
9 In this aim, several ABA poloxamer poly(ethylene glycol)-*b*-poly(propylene oxide)-*b*-
10 poly(ethylene glycol) (PEG-*b*-PPO-*b*-PEG), commercially available under the name pluronic
11 received attention because of their self-assembly in core-shell micelles capable of passing
12 physiological barriers such as the blood-brain barrier.^{5,6} The PPO block ensures the
13 hydrophobic cohesion and subsequently the encapsulation of hydrophobic active compounds
14 while the PEG block is the shell-stabilizing segment. Due to its high water solubility,
15 remarkable stealth properties and apparent low toxicity, many efforts have been carried out to
16 elaborate a large library of amphiphilic copolymers based on PEG. In this way, Kataoka and
17 co-workers^{7,8} performed a one-pot synthesis of α -acetal-poly(ethylene glycol)-*b*-poly(D,L-
18 lactide) (α -acetal-PEG-PLA). Then, the dialysis against water was employed to prepare
19 polymeric micelles of 31 nm, after the solubilization of block copolymer in
20 dimethylacetamide (DMAc), a good solvent for both segments. More recently, amphiphilic
21 copolymers based on PEG and poly(amino acid) block able to form nanometric structures
22 showed a large interest for drug and gene delivery,⁹ in part because polypeptide conformation
23 can influence their aggregation behavior in aqueous medium.^{10,11}

1 However, several studies revealed important drawbacks of PEG. Due to the intensive use of
2 PEG, PEGylated systems have lost their stealth function when applied *in vivo*, because of the
3 existence of non-specific and specific recognition by the immune system. Indeed, specific
4 antibodies were detected in the serum of patients treated with PEG-asparaginase¹² and PEG-
5 uricase.¹³ In addition, PEG-specific antibodies were identified in 25 % of patients who had
6 never received treatment based on PEG, due to the presence of PEG in many food and
7 cosmetic products.^{14,15} These antibodies have a neutralizing effect and cause a significant loss
8 of the expected therapeutic effect.

9 To solve this problem, different polymer families are considered as possible alternatives to
10 PEG for biomedical uses: poly(vinylpyrrolidone) (PVP), poly(*N*-(2-hydroxypropyl)
11 methacrylamide) (PHPMA), synthetic hydrophilic polypeptides and poly(oxazoline)s.¹⁶
12 Particularly poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx) are
13 often compared to PEG because they present similar chain flexibility in physiological
14 media,¹⁷ same stealth behavior towards plasmatic protein¹⁸⁻²⁰ and a better
15 biocompatibility.^{21,22} Amphiphilic diblock and triblock copolymers based on PMeOx have
16 been reported in the literature.²³⁻²⁷ In these works, hydrophobic blocks were of different
17 natures, going from fluid blocks made of low glass transition temperature (T_g) polymers such
18 as polydimethylsiloxane,²³ poly(tetrahydrofuran)²⁴ or poly(2-(1-ethylheptyl)-1,3-oxazoline)²⁵
19 or poly(2-nonyl-2-oxazoline)²⁶ to frozen blocks made of high T_g polymers such as poly(2-
20 phenyl-2-oxazoline).²⁷

21 Despite the well-known interesting and often reported properties of poly(lactide) (PLA), such
22 as biocompatibility and biodegradability,^{28,29} and extensive description within PEG/PLA-
23 based systems both in term of synthesis strategies, biofunctionalization, colloidal stability,
24 physico-chemical characterization and drug encapsulation,^{7,8,30-33} only a few works were

1 related to self-assembly study of amphiphilic block copolymer combining poly(2-oxazoline)
2 with PLA, which behave as frozen block at ambient temperature.³⁴
3 Moreover, the control of the characteristics of polymeric micelles, such as size, shape and
4 surface properties characterization, have crucial importance, to optimize drug encapsulation
5 and then delivery as well as to maintain colloidal stability.^{35,36} In addition, the distribution of
6 drug-loaded polymeric micelles in the body may be determined mainly by their size and
7 morphology and is less affected by the properties of loaded drugs if these are embedded in
8 the inner core of the micelles. Indeed, a nanometric size range from 10 to 200 nm is a crucial
9 factor in determining their body disposition, especially when an enhanced permeation
10 retention effect (EPR effect) is involved.³⁷⁻⁴⁰ Consequently, a thorough characterization of
11 their size and structure at nanometer resolution is a prerequisite.

12 In this context, we propose the elaboration of amphiphilic diblock and triblock copolymers
13 poly(2-methyl-2-oxazoline)-*b*-poly(D,L lactide) (PMeOx-*b*-PLA) and poly(D,L lactide)-*b*-
14 poly(2-methyl-2-oxazoline)-*b*-poly(D,L lactide) ((PLA-*b*-PMeOx-*b*-PLA) and their self-
15 assembly in water. It should be emphasized that contrary to the literature works where the
16 middle block was hydrophobic, the hydrophilic block was set in the middle of the triblock
17 copolymers synthesized, leading to another variety of supramolecular structures. Two
18 processes were used, the nanoprecipitation and the dialysis to rationalize the relationship
19 between copolymer structure, method of self-assembly and the morphology of the micelles
20 by using dynamic light scattering (DLS), small angle neutron scattering (SANS) and cryo-
21 TEM.

22

23 **Materials and methods**

1 **Materials**

2 The high-molecular-mass poly(D,L-lactide) (PLA) was purchased from Cargill Laboratories
3 (Cedar Grove, NJ, USA). The monomer 2-methyl-2-oxazoline (Sigma-Aldrich, Saint-Louis,
4 MO, USA, purity 99 %) were dried overnight over calcium hydride and purified by
5 distillation under an argon atmosphere. Sodium azide (NaN₃) (BioUltra, purity ≥ 99.5 %),
6 anhydrous acetonitrile (ACN) (purity 99,8%), 1-iodobutane (purity 99%), 1,3-diiodopropane
7 (purity 99%) were purchased from Sigma Aldrich and used as received. Dimethylsulfoxide
8 (DMSO), diethyl ether and dichloromethane were purchased from Carlo Erba (Val de Reuil,
9 France) and used without extra purification. *N,N*-Dimethylformamide (DMF) was purchased
10 from VWR (Radnor, Pennsylvania, USA). Copper commercially available nanopowder (Cu
11 NPs) with nominal size from 20 nm to 40 nm was purchased from Alfa Aesar (Haverhill,
12 MA, USA).

13 **Polymers synthesis**

14 *Synthesis of functionalized polymers: alkynyl-terminated poly(D,L-lactide) and azide-*
15 *terminated poly(2-methyl-2-oxazoline)*

16 Alkynyl-terminated poly(D,L-lactide) (PLA≡) oligomers were prepared *via* transesterification
17 of high-molecular-mass PLA ($\overline{M}_n = 8.72 \times 10^4 \text{ g}\cdot\text{mol}^{-1}$, $D = \frac{\overline{M}_w}{\overline{M}_n} = 2$) as previously reported.⁴¹

18 ¹H NMR (400 MHz, CDCl₃): δ = 5.14 (q, 1H, CH(CH₃)), 4.69 (m, 2H, CH₂CCH), 4.31 (m,
19 1H, HOCHCH₃), 2.66 (s, 1H, OH), 2.48 (t, 1H, HCC), 1.56 (d, 3H, CH₃) ppm.

20 FTIR (ATR mode): C-H 2996, 2946 cm⁻¹; C≡C 2124 cm⁻¹; C=O 1754 cm⁻¹; C-O 1180 cm⁻¹.

21

22 α-Azide-terminated poly(2-methyl-2-oxazoline) (PMeOx-N₃) and α-, ω-azide-functionalized
23 poly(2-methyl-2-oxazoline) (N₃-PMeOx-N₃) were synthesized *via* cationic ring-opening

1 polymerization (CROP). Various [monomer]/[initiator] molar ratios were used to obtain
2 polymers with different molecular masses. For PMeOx-N₃, as an example, a solution of 2-
3 methyl-2-oxazoline (4.0 mL, 47.2 mmol) and 1-iodobutane as initiator (230 μL, 2.01 mmol)
4 in dry acetonitrile (ACN) (12.0 mL) was stirred at 80 °C, under dry argon. After 5 hours, the
5 necessary time for total monomer consumption, determined by the kinetic study previously
6 conducted,⁴² polymer was functionalized by adding an excess of NaN₃, and the reaction
7 mixture was stirred for 24 hours still at 80 °C. The polymer obtained was purified by
8 precipitation in diethyl ether, filtered and dried under vacuum at 60 °C to afford a white
9 powder (yield = 95 %). Two polymers were synthesized with different number-average
10 molecular masses \overline{M}_n : 2.2×10^3 and 5.5×10^3 g.mol⁻¹. A similar procedure was used to
11 synthesize telechelic polymers N₃-PMeOx-N₃ using a bifunctional initiator, the 1,3-
12 diiodopropane and knowing the polymerization constant.⁴³ The telechelic polymers obtained
13 had \overline{M}_n of 2.2×10^3 and 8.2×10^3 g.mol⁻¹.

14

15 *Synthesis of amphiphilic copolymers: PMeOx-b-PLA and PLA-b-PMeOx-b-PLA*

16 The amphiphilic diblock and triblock copolymers, poly[(2-methyl-2-oxazoline)-*b*-poly(D,L-
17 lactide)] (PMeOx-*b*-PLA) and poly[(D,L-lactide)-*b*-(2-methyl-2-oxazoline)-*b*-(D,L-lactide)]
18 (PLA-*b*-PMeOx-*b*-PLA), were obtained by coupling the alkynyl-PLA with the azide end
19 group of the functionalized PMeOx-N₃ or N₃-PMeOx-N₃. Typically, to obtain the diblock
20 copolymer (PMeOx₂₂₀₀-*b*-PLA₂₈₀₀), PLA≡ (2.8×10^3 g.mol⁻¹, 100 mg, 3.57×10^{-2} mmol) and
21 1.1 equivalent of PMeOx-N₃ (2.2×10^3 g.mol⁻¹, 87.0 mg, 3.95×10^{-2} mmol), were dissolved in
22 10 mL of dry DMSO and 10 mg of commercially available copper nanoparticles (Cu NPs)
23 were added. The reaction mixture was then subjected to microwave irradiation under stirring
24 at 80 °C for 25 min. Cu NPs were removed by centrifugation at 4000 rpm for 10 min. The
25 supernatant was centrifuged a second time and purified by dialysis against water (molecular

1 weight cut off: 3.5 KDa or 6-8 KDa depending on the PMeOx molecular mass). The diblock
2 copolymer was recovered by freeze-drying (yield = 79 - 85 %). The amphiphilic triblock
3 copolymers PLA-*b*-PMeOx-*b*-PLA were obtained from a similar procedure using the
4 telechelic polymers N₃-PMeOx-N₃ (yield = 73 - 92 %).

5

6 **Nanoprecipitation and dialysis methods**

7 Self-assembly of diblock copolymers were prepared using the nanoprecipitation method: 1 ml
8 of solution of copolymer in acetonitrile (ACN) with a concentration of 2 g.L⁻¹ was stirred and
9 added dropwise in 2 mL of ultrapure water at 25 °C stirred at 220 rpm. The organic solvent
10 was removed from the dispersion by evaporation under reduced pressure at room temperature
11 and the volume was adjusted by addition of ultrapure water to obtain a concentration of 1 g.L⁻¹
12 ¹.

13 Self-assembly of triblock copolymers were prepared with the same nanoprecipitation
14 procedure and by the dialysis method. Concretely, a solution of copolymer in ACN at a
15 concentration of 1 g.L⁻¹ was dialyzed against water for 2 days (Spectra/Por dialysis tubing,
16 molecular weight cut off 6-8000 Da). The volume of water was 500 mL and was renewed 5
17 times per day. Finally, the volume of the suspension was adjusted by evaporation under
18 vacuum to obtain a concentration of 1 g.L⁻¹.

