SUPPORTING INFORMATION

for

Stable self-assemblies of polyhydroxybutyrate-based diblock and triblock copolymers nanoprecipitated in water: influence of their hydrophilic weight fraction

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A) Synthesis of PMLA-*b*-PHB and PMLA-*b*-PHB-*b*-PMLA copolymers.

Typical procedure for the sequential copolymerization of BL and MLA^{Be} to synthesize PHB-*b***-PMLA^{Be} according to the reported procedure. G. Barouti, K. Jarnouen, S. Cammas-Marion, P. Loyer, and S. M. Guillaume,** *Polym. Chem.***, 2015**, *6*, 5414-5429.

In a typical experiment, TBD (35 mg, 0.25 mmol, 1 equiv.) was added to BL (1 g, 0.01 mol, 40 equiv.). The neat mixture was then stirred in bulk at 60 °C for the appropriate reaction time (reaction times were not systematically optimized). At the end of the polymerization, the BL conversion was determined from ¹H NMR analysis of an aliquot of the reaction mixture in CDCl₃. The polymerization was then quenched with addition of an excess of acetone (ca. 2 mL) and the resulting mixture was concentrated to dryness under vacuum. The crude polymer was then dissolved in acetone (5 mL) and dialyzed for 24 h with a dialysis membrane (MWCO of 3500 g mol⁻¹ or 1000 g mol⁻¹, depending of the polymer molar mass) using 500 mL of acetone which was renewed twice. Finally, the resulting polymer solution was dried under vacuum overnight (80 wt% typical isolated yield). The recovered PHB homopolymer was then analyzed by NMR and SEC analyses, which were in agreement with literature data (refer to C. G. Jaffredo, J.-F. Carpentier and S. M. Guillaume, *Macromol. Rapid Commun.*, **2012**, *33*, 1