19

20 **Instrumentation and Measurements**

21 Fourier-transform infrared spectroscopy (FTIR) spectra were recorded on a Bruker Tensor 27
22 instrument equipped with a Digi Tect DLaTGS detector, 32 scans were collected at a
23 resolution of 1 cm⁻¹ using an ATR accessory (single-reflection horizontal ATR, Miracle, Pike

1 Technologies). Chemical structures of the polymers were investigated using a Raman
2 apparatus Xplora from Horiba Jobin Yvon (Longjumeau, France) equipped with a laser
3 emitting at 638 nm. The acquisition time was fixed at 1 min. ^1H nuclear magnetic resonance
4 spectroscopy (NMR) experiments were performed in D_2O , CDCl_3 or DMSO-d_6 at $25\text{ }^\circ\text{C}$ on a
5 Bruker NMR spectrometer operating at 400 MHz equipped with a Bruker multinuclear z-
6 gradient direct probe head capable of producing gradients in the z direction with 53.5 G.cm^{-1}
7 strength. ^1H NMR spectra were recorded with 64 scans and a relaxation time of 4 sec. Size
8 exclusion chromatography (SEC) of polymers was performed in two different solvents:
9 aqueous eluent ($0.1\text{ mol.L}^{-1}\text{ LiNO}_3$) for the azide-terminated poly(2-methyl-2-oxazoline) and
10 *N,N*-Dimethylformamide (DMF) for the other polymers. In the case of aqueous eluent (0.1
11 $\text{mol.L}^{-1}\text{ LiNO}_3$), the chromatograph is equipped with a pump P 100 (Spectra-Physics,
12 Fremont, CA, USA) and a 1260 Infinity automatic injector (Agilent Technologies, Santa
13 Clara, CA, USA). For the analysis of polymers in aqueous eluent ($0.1\text{ mol.L}^{-1}\text{ LiNO}_3$), a set
14 of two columns PL-aquagel OH-30 and OH-40 (Polymer Laboratories, Shropshire, UK) was
15 used. Two detectors were connected in series at the end of the columns: a Dawn Optilab Rex
16 differential refractometer and a Dawn Heleos 8 light scattering detector (Wyatt Technology,
17 Santa Barbara, CA, USA). In the case of DMF eluent, SEC was performed on a Waters
18 equipment with refractive index detector and a Shodex KD-806M column that was calibrated
19 with poly(ethylene oxide) standards. The chromatographic analysis of polymers was done
20 with all polymer solutions at a concentration of 10 mg mL^{-1} .

21 Thermogravimetric analyses (TGA) were performed on a Setaram Setsys Evolution 16
22 apparatus by heating the samples at a rate of $10\text{ }^\circ\text{C.min}^{-1}$ from $20\text{ }^\circ\text{C}$ to $800\text{ }^\circ\text{C}$ under argon
23 atmosphere.

24 To determine the hydrodynamic radius (R_h) and the polydispersity index (PDI, a
25 dimensionless measure of the broadness of the size distribution), dynamic light scattering

1 (DLS) measurements were performed on a Malvern Instruments Zetasizer Nano ZS operating
2 with a He-Ne laser source at the wavelength of 633 nm. All measurements were performed at
3 25 °C and at a scattering angle of 173°. The concentration of the solutions was 1 mg.mL⁻¹.
4 The autocorrelation functions ($g_1(t)$) were analyzed in terms of relaxation time distribution (τ)
5 (equation 1).

$$6 \quad g_1(t) = \int A_{(\tau)} \exp\left(-\frac{t}{\tau}\right) d\tau \quad (1)$$

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

$$8 \quad R_h = \frac{k_B T}{6\pi\eta_s D_0} \quad (2)$$

9 Where D_0 is diffusion coefficient, η_s is the viscosity of the solvent, T is absolute temperature
10 and k_B the Boltzmann constant. The correlation functions were also analyzed using the
11 cumulants method to derive the mean radii and polydispersity index (PDI) of the
12 nanoparticles. In case of PDI lower than 0.3, the distribution width, σ_{DLS} , can be
13 approximated as $(PDI)^{1/2}$.

14 For the cryo-transmission electron microscopy (Cryo-TEM), a drop of NPs suspension was
15 deposited on "quantifoil"® (Quantifoil Micro Tools GmbH, Germany) carbon membrane.
16 The excess of liquid on the membrane was blotted out with a filter paper and, before
17 evaporation, the membrane was quenched-frozen in liquid ethane to form a thin vitreous ice
18 film in which NPs were captive. Once mounted in a Gatan 626 cryo-holder cooled with liquid
19 nitrogen, the samples were transferred in the microscope and observed at low temperature (-
20 180 °C). Cryo-TEM images were recorded on an ultrascan 2k ccd camera (Gatan, USA),
21 using a LaB6 JEOL 2100 (JEOL, Japan) cryo-microscope operating at 200kV with a JEOL
22 low dose system (Minimum Dose System, MDS) to protect the thin ice film from any
23 irradiation before imaging and reduce the irradiation during the image capture.

1 Small-angle neutron scattering (SANS) measurements were performed at the Laboratoire
2 Léon Brillouin (“ORPHEE” reactor, CEA Saclay) on the “PACE” spectrometer. The
3 experimental scattering vector q ($q = (4\pi / \lambda) \sin(\theta/2)$) range was $0.0032 < q$ (\AA^{-1}) < 0.44 and
4 was covered by three sample-to-detector distances and two different wavelengths (1 or 3 m at
5 the neutron wavelength of 5 \AA and 4.5 m at 13 \AA). The samples were loaded into Hellma
6 quartz cells with a 2 mm optical path length. The cells were placed in a sample changer, and
7 the scattering for each sample was measured for about 1.5 hour at room temperature.
8 Scattering intensities from solutions were corrected for empty cell scattering and sample
9 transmission. $I(q)$ is in absolute scale (cm^{-1}). For all samples, the solvent scattering intensity
10 was subtracted, but we have not succeeded in completely removing the sample background
11 (which is inferior to $1 \times 10^{-3} \text{ cm}^{-1}$). All colloidal suspensions were prepared as previously
12 explained and then dialyzed against D_2O , and the concentration was 1 g.L^{-1} unless specified.

13 Fits: 2 types of model fitting provided by SasView software (<http://www.sasview.org>) were
14 used. Polydisperse sphere model describes a sphere with a lognormal distribution on the
15 radius r_s (Equation 3).

$$16 \quad I(q, r) = \frac{I_0}{V} [3V(\rho_s - \rho_{\text{solvent}})P_{sp}(q, r)]^2 \quad (3)$$

17 Where $P_{sp}(q, r)$ is the form factor for a monodisperse spherical particle with uniform
18 scattering length density ρ_s , ρ_s value has been fixed as the scattering length density of PLA.

19 Polydisperse core-multishell model (Equation 4) consists in a core of radius r_c , spherical
20 shells with thicknesses δ_1 ($\delta_1 = r_{s1} - r_c$) and δ_2 ($\delta_2 = r_{s2} - r_{s1}$), r_{s1} and r_{s2} being respectively the
21 radius of the first and the second shell. We applied a lognormal distribution for r_c , r_{s1} and r_{s2} .

$$22 \quad I(q, r_c, r_{s1}, r_{s2}) = \frac{I_0}{V_s} \left[3V_c(\rho_c - \rho_{s1})P_{sp}(q, r_c) + 3V_{s1}(\rho_{s1} - \rho_{s2})[V_{s1}P_{sp}(q, r_{s1}) - \right. \\ 23 \quad \left. V_cP_{sp}(q, r_c)] + 3V_{s2}(\rho_{s2} - \rho_{\text{solvent}})[V_{s2}P_{sp}(q, r_{s2}) - V_{s1}P_{sp}(q, r_{s1})] \right]^2 \quad (4)$$

1 Where $P_{sp}(q, r)$ has been previously defined. ρ_c , ρ_{s1} , ρ_{s2} , ρ_{sol} are respectively the neutron
2 scattering length density of the core, the shell 1, the shell 2 and the solvent.

3

4

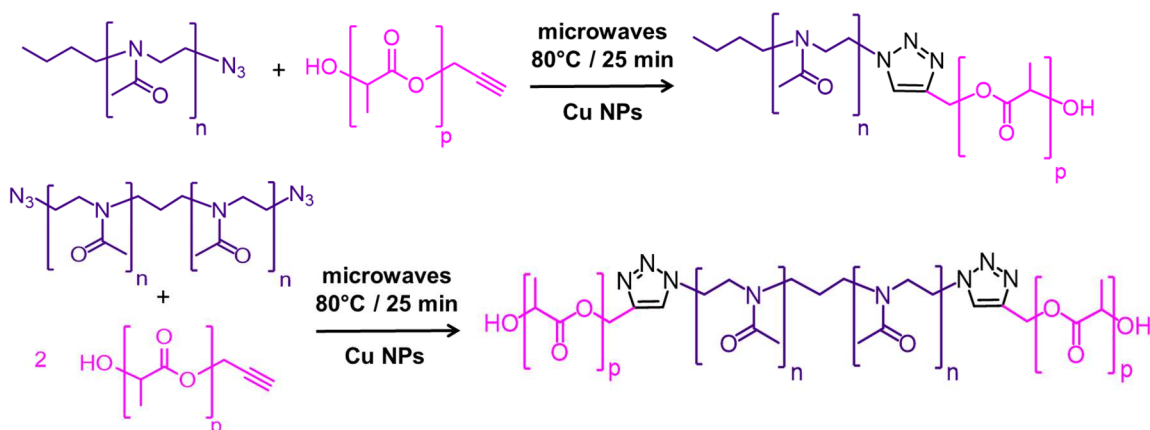
5 **Results and discussion**

6 **Synthesis and characterization of di- and triblock copolymers.**

7 First, the functionalized polymers were prepared by cationic ring opening polymerization
8 (CROP) for poly(2-methyl-2-oxazoline) (PMeOx) and by transesterification for poly(D,L-
9 lactide) (PLA). In a second step, the block copolymers were built by “click” chemistry. The
10 CROP of 2-methyl-2-oxazoline was performed, as described previously,^{27, 25} in anhydrous
11 ACN at 80 °C, using 1-iodobutane or 1,3-diiodopropane as initiator and NaN₃ as termination
12 agent to obtain α -azide-terminated poly(2-methyl-2-oxazoline) (PMeOx-N₃) and α -, ω -azide-
13 functionalized poly(2-methyl-2-oxazoline) (N₃-PMeOx-N₃) respectively. The polymers were
14 fully characterized by ¹H NMR and SEC. The ¹H NMR spectra of the PMeOx-N₃ and N₃-
15 PMeOx-N₃ (Figure S1 and S2) were performed in D₂O and CDCl₃ respectively. The degrees
16 of polymerization and consequently the number-average molecular masses (\overline{M}_n^{NMR}) of the
17 polymers were calculated using the integration of the peaks **1** at 2.08 ppm, corresponding to
18 the repetitive unit and the integration of the peak **a** at 0.92 ppm corresponding to the protons
19 of methyl end group in the case of PMeOx-N₃ or the integration of the peaks **a** at 1.18 ppm
20 corresponding to the methylene protons of the bifunctional initiator in the case of N₃-PMeOx-
21 N₃. The polymers obtained were characterized by SEC in aqueous solution with LS detector
22 to obtain absolute number-average molecular masses \overline{M}_n^{SEC} reported in Table S1. A good
23 agreement was observed between the \overline{M}_n^{NMR} , \overline{M}_n^{SEC} and the expected molecular masses

1 calculated from the feed ratio $\overline{M}_n^{expected}$. Chromatograms highlighted monomodal
 2 distributions (Figure S3) with narrow dispersities indicating the controlled character of the
 3 polymerizations. The presence of the azide end groups, for all polymers, was confirmed by
 4 FTIR with the band at 2097 cm^{-1} (Figure S4). Alkynyl-terminated PLA (PLA \equiv) was
 5 previously prepared by direct transesterification reaction as reported elsewhere.²⁵ This
 6 method has the major advantage to produce oligomers with an alkynyl end group in a one-
 7 step reaction. The average-number molecular mass and the chemical structure of the alkynyl-
 8 terminated PLA were previously confirmed by SEC, ¹H NMR and Raman spectroscopy.⁴⁴ A
 9 series of five oligomers were prepared with number-average molecular masses ranging from
 10 1.2×10^3 to 5.2×10^3 $\text{g}\cdot\text{mol}^{-1}$ with dispersity values from 1.3 to 1.5.

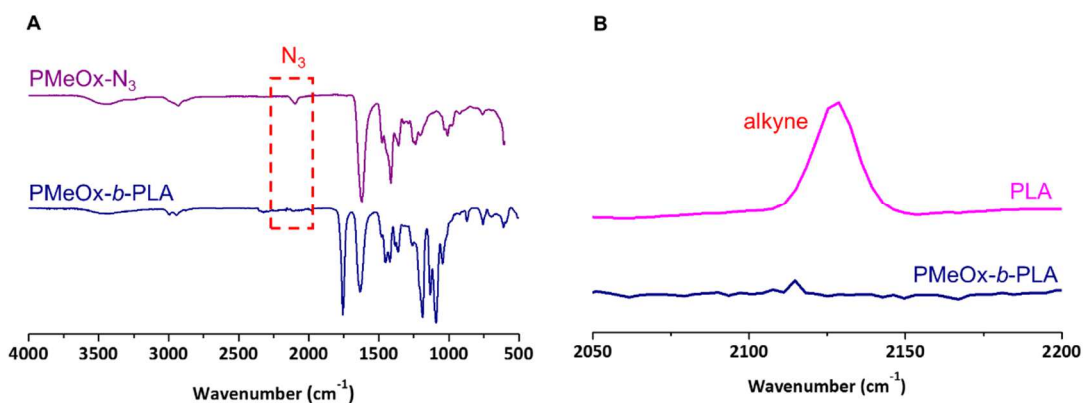
11 The di- and triblock copolymers were prepared using the copper-catalyzed Huisgen [3+2]
 12 cycloaddition “click” chemistry between azide and alkyne (CuAAC) (Scheme 1). The “click”
 13 reaction was catalyzed by copper nanoparticles (Cu NPs) in dimethylsulfoxide (DMSO) at 80
 14 °C. The $[\text{N}_3]/[\text{C}\equiv\text{C}]$ feed ratio was maintained at 1.1, and the reaction was conducted for 25
 15 min under microwave irradiation. The purification of the block copolymers was simplified
 16 using copper nanoparticles as the Cu NPs could be easily removed by centrifugation.



17
 18 Scheme 1. Synthesis of PMeOx *b*-PLA and PLA-*b*-PMeOx *b*-PLA by Huisgen 1,3-dipolar
 19 cycloaddition with copper-nanoparticles (Cu NPs) as catalyst.

1 The copolymers were characterized by SEC in DMF using a poly(ethylene oxide) (PEO)
2 calibration curve. The chromatograms have shown monomodal distributions, as reported in
3 Figure S5 for a diblock and a triblock copolymer. This is a first indication of the coupling
4 efficiency between the different blocks. The quantitative results are reported in Table S2. For
5 comparison, the homopolymers PLA≡, PMeOx-N₃ and N₃-PMeOx-N₃ were also analyzed in
6 DMF. For PLA≡, there is a good agreement between the \overline{M}_n^{SEC} and $\overline{M}_n^{expected}$ but the
7 molecular weights determined for PMeOx are lower than the expected values and are also
8 lower than the molecular weights determined by light scattering detector in aqueous eluent
9 (Table S1). This discrepancy could be attributed to a more compact conformation of PMeOX
10 chains than PEO standards. The molecular weights determined for the copolymers indicated
11 also a similar discrepancy between the molecular weights determined from the PEO
12 calibration curve and $\overline{M}_n^{expected}$ and this can be attributed to the more compact
13 conformations of the copolymers in DMF than PEO chains. Nevertheless, the molecular
14 weights increased, as expected, with the molar masses of each block.

15 The efficiency of the coupling reaction between the azido and alkynyl end groups was then
16 demonstrated by FTIR and Raman spectroscopy, two perfectly complementary techniques.
17 The success of the cycloaddition reaction, for the diblock and triblock copolymers, was
18 confirmed by FTIR with the total disappearance of the azide signal at 2097 cm⁻¹ (Figure 1A)
19 and by Raman spectroscopy (Figure 1B) with the disappearance of the signal corresponding
20 to the alkyne end function of the PLA at 2131 cm⁻¹.



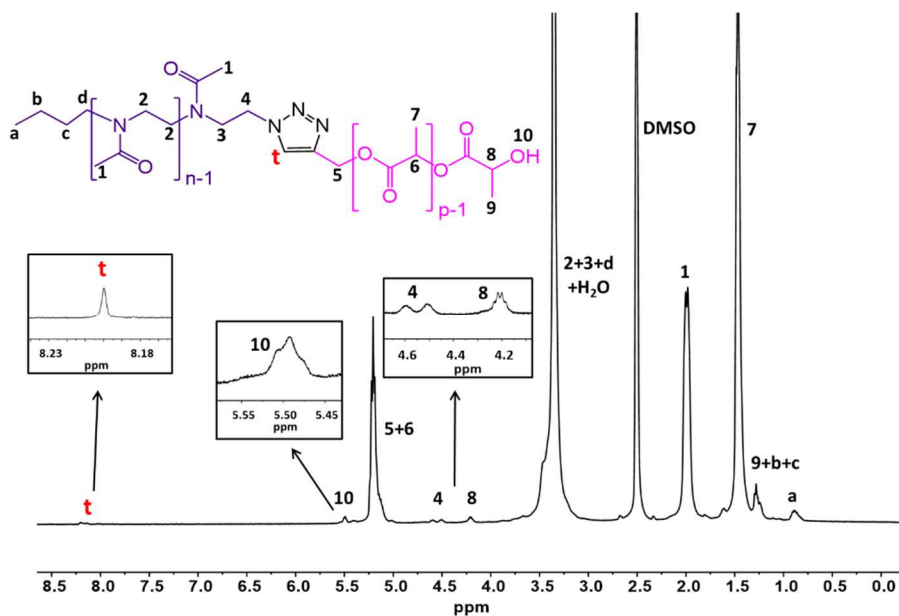
1

2 Figure 1. (A) FTIR of spectra of $\text{PMeOx}_{2200}\text{-N}_3$ and $\text{PMeOx}_{2200}\text{-}b\text{-PLA}_{2800}$ and (B) RAMAN
 3 spectra of $\text{PLA}\equiv$ and $\text{PMeOx}_{2200}\text{-}b\text{-PLA}_{2800}$

4

5 The chemical structure of diblock copolymers $\text{PMeOx-}b\text{-PLA}$ was investigated by ^1H NMR
 6 in DMSO-d_6 (Figure 2). Coupling was attested by the appearance of the peak of resonance **t**
 7 at 8.20 ppm corresponding to the triazole proton **t** resulting from the CuAAC, and by the
 8 expected downfield shift of the peak **4** observed for the methylene protons in α of triazole
 9 compared to the one in α of the azide group (from 3.32 to 4.55 ppm). Furthermore, in the
 10 DMSO-d_6 , the protons of this signal **4**, are not equivalent due to the lack of mobility in α of
 11 the triazole ring, so the signal observed is a doublet. Finally, the total shift of the peak **5**
 12 corresponding to the proton on the carbon of the PLA in α of the triazole ring (from 4.85 in
 13 $\text{PLA}\equiv$ to 5.20 ppm in coupled PLA) confirmed the success of the click reaction.

14

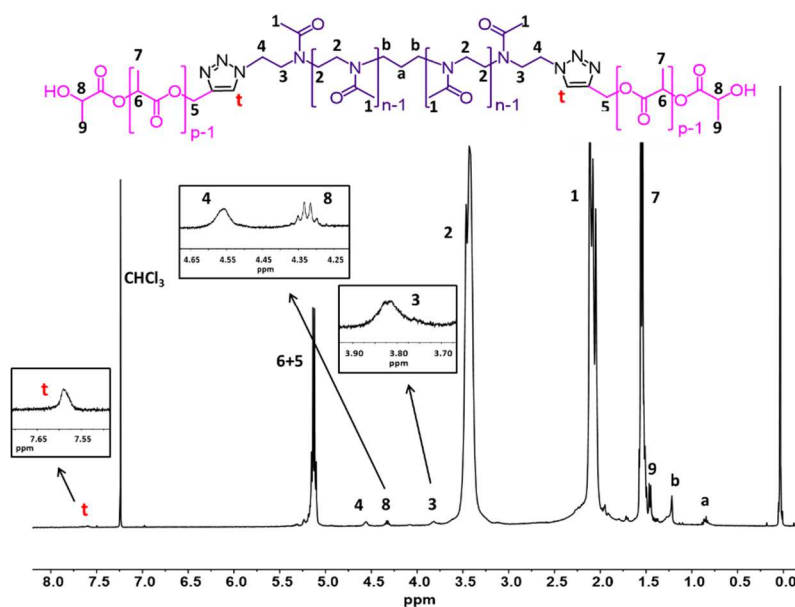


1

2 Figure 2. ^1H NMR spectrum of a diblock copolymer $\text{PMeOx}_{2200}\text{-}b\text{-PLA}_{2800}$ in DMSO-d_6

3

4 The ^1H NMR spectra of the triblock copolymers $\text{PLA-}b\text{-PMeOx-}b\text{-PLA}$ were performed in
 5 CDCl_3 (Figure 3). The presence of the peak of resonance **t** at 7.59 ppm corresponding to the
 6 proton of the triazole rings as well as the downfield shift of the peaks **4** at 4.55 ppm and **3** at
 7 3.80 ppm in α and β of the triazole with respect to the signal in α and β of azide group
 8 showed the coupling.



1

2 Figure 3. ^1H NMR spectrum of a triblock copolymer $\text{PLA}_{2200}\text{-}b\text{-PMeOx}_{2200}\text{-}b\text{-PLA}_{2200}$ in
 3 CDCl_3 .

4

5 It is interesting to note that the signal **4**, for the diblock as for the triblock copolymers, is a
 6 singlet in the CDCl_3 and a doublet in DMSO-d_6 . Finally, the total shift of the signal **5**
 7 corresponding to the proton on the carbon of the PLA in α of the triazole ring (from 4.70 to
 8 5.10 ppm) and, in COSY NMR (Figure S6), the absence of correlation signal corresponding
 9 to the methylene proton adjacent to terminal alkyne group before the cycloaddition confirmed
 10 the success of the click reaction and the absence of free alkynyl-terminated PLA. The
 11 characterization of the four synthesized diblock copolymers and of the six triblock
 12 copolymers including number-average molecular masses of each block and the fraction of the
 13 molecular mass of the hydrophilic part to the total molecular mass (f) are summarized in
 14 Tables 1 and 2.

15

16

1 Table 1. Diblock copolymers PMeOx-*b*-PLA: molecular characteristics and weight fractions
 2 of PLA.

Copolymers	\overline{M}_n^{PMeOx}	\overline{M}_n^{PLA}	$\overline{M}_n^{diblock}$	PLA	PLA	PLA	<i>f</i> (%)
	(g.mol ⁻¹)	(g.mol ⁻¹)	(g.mol ⁻¹)	wt. % expected	wt. % NMR	wt. % TGA	
PMeOx ₂₂₀₀ - <i>b</i> -PLA ₂₈₀₀	2.2×10 ³	2.8×10 ³	5.0×10 ³	56	58	57	44
PMeOx ₂₂₀₀ - <i>b</i> -PLA ₅₂₀₀	2.2×10 ³	5.2×10 ³	7.4×10 ³	70	75	76	30
PMeOx ₅₅₀₀ - <i>b</i> -PLA ₂₈₀₀	5.5×10 ³	2.8×10 ³	8.3×10 ³	34	34	32	66
PMeOx ₅₅₀₀ - <i>b</i> -PLA ₅₂₀₀	5.5×10 ³	5.2×10 ³	10.7×10 ³	48	52	50	52

3 *f*: hydrophilic weight ratio (ratio of the mass of the hydrophilic part to the total mass)

4

5

6

7

8

9

10

11

12

- 1 Table 2. Triblock copolymers PLA-*b*-PMeOx-*b*-PLA: molecular characteristics and weight
 2 fractions of PLA.

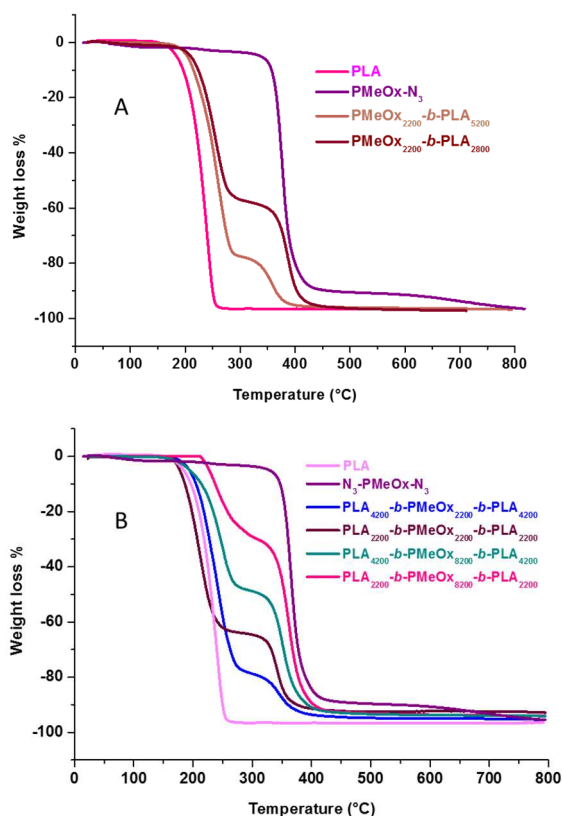
Copolymers	\overline{M}_n^{PMeOx}	\overline{M}_n^{PLA}	$\overline{M}_n^{triblock\ calculated}$	PLA	PLA	PLA	<i>f</i> (%)
	(g.mol ⁻¹)	(g.mol ⁻¹)	(g.mol ⁻¹)	wt. % expected	wt. % NMR	wt. % TGA	
PLA ₁₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₁₂₀₀	2.2×10 ³	1.2×10 ³	4.6×10 ³	52	55	54	48
PLA ₂₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₂₂₀₀	2.2×10 ³	2.2×10 ³	6.6×10 ³	66	69	65	34
PLA ₄₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₄₂₀₀	2.2×10 ³	4.2×10 ³	10.6×10 ³	79	81	78	21
PLA ₁₂₀₀ - <i>b</i> -PMeOx ₈₂₀₀ - <i>b</i> -PLA ₁₂₀₀	8.2×10 ³	1.2×10 ³	10.6×10 ³	23	25	25	77
PLA ₂₂₀₀ - <i>b</i> -PMeOx ₈₂₀₀ - <i>b</i> -PLA ₂₂₀₀	8.2×10 ³	2.2×10 ³	12.6×10 ³	34	35	30	67
PLA ₄₂₀₀ - <i>b</i> -PMeOx ₈₂₀₀ - <i>b</i> -PLA ₄₂₀₀	8.2×10 ³	4.2×10 ³	16.6×10 ³	50	52	48	50

3 *f*: hydrophilic weight ratio (ratio of the mass of the hydrophilic part to the total mass)

4

5 Thermal stability of the diblock and triblock copolymers was studied by thermogravimetric
 6 analyses (TGA) (Figure 4) and compared with the degradation profiles of the PMeOx-N₃ or
 7 N₃-PMeOx-N₃ and the PLA≡.

8



1

2 Figure 4. TGA curves for PLA≡, PMeOx-N₃ and PMeOx *b*-PLA (A) and for PLA≡ and N₃-
 3 PMeOx-N₃ and PLA-*b*-PMeOx *b*-PLA (B).

4

5 The obtained results showed a two-step degradation behavior with a first step from 200 to
 6 280 °C (or 150 to 250 °C) corresponding to the degradation of the PLA block and a second
 7 step from 280 to 440 °C (or 250 to 450 °C) corresponding to the degradation of PMeOx
 8 block. The weight fraction of PLA in each copolymer was calculated and the results
 9 (summarized in Tables 1 and 2) demonstrated a good correlation with those obtained by ¹H
 10 NMR and with the expected values. Thereby, these results confirmed the efficiency of the
 11 coupling reaction for the preparation of amphiphilic diblock and triblock copolymers
 12 PMeOx-*b*-PLA and PLA-*b*-PMeOx-*b*-PLA with various hydrophilic weight ratios (*f*).

13

1 Self-assembly study of the diblock copolymers

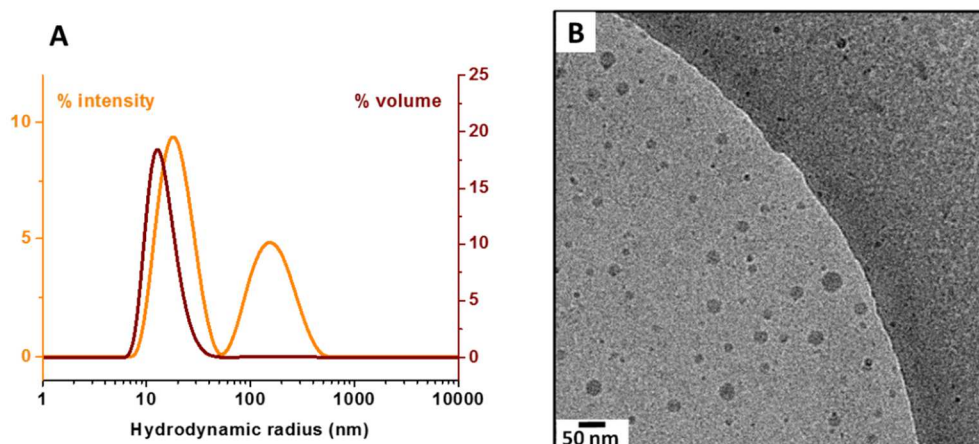
2 Self-assemblies of the diblock copolymers were prepared by nanoprecipitation, that is
3 commonly known for resulting in kinetically trapped structures due to rapid solvent
4 exchange.⁴⁵ First, the PMeOx-*b*-PLA diblock copolymers were dissolved in acetonitrile
5 (ACN) (2 g L⁻¹), which is a solvent for both PMeOx and PLA polymers and has a good
6 miscibility with water. The solution was added dropwise to deionized water, under moderate
7 stirring, inducing the self-assembly process. The organic solvent was then removed under
8 vacuum and water added to reach the desired concentration. Herein, the different diblock
9 copolymers used were, in increasing order of hydrophilic weight ratio *f*: PMeOx₂₂₀₀-*b*-
10 PLA₅₂₀₀ (*f* = 30 %), PMeOx₂₂₀₀-*b*-PLA₂₈₀₀ (*f* = 44 %), PMeOx₅₅₀₀-*b*-PLA₅₂₀₀ (*f* = 52 %) and
11 PMeOx₅₅₀₀-*b*-PLA₂₈₀₀ (*f* = 66 %).

12 The resulting suspensions were characterized by dynamic light scattering (DLS) at 173°, the
13 results showed the presence of nano-objects with hydrodynamic radius (R_h) between 14 nm
14 and 24 nm according to the composition of the amphiphilic copolymer, as well as objects of
15 several hundred nanometers (as shown in the Figure 5.A for the distribution based on
16 PMeOx₂₂₀₀-*b*-PLA₂₈₀₀). The distribution widths (σ_{DLS}) are high, of the order of 0.5, because
17 of the bimodal size distribution (only in intensity, volume size distributions showed a
18 unimodal distribution). The R_h (from volume size distribution) and σ_{DLS} values are reported in
19 Table 3.

20 The cryo-TEM micrographs of the suspension based on PMeOx₂₂₀₀-*b*-PLA₂₈₀₀ (Figure 5.B)
21 allowed observing spherical objects with an average radius of 15 nm (average radius was
22 calculated on the basis of at least 100 nano-objects using imageJ software) which is
23 extremely close to the R_h obtained by DLS. It should be noted the absence of large
24 aggregates, around about 200 nm, in cryo-TEM, suggesting that they are few, which is

1 consistent with their presence in DLS only in intensity, not in volume.

2

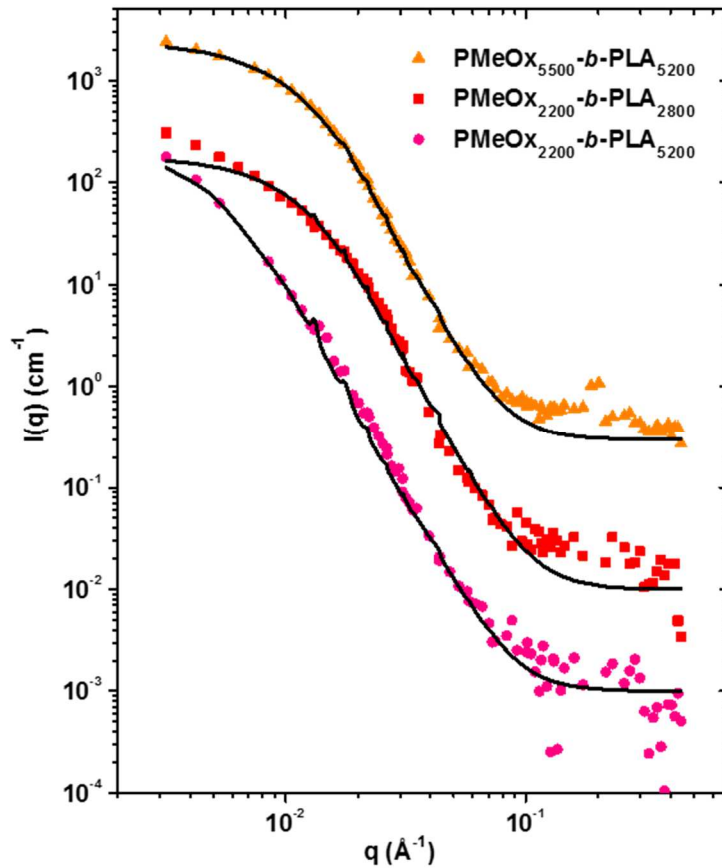


3

4 Figure 5. (A) DLS size distribution and (B) cryo-TEM micrograph of nano-objects of
5 $\text{PMeO}_{x2200}\text{-}b\text{-PLA}_{2800}$ ($f = 44\%$) obtained by nanoprecipitation.

6

7 Small-angle neutron scattering (SANS) was used to probe more precisely and quantitatively
8 the structure of the nano-objects and to measure better averaged nanoscopic dimensions of
9 the objects through an appropriate model. As said before, the suspensions were analyzed in
10 D_2O at a concentration of 1 g.L^{-1} at room temperature. Figure 6 shows the scattered intensity
11 as a function of scattering vector (q) for nano-objects based on $\text{PMeO}_{x2200}\text{-}b\text{-PLA}_{5200}$ ($f =$
12 30%) (pink circle), $\text{PMeO}_{x2200}\text{-}b\text{-PLA}_{2800}$ ($f = 44\%$) (red square) and $\text{PMeO}_{x5500}\text{-}b\text{-PLA}_{52000}$
13 ($f = 52\%$) (orange triangle) (for better visibility, scattered intensities of red and orange curves
14 were multiplied by a power of 10 on the graph).



1

2 Figure 6. Scattered intensity as a function of scattering vector for nano-objects of PMeOx₂₂₀₀-
 3 *b*-PLA₅₂₀₀ ($f = 30\%$), PMeOx₂₂₀₀-*b*-PLA₂₈₀₀ ($f = 44\%$), and PMeOx₅₅₀₀-*b*-PLA₅₂₀₀ ($f = 52\%$)
 4 (the black line is the fitting curve by a polydisperse sphere model).

5

6 The general appearance of the three curves was similar for large and medium q values.
 7 Indeed, the intermediate q value regime ($2.0 \times 10^{-2} < q \text{ (Å}^{-1}\text{)} < 8.0 \times 10^{-2}$) is characterized by a
 8 variation in q^{-4} and might correspond to a compact structure. While for larger values of q
 9 ($8.0 \times 10^{-2} < q \text{ (Å}^{-1}\text{)} < 4.4 \times 10^{-1}$), the decrease of $\log I$ was less abrupt. This variation might be
 10 characteristic of an interface of dangling polymer chains. However, the absence of a variation
 11 in q^{-2} was due to the impossibility to subtract more incoherent from the solvent. Nevertheless,
 12 for small values of scattering vector q , it is possible to observe, for samples based on
 13 PMeOx₂₂₀₀-*b*-PLA₂₈₀₀ and PMeOx₅₅₀₀-*b*-PLA₅₂₀₀, a plateau (Guinier regime) characteristic of

1 a size approachable by SANS, while for samples based on PMeOx₂₂₀₀-*b*-PLA₅₂₀₀ this plateau
 2 was not reached which might correspond to a greater polydispersity and / or larger radius of
 3 gyration.

4 The SANS data were fitted using a polydisperse sphere model. The dispersities due to the
 5 detector, on the I and q values, have been considered. The traces of the fits obtained are in
 6 black on the Figure 6. The parameters of the fits (R_{SANS} and σ_{SANS}) are reported in Table 3.

7

8 Table 3. Characteristics of PMeOx-*b*-PLA self-assemblies obtained by nanoprecipitation.

Copolymers	f (%)	$R_h^{(a)}$ (nm)	$\sigma_{DLS}^{(a)}$	$R_{SANS}^{(b)}$ (nm)	$\sigma_{SANS}^{(b)}$	Lc(PMeOx) (nm)	Lc(PLA) (nm)	$N_{agg}^{(c)}$
PMeOx ₂₂₀₀ - <i>b</i> -PLA ₅₂₀₀	30	23.5	0.59	16.7	0.44	9.0	26.0	2800
PMeOx ₂₂₀₀ - <i>b</i> -PLA ₂₈₀₀	44	14.5	0.67	9.9	0.40	9.0	14.0	1090
PMeOx ₅₅₀₀ - <i>b</i> -PLA ₅₂₀₀	52	24.5	0.47	9.5	0.37	22.6	26.0	520
PMeOx ₅₅₀₀ - <i>b</i> -PLA ₂₈₀₀	66	22.0	0.55	-	-	22.6	14.0	-

9 (a) Hydrodynamic radii and σ_{DLS} values determined by DLS

10 (b) Determined by fitting the SANS curves using a polydisperse sphere model.

11 (c) Aggregation number.

12

13 One can notice that R_{SANS} were always lower than R_h for all the samples analyzed by the two
 14 methods. For instance, for PMeOx₂₂₀₀-*b*-PLA₂₈₀₀, $R_{SANS} = 9.9$ nm which was lower than $R_h =$
 15 14.5 nm whereas the distribution widths were comparable from both techniques. This is in

1 agreement with a core-shell structure of the nano-assemblies, *i.e.* a denser polymer core and a
2 corona made of hydrated PMeOx chains. Indeed, the hydrodynamic radius includes the
3 solvent displaced by the nano-object whereas SANS should probe only the dense core, the
4 scattering length density of the hydrated corona layer being very close to the one of D₂O. It is
5 thus reasonable to assume that R_{SANS} corresponds to the core radius.

6 It was possible to calculate the contour length of each block $L_C(\text{PLA})$ and $L_C(\text{PMeOx})$, to
7 verify the possibility of a micellar core-shell structure by determining the maximum radius
8 that could have a core only made of PLA and the maximum extension of a PMeOx external
9 layer (equation 5):

$$10 \quad L_C(\text{PLA}) = a_{\text{PLA}} \times \text{DP}_{\text{PLA}} \quad \text{and} \quad L_C(\text{PMeOx}) = a_{\text{PMeOx}} \times \text{DP}_{\text{PMeOx}} \quad (5)$$

11 In which DP_{PLA} and DP_{PMeOx} are the degrees of polymerization of PLA and PMeOx
12 respectively, and a_{PLA} and a_{PMeOx} are the lengths of a repeating unit in PLA and PMeOx, both
13 approximated to 3.5 Å (0.35 nm). Values are reported in Table 3. In all cases, R_{SANS} was
14 lower than $L_C(\text{PLA})$. Moreover, the thickness of the corona layer, roughly estimated from the
15 difference between R_h and R_{SANS} were also always lower than $L_C(\text{PMeOx})$. The aggregates
16 had thus likely a micellar core-shell structure with a core made of PLA blocks' self-assembly
17 and a corona of non-extended PMeOx.

18 The results showed that with the same size of the hydrophilic PMeOx block, the core radius
19 increased with an increase of the hydrophobic PLA block polymer length, from 9.9 nm for
20 PMeOx₂₂₀₀-*b*-PLA₂₈₀₀ to 16.7 nm for PMeOx₂₂₀₀-*b*-PLA₅₂₀₀. Moreover, the thickness of the
21 PMeOx shell increased with the PMeOx length, as roughly represented by the difference R_h -
22 R_{SANS} which was about 5.0 – 7.0 nm for PMeOx₂₂₀₀ and 16.0 nm for PMeOx₅₂₀₀. These
23 observations are consistent with previous studies on other linear amphiphilic diblock
24 copolymers.⁴⁶

1 In order to demonstrate the coherence of this analysis, aggregation numbers N_{agg} were
2 estimated assuming that the core was made of only PLA blocks and contained no solvent
3 (equation 6):

$$4 \quad N_{agg} = \frac{N_A d}{\overline{M}_{nPLA}} \left(\frac{4\pi r_{core}^3}{3} \right) \quad (6)$$

5 Where \overline{M}_{nPLA} was the PLA number-average molecular mass obtained by ^1H NMR, N_A the
6 Avogadro number, d the density of the PLA block equal to 1.25 g.cm^{-3} and r_{core} estimated as
7 R_{SANS} .

8 The data are presented in Table 3. N_{agg} are ranging from 500 to 2800. These are relatively
9 high values compared to the ones reported for micellar surfactants (less than few hundred).⁴⁷

10 The unusually high N_{agg} values were mainly due to the formation of “irregular” micelles
11 made of a frozen PLA core. However, these values were always lower than the maximum
12 N_{agg} values calculated for a given PLA block and obtained by replacing r_{core} by $L_c(\text{PLA})$ in
13 equation 6. (N_{agg}) max were equal to 1700 and 10600 for PLA blocks of 2800 and 5200
14 g.mol^{-1} respectively. More quantitatively, N_{agg} decreased when the hydrophilic weight
15 fraction f increased.

16 Discher^{48,49} proposed an unified empirical rule based on the ratio f of the molecular mass of
17 the hydrophilic part to the total molecular mass: polymersomes are generally observed in the f
18 range 25 % - 40 %, worm like micelles in the f range 40 % - 50 %, and spherical micelles for
19 higher f values. Several authors verified this rule and reported the formation of core-shell
20 micellar structures for PMeOx-based diblock copolymers in which the hydrophilic weight
21 ratio f was larger than 50 %^{26,50} but also for triblock copolymers with a hydrophobic middle
22 block. For instance the self-assembly of poly(2-methyl-2-oxazoline)-*b*-poly(tetrahydrofuran)-
23 *b*-poly(2-methyl-2-oxazoline) (PMeOx-*b*-PTHF-*b*-PMeOx) amphiphilic triblock copolymers
24 was previously reported by Rasolonjatovo *et al.*,²⁴ they showed the increase of the

1 hydrodynamic radius of micelles when the PMeOx length increased and the PTHF length
2 remained constant. On the other hand, well defined polymersome morphologies have been
3 obtained with PMeOx-*b*-PDMS diblock copolymers as well as PMeOx-*b*-PDMS-*b*-PMeOx
4 triblock copolymers within the expected hydrophilic weight ratio range, between 20 and 30
5 %.^{23,51} When the hydrophobic middle block (poly(2-phenyl-2-oxazoline)) was glassy,
6 polymersomes were also observed, for a hydrophilic weight ratio of 50 % but in coexistence
7 with micelles.⁵²

8 Le Meins *et al.*⁵³ recalled that for polymers, the obtained morphologies not only results from
9 geometrical parameters but an entropy and enthalpy loss must be taken into account during
10 the self-assembling process. In our case, multiple morphologies were expected as *f* was
11 varied between 30 and 66 %. However, only the spherical micelles morphologies were
12 clearly identified in this work. The other morphologies (vesicles, wormlike micelles) were
13 not reached. This could be due to the kinetically trapped system led by the kinetic of solvent
14 diffusion faster than the kinetics of self-assembly (by nanoprecipitation), leading to frozen
15 morphologies. Similar findings have also been reported of PEG-*co*-PLA copolymers with *f*
16 ratio close to 0.2 using a flash nanoprecipitation⁴⁶ method whereas micrometric sized vesicles
17 where observed using a slow water dissolution process with *f* in the range 0.2 – 0.42.⁵⁴ This
18 phenomenon confirms that even if it is reported that the morphologies are in part
19 predetermined by the macromolecular composition, the material morphology can also be
20 controlled by the self-assembly process used.^{55,56}

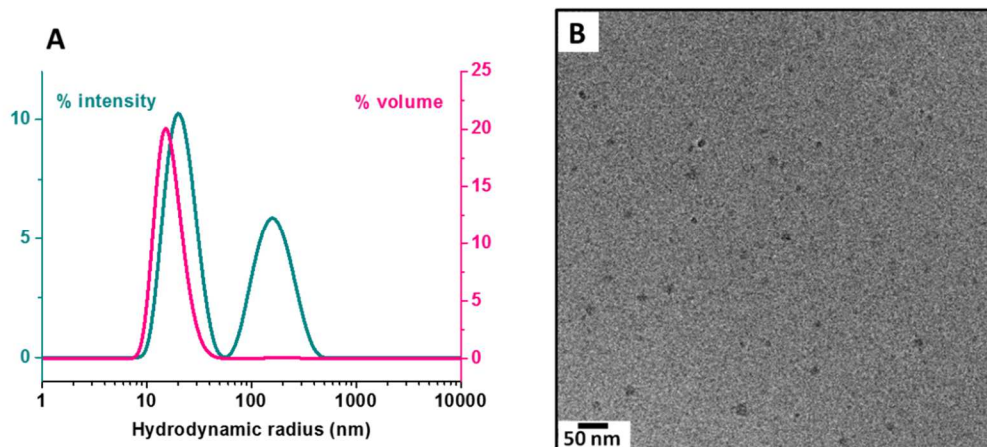
21

22

23 **Self-assembly study of the triblock copolymers**

24 Next we studied the self-assembly behavior of the ABA triblock copolymers, in a B-selective
25 solvent. For this purpose, six copolymers with different compositions were synthesized: three

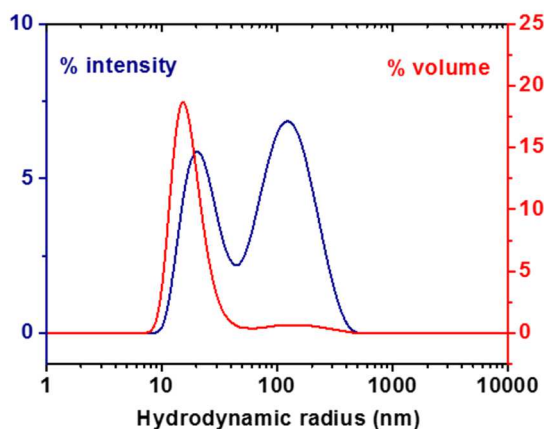
1 copolymers having a hydrophilic middle block of $8.2 \times 10^3 \text{ g.mol}^{-1}$: PLA₁₂₀₀-*b*-PMeOx₈₂₀₀-*b*-
 2 PLA₁₂₀₀ ($f = 77 \%$), PLA₂₂₀₀-*b*-PMeOx₈₂₀₀-*b*-PLA₂₂₀₀ ($f = 67 \%$), PLA₄₂₀₀-*b*-PMeOx₈₂₀₀-*b*-
 3 PLA₄₂₀₀ ($f = 50 \%$) and three copolymers having a hydrophilic middle block of 2.2×10^3
 4 g.mol^{-1} : PLA₁₂₀₀-*b*-PMeOx₂₂₀₀-*b*-PLA₁₂₀₀ ($f = 48 \%$), PLA₂₂₀₀-*b*-PMeOx₂₂₀₀-*b*-PLA₂₂₀₀ ($f = 34$
 5 $\%$), PLA₄₂₀₀-*b*-PMeOx₂₂₀₀-*b*-PLA₄₂₀₀ ($f = 21 \%$). Self-assembly of PLA-*b*-PMeOx-*b*-PLA
 6 triblock copolymers was triggered by nanoprecipitation in water from ACN solution but also
 7 by dialysis from ACN (see experimental part) for some of the samples, a slower solvent
 8 mixing process, in order to compare nano-structures obtained from both techniques.
 9 The suspensions were firstly characterized by DLS at 173°. The results showed nano-objects
 10 with hydrodynamic radii between 14 nm and 30 nm depending on the composition as well as
 11 objects of several hundred nanometers (as shown in the Figure 7.A for the distribution based
 12 on PLA₂₂₀₀-*b*-PMeOx₈₂₀₀-*b*-PLA₂₂₀₀ by nanoprecipitation).



13
 14 Figure 7. (A) DLS size distribution and (B) cryo-TEM micrograph of nano-objects of
 15 PLA₂₂₀₀-*b*-PMeOx₈₂₀₀-*b*-PLA₂₂₀₀ ($f = 67 \%$) obtained by nanoprecipitation.

16 The distribution widths σ_{DLS} are high, of the order of 0.6 and in the same range as for the
 17 diblock copolymers, because of the bimodal size distribution (only in intensity, volume size
 18 distributions showed a unimodal distribution). R_h and σ_{DLS} values are reported in Table 4.

1 It should be outlined that the contribution of the large size aggregates on the intensity signal
2 is somewhat larger in the case of the samples obtained by the dialysis method. Moreover, a
3 discreet signal corresponding to these large aggregates is persistent on the volume signal
4 (Figure 8, PLA₂₂₀₀-*b*-PMeOx₂₂₀₀-*b*-PLA₂₂₀₀).

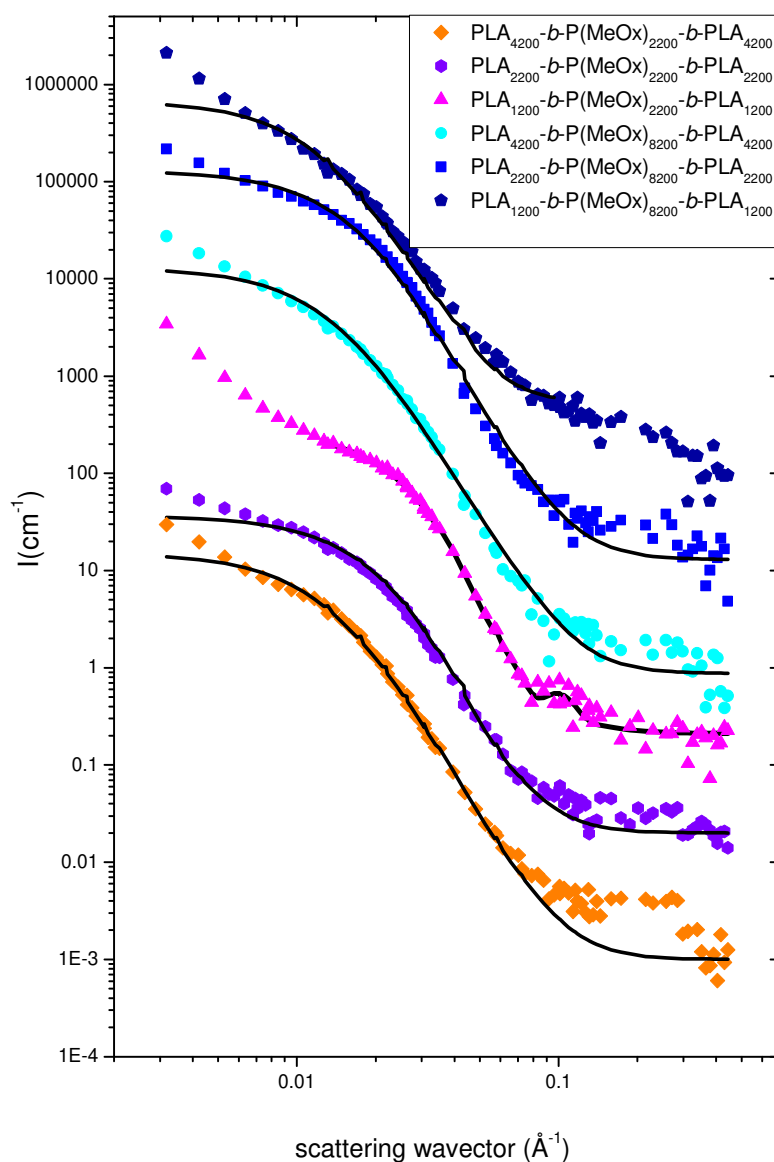


5
6 Figure 8. DLS size distribution of nano-objects of PLA₂₂₀₀-*b*-PMeOx₈₂₀₀-*b*-PLA₂₂₀₀ ($f = 67$
7 %) obtained by dialysis.

8
9 The intensity representation gives a strong importance to the aggregates, so we chose to
10 retain the R_h value in volume distribution in order to characterize the sizes of the majority of
11 objects.

12 The cryo-TEM micrographs of the suspension based on PLA₂₂₀₀-*b*-PMeOx₈₂₀₀-*b*-PLA₂₂₀₀
13 copolymer (Figure 7.B) showed spherical structures with an average radius of 9.5 nm which
14 was smaller than the hydrodynamic radius obtained by DLS (17.0 nm). This can be explained
15 by the influence of the largest structures on the hydrodynamic diameter obtained by DLS, but
16 also one should consider that hydrodynamic diameters take into account the solvent displaced
17 by the nano-objects and this could result in an overestimate of the size.

1 The self-assemblies from triblock copolymers were characterized by SANS in D₂O at 1 g.L⁻¹
 2 and at room temperature. Figure 9 and Figure S7 show the plots of the scattered intensity as a
 3 function of q from the different samples obtained by nanoprecipitation and dialysis
 4 respectively. For better visibility, intensity was multiplied by a power of 10 on the graph.
 5



6
 7 Figure 9. Scattered intensity as a function of scattering vector q for nano-objects of PLA-*b*-
 8 PMeOx-*b*-PLA obtained by nanoprecipitation (the solid black lines are the fitting curves).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Similar to the SANS results obtained for the diblocks, the six curves were characterized by a variation in q^{-4} in the intermediate q value regime ($2.0 \times 10^{-2} < q \text{ (\AA}^{-1}) < 8.0 \times 10^{-2}$) that may correspond to a compact structure. While for larger values of q ($8.0 \times 10^{-2} < q \text{ (\AA}^{-1}) < 4.4 \times 10^{-1}$), the decrease of $\log I$ was less abrupt. This variation might be characteristic of an interface coated with hydrated polymer chains. However, the absence of a variation in q^{-2} was due to the impossibility to subtract more incoherent from the solvent.

The low q behavior ($3.0 \times 10^{-3} < q \text{ (\AA}^{-1}) < 2.0 \times 10^{-2}$) was quite different from the one observed for the diblock copolymers. In all cases, there was an intensity increase as q decreased which should be characteristic of an aggregation phenomenon. The self-assemblies were thus made of finite size aggregates, as can be extrapolated from the combination of DLS, cryo-TEM and SANS results.

1

2 Table 4. Characteristics of PLA-*b*-PMeOx-*b*-PLA nano-objects

Copolymers	Method ^(a)	<i>f</i> (%)	R_h ^(b) (nm)	σ_{DLS} ^(a)	R_{SANS}	σ_{SANS}	N_{agg} ^(d)
					or $R^c_{SANS}, \delta^1_{SANS}, \delta^2_{SANS}$ ^(c) (nm)	or $\sigma^c_{SANS}, \sigma^1_{SANS}, \sigma^2_{SANS}$ ^(c)	
PLA ₄₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₄₂₀₀	1	21	31.0	0.48	6.4	0.42	98
PLA ₂₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₂₂₀₀	1	34	15.0	0.73	6.9	0.33	242
PLA ₁₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₁₂₀₀	1	48	14.5	0.57	3.4/1.5/1.7	0.25/0.4/0.4	275
PLA ₄₂₀₀ - <i>b</i> -PMeOx ₈₂₀₀ - <i>b</i> -PLA ₄₂₀₀	1	50	31.0	0.53	6.5	0.45	109
PLA ₂₂₀₀ - <i>b</i> -PMeOx ₈₂₀₀ - <i>b</i> -PLA ₂₂₀₀	1	67	17.0	0.68	8.2	0.31	410
PLA ₁₂₀₀ - <i>b</i> -PMeOx ₈₂₀₀ - <i>b</i> -PLA ₁₂₀₀	1	77	-*	-*	6.0	0.55	283
PLA ₄₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₄₂₀₀	2	21	25.0	0.63	11.5	0.35	593
PLA ₂₂₀₀ - <i>b</i> -PMeOx ₂₂₀₀ - <i>b</i> -PLA ₂₂₀₀	2	34	16.0	0.60	6.9	0.45	244
PLA ₂₂₀₀ - <i>b</i> -PMeOx ₈₂₀₀ - <i>b</i> -PLA ₂₂₀₀	2	67	18.0	0.69	10.4	0.17	830

3 (a) Method 1 is nanoprecipitation, method 2 is dialysis

4 (b) Hydrodynamic radii (R_h) and σ_{DLS} values determined by DLS5 (c) Determined by fitting the SANS curves using a polydisperse sphere or a core multishell
6 model.

7 (d) Aggregation number.

1 *These values are not accessible by DLS measurement, the correlogram is not correct due to
2 the large presence of the aggregates

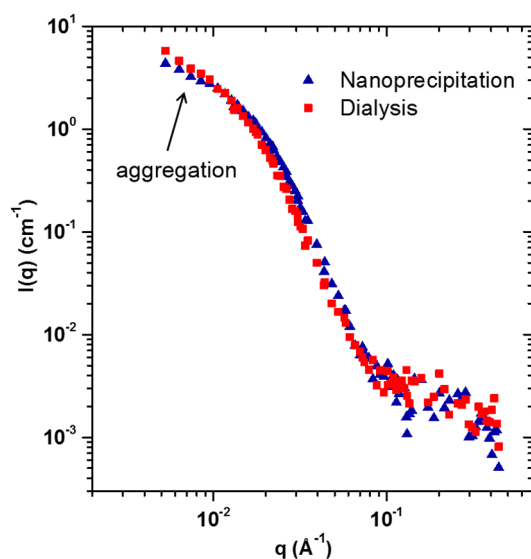
3

4 The SANS data were fitted and the traces of the fits obtained are in black in Figure 9. A
5 polydisperse sphere model was used in most cases. As was highlighted in the diblock self-
6 assembly study, SANS probes mainly the dense PLA core because the hydrated PMeOx
7 corona has a contrast length density close to the one of D₂O. Consequently, the fits
8 parameters, R_{SANS} and σ_{SANS} , are thus characteristic of the core radius of the micelles. R_{SANS}
9 were in a close range 6.0 – 8.2 nm for the nanoprecipitation method and 6.9 – 11.5 nm for the
10 dialysis method, without a marked dependence upon the PLA chain length. Interestingly,
11 R_{SANS} for the triblocks were smaller than the ones determined for the diblocks (9.5- 16.7 nm)
12 whereas R_h of the triblocks had comparable values to the ones of the diblocks. One can notice
13 that R_{SANS} were always lower than R_h as expected, the latter being sensitive to the hydrated
14 micellar corona and to the aggregation phenomenon. Interestingly, for PLA₁₂₀₀-*b*-PMeOx₂₂₀₀-
15 *b*-PLA₁₂₀₀, a polydisperse core-multishell model was found to be the most appropriate model
16 to fit the SANS data. It consists in a dense PLA core (ρ_{PLA} , R^c_{SANS} and σ^c_{SANS}), a hydrated
17 shell (ρ_{D2O} , R^1_{SANS} and σ^1_{SANS}) and an external PLA shell (ρ_{PLA} , R^2_{SANS} and σ^2_{SANS}). Again,
18 in this case, the total radius ($R^2_{SANS}= 7.1$ nm) was in the same range as for the other triblocks
19 and much lower than R_h .

20 In all the cases, the aggregation number has been deduced from a similar calculation as for
21 the diblock copolymers (equation 6). In the case of the analysis by a core-multishell model,
22 the term $4/3\pi(r_c)^3$ has been replaced in the same equation by the total volume of the PLA
23 domains (in the core and in the shell). As was expected from the values of R_{SANS} , N_{agg} were
24 smaller than the ones estimated for the diblock copolymers. Values depended on the
25 preparation method. N_{agg} were in the range 100- 400 for the nanoprecipitated samples and
26 were generally larger for the dialyzed samples 240-830.

1 The dialysis method brought about similar results as from the nanoprecipitation method,
2 except that R_{SANS} , N_{agg} were generally larger in the former method. Figure 10 shows the
3 overlay of the SANS plots obtained from the 2 methods for a same sample (PLA₂₂₀₀-*b*-
4 PMeOx₂₂₀₀-*b*-PLA₂₂₀₀).

5



6

7 Figure 10. Scattered intensity as a function of q for nano-objects of PLA₂₂₀₀-*b*-PMeOx₂₂₀₀-*b*-
8 PLA₂₂₀₀ ($f = 34\%$) obtained by nanoprecipitation and by dialysis.

9

10 Whereas these two samples showed very similar SANS signals, the aggregation was stronger
11 in the sample prepared from dialysis method as the scattered intensity at low q increased
12 more steeply. Indeed, when the self-assembly took place more slowly, here *via* a dialysis
13 process, the micelles which were close enough, could form more bridges between them.

14 The self-assembly of triblock copolymers PLA-*b*-PMeOx-*b*-PLA, by two relatively rapid
15 processes compare to other common methods such as direct solubilization or slow addition of
16 water in organic solution for example, thus allowed the formation of micelles. Average

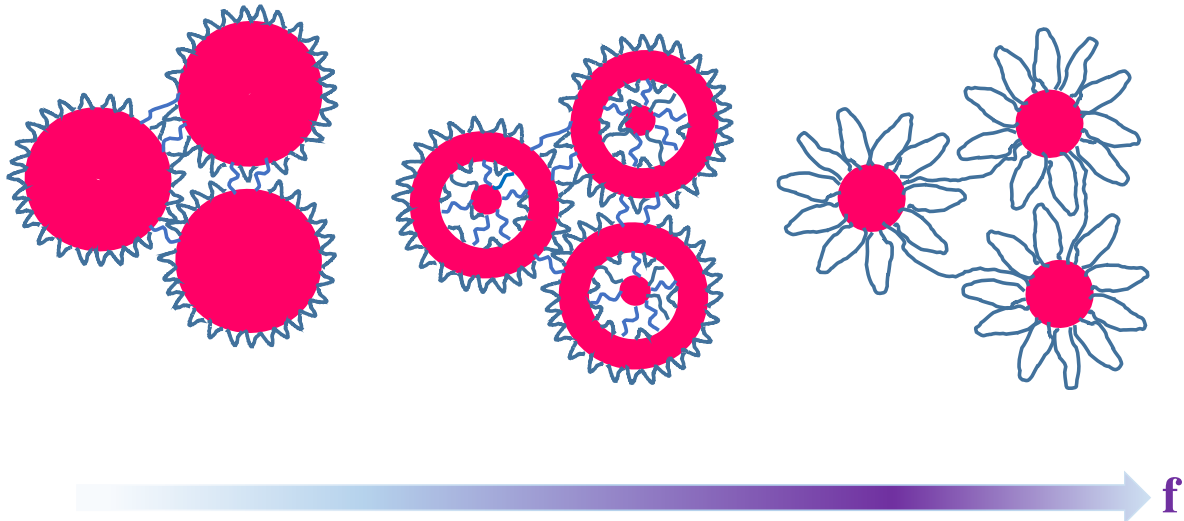
1 aggregate radii were in the range 15.0 – 30.0 nm although DLS highlighted the presence of a
2 small proportion of larger objects of several hundred nanometers. The SANS and cryo-TEM
3 measurements revealed the compact structure of the micellar cores and SANS showed their
4 aggregation into connected structures. Several authors have reported upon the self-assemblies
5 of ABA triblock copolymers in a B-selective solvent.^{57,58} Two types of association
6 mechanisms were described: (i) a closed association mechanism leading to flower-like
7 micelles where the hydrophilic middle block is looped at the outer rim of the micelle, the core
8 being composed of the hydrophobic block; and (ii) an open association mechanism with the
9 formation of loose aggregates. Formation of micelles was reported using a crystalline PLLA
10 block in PLLA-*b*-PEtOx-*b*-PLLA by Hsiue and co-workers⁵⁹. In the case of the same
11 amorphous PLA blocks as in the present study, a closed association mechanism was also
12 reported as micelles were detected and analyzed in PLA-*b*-PEO-*b*-PLA copolymers of *f* ratio
13 larger than 0.5.^{57,58} The results of Bhatia and co-workers were also in good agreement with
14 the formation of flower-like micelles where the core sizes and aggregation numbers increased
15 with the PLA molecular mass. It should be noted that the core sizes were on the same order
16 as for the micelles of this study (for comparable PLA number-average molecular mass).
17 Moreover, networks of bridged micelles were reported as the concentration was increased
18 above 100 g.L⁻¹ but there was no report of the formation aggregates of bridged micelles at
19 concentration as low as 1 g.L⁻¹ as in the present work.

20 As for the diblocks, there is the question of the micelles' morphology as a function of the *f*
21 parameter . Additionnaly to the geometrical and energetic parameters already mentioned for
22 the diblocks, one must take into account the entropy loss associated to the looping of the
23 middle block. This last constraint might be less strong when the middle block has a larger
24 molecular mass. In the experiments, there were two series of triblock copolymers, three
25 copolymer samples with a short PMeOx block (2200 g.mol⁻¹) and three other samples with a

1 PMeOx block more than three times longer (8200 g.mol⁻¹). As f is larger than 50 % for these
2 last three copolymers, spherical micelles were expected, and the results were in good
3 agreement whatever the preparation method chosen. The lower core radius, in comparison to
4 the ones of the diblocks, could be attributed to the looping penalty of the middle block. The
5 series with shorter PMeOx block had f values in a 21-48 % range. For PLA₄₂₀₀- b -PMeOx₂₂₀₀-
6 b -PLA₄₂₀₀, $f = 21$ %, spherical micellar morphologies were obtained using nanoprecipitation
7 and dialysis method. This is in good agreement with the Discher morphology rules^{48,49}. For
8 the two other triblock copolymers with f values of 34 and 48 %, vesicular morphologies could
9 be expected following the same rule. A core-multishell morphology have been detected for
10 PLA₁₂₀₀- b -PMeOx₂₂₀₀- b -PLA₁₂₀₀ using the nanoprecipitation preparation method and
11 appearing as a shoulder in the intermediate q -range of the plot. This morphology might exist
12 for other samples (such as samples with $f = 34\%$) but could not be always detected by SANS
13 due to smoothing polydispersity effects. The core-multishell morphologies were not observed
14 for the diblocks, neither from the SANS plots nor from the cryo-TEM pictures. In the case of
15 the triblocks, the formation of core-multishell structures should be favoured because of the
16 looping penalty of the short PMeOx block.

17 The following association behavior is thus proposed as a function of the parameter f in
18 Scheme 2. Whatever the value of f , bridged micelles of finite size were formed but those
19 might coexist with isolated micelles which have not been represented in Scheme 2 for the
20 sake of simplicity. The micellar core may have an inner structure depending on f as well as
21 the middle block molecular mass. Indeed, for very close f values, 48 and 50 %, core-
22 multishell and core-shell structures were respectively observed due to the PMeOx block
23 length increase.

24



1

2 Scheme 2: Association scheme of the triblock copolymers as a function of the hydrophilic
 3 ratio f . Isolated micelles may coexist with bridged micelles in each case but for simplicity it
 4 has not been drawn.

5

6 However, the technique of self-assembly used has a great influence on the self-assembly
 7 process. Systematic experiments provided information about the location of the “Ouzo
 8 domain”, in which only nanoparticles are obtained, and identified the parameters controlling
 9 the size, polydispersity and the crystallinity of nanoparticles.⁶⁰ The copolymer composition
 10 has a significant influence but the mixing time of the organic solution containing the
 11 amphiphilic copolymer with the aqueous phase is a parameter of first importance. When the
 12 kinetics of self-assembly is slow down by a rapid solvent mixing, here by nanoprecipitation
 13 or dialysis, copolymers do not have enough mobility during the self-assembly to reach the
 14 most stable morphology, the system is trapped under a metastable structure, often of reduced
 15 mean size, as the polydisperse core-multishell and micellar structures obtained in this study.
 16 Consequently, these structures were obtained by the combination of parameters, among them,
 17 the copolymer characteristics and the used self-assembly routes.

1 **Conclusion**

2 Novel well defined diblock and triblock amphiphilic copolymers PMeOx-*b*-PLA and PLA-*b*-
3 PMeOx-*b*-PLA were successfully synthesized *via* CROP and copper-catalyzed Huisgen 1,3-
4 dipolar cycloaddition. In that way, two series of copolymers with varying PLA as well as
5 PMeOx chain length were prepared allowing reaching hydrophilic weight ratio (*f*) in a wide
6 range (21 to 77 %). These copolymers were self-assembled in water *via* solvent displacement
7 methods and in all cases, “irregular” micelles with quite high aggregation numbers were
8 obtained due to the frozen nature of the PLA blocks. For the diblock copolymers, core-shell
9 micellar structures with a dense PLA core and a hydrated PMeOx shell were obtained in the
10 whole range of *f* values. Although vesicular structures were reported in parent diblock
11 copolymers such as PEO-*b*-PLA, their absence in the present study was attributed to the fast
12 preparation method by nanoprecipitation. An original association behavior was evidenced for
13 the triblock copolymers. At concentration as low as 1 g.L⁻¹, they formed spherical micelle-
14 like structure with a few aggregates of finite sizes made of bridged micelles, the micelles
15 being connected by some of the PLA end-blocks. This finding has been reported for the
16 parent PLA-*b*-PEO-*b*-PLA copolymers but it occurred only at much larger concentrations
17 than in the present study. Moreover, the micelles displayed original nanostructures with the
18 formation of core-multishell structures in a specific range of *f* values (close to 48 %) never
19 reported for this kind of triblock copolymers. This process could be favored to reduce the
20 entropy loss coming from the looping of the PMeOx middle blocks when forming simple
21 flower-like micelles. The amphiphilic copolymers of this study displayed a rich variety of
22 controlled self-assembled morphologies which could be promising candidates for drug
23 delivery applications.

24

1 **Acknowledgement**

2 We are grateful to Sena Hamadi for SEC analysis and ICP-OES measurements as well as
3 Mohamed Guerrouache for Raman spectroscopy. We would like to thank the LLB-Orphée
4 large-scale facility for giving us access to SANS measurements and Fabrice Cousin for
5 discussions and help during experiments. We would like to thank Nathalie Jarroux,
6 Laboratoire Analyse et Modélisation pour la Biologie et l'Environnement (LAMBE),
7 Université d'Evry Val d'Essonne for her help for SEC analysis in DMF.

8

REFERENCES

- (1) Adams, M. L.; Lavasanifar, A.; Kwon, G. S. Amphiphilic block copolymers for drug delivery. *Journal of pharmaceutical sciences* **2003**, *92*, 1343-1355.
- (2) Kwon, G. S.; Kataoka, K. Block copolymer micelles as long-circulating drug vehicles. *Advanced drug delivery reviews* **1995**, *16*, 295-309.
- (3) Kataoka, K.; Harada, A.; Nagasaki, Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. *Advanced drug delivery reviews* **2012**, *64*, 37-48.
- (4) Biswas, S.; Kumari, P.; Lakhani, P. M.; Ghosh, B. Recent advances in polymeric micelles for anti-cancer drug delivery. *European Journal of Pharmaceutical Sciences* **2016**, *83*, 184-202.
- (5) Kabanov, A. V.; Batrakova, E. V.; Melik-Nubarov, N. S.; Fedoseev, N. A.; Dorodnich, T. Y.; Alakhov, V. Y.; Chekhonin, V. P.; Nazarova, I. R.; Kabanov, V. A. A new class of drug carriers: micelles of poly (oxyethylene)-poly (oxypropylene) block copolymers as microcontainers for drug targeting from blood in brain. *Journal of Controlled Release* **1992**, *22*, 141-157.
- (6) Meng, X.; Liu, J.; Yu, X.; Li, J.; Lu, X.; Shen, T. Pluronic F127 and D- α -Tocopheryl Polyethylene Glycol Succinate (TPGS) Mixed Micelles for Targeting Drug Delivery across The Blood Brain Barrier. *Scientific Reports* **2017**, *7*, 2964.
- (7) Scholz, C.; Iijima, M.; Nagasaki, Y.; Kataoka, K. A novel reactive polymeric micelle with aldehyde groups on its surface. *Macromolecules* **1995**, *28*, 7295-7297.
- (8) Nagasaki, Y.; Okada, T.; Scholz, C.; Iijima, M.; Kato, M.; Kataoka, K. The reactive polymeric micelle based on an aldehyde-ended poly (ethylene glycol)/poly (lactide) block copolymer. *Macromolecules* **1998**, *31*, 1473-1479.
- (9) Osada, K.; Christie, R. J.; Kataoka, K. Polymeric micelles from poly (ethylene glycol)-poly (amino acid) block copolymer for drug and gene delivery. *Journal of The Royal Society Interface* **2009**, *6*, S325-S339.
- (10) Lin, J.; Zhu, G.; Zhu, X.; Lin, S.; Nose, T.; Ding, W. Aggregate structure change induced by intramolecular helix-coil transition. *Polymer* **2008**, *49*, 1132-1136.
- (11) Priftis, D.; Leon, L.; Song, Z.; Perry, S. L.; Margossian, K. O.; Tropnikova, A.; Cheng, J.; Tirrell, M. Self-Assembly of α -Helical Polypeptides Driven by Complex Coacervation. *Angewandte Chemie* **2015**, *127*, 11280-11284.
- (12) Armstrong, J. K.; Hempel, G.; Koling, S.; Chan, L. S.; Fisher, T.; Meiselman, H. J.; Garratty, G. Antibody against poly (ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients. *Cancer* **2007**, *110*, 103-111.
- (13) Sherman, M. R.; Saifer, M. G.; Perez-Ruiz, F. PEG-uricase in the management of treatment-resistant gout and hyperuricemia. *Advanced drug delivery reviews* **2008**, *60*, 59-68.
- (14) Leger, R.; Arndt, P.; Garratty, G.; Armstrong, J.; Meiselman, H.; Fisher, T. In *Tilte2001*; AMER ASSOC BLOOD BANKS 8101 GLENBROOK RD, BETHESDA, MD 20814-2749 USA.
- (15) Armstrong, J.; Leger, R.; Wenby, R.; Meiselman, H.; Garratty, G.; Fisher, T. In *Tilte2003*; AMER SOC HEMATOLOGY 1900 M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA.
- (16) Barz, M.; Luxenhofer, R.; Zentel, R.; Vicent, M. J. Overcoming the PEG-addiction: well-defined alternatives to PEG, from structure-property relationships to better defined therapeutics. *Polymer Chemistry* **2011**, *2*, 1900-1918.
- (17) Gubarev, A. S.; Monnery, B. D.; Lezov, A. A.; Sedlacek, O.; Tsvetkov, N. V.; Hoogenboom, R.; Filippov, S. K. Conformational properties of biocompatible poly (2-ethyl-2-oxazoline) s in phosphate buffered saline. *Polymer Chemistry* **2018**, *9*, 2232-2237.
- (18) Hoogenboom, R. Poly (2-oxazoline) s: a polymer class with numerous potential applications. *Angewandte Chemie International Edition* **2009**, *48*, 7978-7994.
- (19) Adams, N.; Schubert, U. S. Poly (2-oxazolines) in biological and biomedical application contexts. *Advanced drug delivery reviews* **2007**, *59*, 1504-1520.

- 1 (20) Luxenhofer, R.; Han, Y.; Schulz, A.; Tong, J.; He, Z.; Kabanov, A. V.; Jordan, R. Poly (2-
2 oxazoline) s as Polymer Therapeutics. *Macromolecular rapid communications* **2012**, *33*, 1613-1631.
- 3 (21) Luxenhofer, R.; Sahay, G.; Schulz, A.; Alakhova, D.; Bronich, T. K.; Jordan, R.;
4 Kabanov, A. V. Structure-property relationship in cytotoxicity and cell uptake of poly (2-oxazoline)
5 amphiphiles. *Journal of Controlled Release* **2011**, *153*, 73-82.
- 6 (22) Bauer, M.; Lautenschlaeger, C.; Kempe, K.; Tauhardt, L.; Schubert, U. S.; Fischer, D.
7 Poly (2-ethyl-2-oxazoline) as Alternative for the Stealth Polymer Poly (ethylene glycol): Comparison
8 of in vitro Cytotoxicity and Hemocompatibility. *Macromolecular bioscience* **2012**, *12*, 986-998.
- 9 (23) Nardin, C.; Hirt, T.; Leukel, J.; Meier, W. Polymerized ABA triblock copolymer
10 vesicles. *Langmuir* **2000**, *16*, 1035-1041.
- 11 (24) Rasolonjatovo, B.; Gomez, J.-P.; Mème, W.; Gonçalves, C.; Huin, C. c.; Bennevault-
12 Celton, V. r.; Le Gall, T.; Montier, T.; Lehn, P.; Cheradame, H.; Midoux, P.; Guégan, P. Poly (2-methyl-
13 2-oxazoline)-b-poly (tetrahydrofuran)-b-poly (2-methyl-2-oxazoline) Amphiphilic Triblock
14 Copolymers: Synthesis, Physicochemical Characterizations, and Hydrosolubilizing Properties.
15 *Biomacromolecules* **2015**, *16*, 748-756.
- 16 (25) Kempe, K.; Hoogenboom, R.; Hoepfener, S.; Fustin, C.-A.; Gohy, J.-F.; Schubert, U. S.
17 Discovering new block terpolymer micellar morphologies. *Chemical Communications* **2010**, *46*, 6455-
18 6457.
- 19 (26) Bonné, T. B.; Lüdtkke, K.; Jordan, R.; Štěpánek, P.; Papadakis, C. M. Aggregation
20 behavior of amphiphilic poly (2-alkyl-2-oxazoline) diblock copolymers in aqueous solution studied by
21 fluorescence correlation spectroscopy. *Colloid and Polymer Science* **2004**, *282*, 833-843.
- 22 (27) Krumm, C.; Fik, C. P.; Meuris, M.; Dropalla, G. J.; Geltenpoth, H.; Sickmann, A.; Tiller,
23 J. C. Well-Defined Amphiphilic Poly (2-oxazoline) ABA-Triblock Copolymers and Their Aggregation
24 Behavior in Aqueous Solution. *Macromolecular rapid communications* **2012**, *33*, 1677-1682.
- 25 (28) Anderson, J. M.; Shive, M. S. Biodegradation and biocompatibility of PLA and PLGA
26 microspheres. *Advanced drug delivery reviews* **1997**, *28*, 5-24.
- 27 (29) Ramot, Y.; Haim-Zada, M.; Domb, A. J.; Nyska, A. Biocompatibility and safety of PLA
28 and its copolymers. *Advanced drug delivery reviews* **2016**, *107*, 153-162.
- 29 (30) Riley, T.; Govender, T.; Stolnik, S.; Xiong, C.; Garnett, M.; Illum, L.; Davis, S. Colloidal
30 stability and drug incorporation aspects of micellar-like PLA-PEG nanoparticles. *Colloids surfaces B:*
31 *Biointerfaces* **1999**, *16*, 147-159.
- 32 (31) Riley, T.; Stolnik, S.; Heald, C.; Xiong, C.; Garnett, M.; Illum, L.; Davis, S.; Purkiss, S.;
33 Barlow, R.; Gellert, P. Physicochemical evaluation of nanoparticles assembled from Poly (lactic acid)-
34 Poly (ethylene glycol)(PLA- PEG) block copolymers as drug delivery vehicles. *Langmuir* **2001**, *17*,
35 3168-3174.
- 36 (32) Salem, A.; Cannizzaro, S.; Davies, M.; Tendler, S.; Roberts, C.; Williams, P.;
37 Shakesheff, K. Synthesis and characterisation of a degradable poly (lactic acid)- poly (ethylene
38 glycol) copolymer with biotinylated end groups. *Biomacromolecules* **2001**, *2*, 575-580.
- 39 (33) Riley, T.; Heald, C.; Stolnik, S.; Garnett, M.; Illum, L.; Davis, S.; King, S.; Heenan, R.;
40 Purkiss, S.; Barlow, R. Core- shell structure of PLA- PEG nanoparticles used for drug delivery.
41 *Langmuir* **2003**, *19*, 8428-8435.
- 42 (34) Wang, C. H.; Hsiue, G. H. Synthesis and characterization of temperature-and pH-
43 sensitive hydrogels based on poly (2-ethyl-2-oxazoline) and poly (D, L-lactide). *Journal of Polymer*
44 *Science Part A: Polymer Chemistry* **2002**, *40*, 1112-1121.
- 45 (35) Decuzzi, P.; Godin, B.; Tanaka, T.; Lee, S.-Y.; Chiappini, C.; Liu, X.; Ferrari, M. Size and
46 shape effects in the biodistribution of intravascularly injected particles. *Journal of Controlled Release*
47 **2010**, *141*, 320-327.
- 48 (36) Yang, Y.; Nie, D.; Liu, Y.; Yu, M.; Gan, Y. Advances in particle shape engineering for
49 improved drug delivery. *Drug Discovery Today* **2018**.
- 50 (37) Yokoyama, M.; Miyauchi, M.; Yamada, N.; Okano, T.; Sakurai, Y.; Kataoka, K.; Inoue,
51 S. Characterization and anticancer activity of the micelle-forming polymeric anticancer drug

1 adriamycin-conjugated poly (ethylene glycol)-poly (aspartic acid) block copolymer. *Cancer Research*
2 **1990**, *50*, 1693-1700.

3 (38) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. Tumor vascular permeability
4 and the EPR effect in macromolecular therapeutics: a review. *Journal of Controlled Release* **2000**, *65*,
5 271-284.

6 (39) Kobayashi, H.; Watanabe, R.; Choyke, P. L. Improving conventional enhanced
7 permeability and retention (EPR) effects; what is the appropriate target? *Theranostics* **2014**, *4*, 81.

8 (40) Matsumura, Y.; Maeda, H. A new concept for macromolecular therapeutics in cancer
9 chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent
10 smancs. *Cancer research* **1986**, *46*, 6387-6392.

11 (41) Lav, T.-X.; Lemechko, P.; Renard, E.; Amiel, C.; Langlois, V.; Volet, G. Development of
12 a new azido-oxazoline monomer for the preparation of amphiphilic graft copolymers by combination
13 of cationic ring-opening polymerization and click chemistry. *Reactive and Functional Polymers* **2013**,
14 *73*, 1001-1008.

15 (42) Le Fer, G.; Amiel, C.; Volet, G. Copolymers based on azidopentyl-2-oxazoline:
16 synthesis, characterization and LCST behavior. *European Polymer Journal* **2015**, *71*, 523-533.

17 (43) Volet, G.; Lav, T. X.; Babinot, J.; Amiel, C. Click-Chemistry: An Alternative Way to
18 Functionalize Poly (2-methyl-2-oxazoline). *Macromolecular Chemistry and Physics* **2011**, *212*, 118-
19 124.

20 (44) Le Fer, G. I.; Le Cœur, C. m.; Guigner, J.-M.; Amiel, C.; Volet, G. I. Biocompatible Soft
21 Nanoparticles with Multiple Morphologies Obtained from Nanoprecipitation of Amphiphilic Graft
22 Copolymers in a Backbone-Selective Solvent. *Langmuir* **2017**, *33*, 2849-2860.

23 (45) Lepeltier, E.; Bourgaux, C.; Couvreur, P. Nanoprecipitation and the “Ouzo effect”:
24 Application to drug delivery devices. *Advanced drug delivery reviews* **2014**, *71*, 86-97.

25 (46) Szymusiak, M.; Kalkowski, J.; Luo, H.; Donovan, A. J.; Zhang, P.; Liu, C.; Shang, W.;
26 Irving, T.; Herrera-Alonso, M.; Liu, Y. Core-Shell Structure and Aggregation Number of Micelles
27 Composed of Amphiphilic Block Copolymers and Amphiphilic Heterografted Polymer Brushes
28 Determined by Small-Angle X-ray Scattering. *ACS macro letters* **2017**, *6*, 1005-1012.

29 (47) Israelachvili, J. N.: *Intermolecular and surface forces*; Academic press, 2011.

30 (48) Discher, D. E.; Eisenberg, A. Polymer vesicles. *Science* **2002**, *297*, 967-973.

31 (49) Discher, D. E.; Ahmed, F. Polymersomes. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 323-341.

32 (50) Ivanova, R.; Komenda, T.; Bonné, T. B.; Lüdtkke, K.; Mortensen, K.; Pranzas, P. K.;
33 Jordan, R.; Papadakis, C. M. Micellar Structures of Hydrophilic/Lipophilic and
34 Hydrophilic/Fluorophilic Poly (2-oxazoline) Diblock Copolymers in Water. *Macromolecular Chemistry*
35 *and Physics* **2008**, *209*, 2248-2258.

36 (51) Dieu, L.-H.; Wu, D.; Palivan, C. G.; Balasubramanian, V.; Huwyler, J. Polymersomes
37 conjugated to 83-14 monoclonal antibodies: in vitro targeting of brain capillary endothelial cells.
38 *European journal of pharmaceuticals and biopharmaceutics* **2014**, *88*, 316-324.

39 (52) Krumm, C.; Fik, C. P.; Meuris, M.; Dropalla, G. J.; Geltenpoth, H.; Sickmann, A.; Tiller,
40 J. C. J. M. r. c. Well-Defined Amphiphilic Poly (2-oxazoline) ABA-Triblock Copolymers and Their
41 Aggregation Behavior in Aqueous Solution. **2012**, *33*, 1677-1682.

42 (53) Le Meins, J.-F.; Sandre, O.; Lecommandoux, S. Recent trends in the tuning of
43 polymersomes' membrane properties. *The European Physical Journal E* **2011**, *34*, 14.

44 (54) Discher, D. E.; Ahmed, F. Polymersomes. *Annual Review of Biomedical Engineering*
45 **2006**, *8*, 323-341.

46 (55) Huang, J.; Bonduelle, C.; Thévenot, J.; Lecommandoux, S. b.; Heise, A. Biologically
47 active polymersomes from amphiphilic glycopeptides. *Journal of the American Chemical Society*
48 **2011**, *134*, 119-122.

49 (56) Le Fer, G.; Portes, D.; Goudounet, G.; Guigner, J.-M.; Garanger, E.; Lecommandoux,
50 S. Design and self-assembly of PBLG-b-ELP hybrid diblock copolymers based on synthetic and elastin-
51 like polypeptides. *Organic and biomolecular chemistry* **2017**, *15*, 10095-10104.

- 1 (57) Liu, T.; Kim, K.; Hsiao, B. S.; Chu, B. Regular and irregular micelles formed by A LEL
2 triblock copolymer in aqueous solution. **2004**, *45*, 7989-7993.
- 3 (58) Agrawal, S. K.; Sanabria-DeLong, N.; Tew, G. N.; Bhatia, S. R. Structural
4 Characterization of PLA- PEO- PLA Solutions and Hydrogels: Crystalline vs Amorphous PLA Domains.
5 **2008**, *41*, 1774-1784.
- 6 (59) Wang, C.-H.; Hsiue, G.-H. New amphiphilic poly (2-ethyl-2-oxazoline)/poly (L-lactide)
7 triblock copolymers. *Biomacromolecules* **2003**, *4*, 1487-1490.
- 8 (60) Pucci, C.; Cousin, F.; Dole, F.; Chapel, J.-P.; Schatz, C. Impact of the formulation
9 pathway on the colloidal state and crystallinity of poly- ϵ -caprolactone particles prepared by solvent
10 displacement. *Langmuir* **2018**, *34*, 2531-2542.

11

Graphical abstract

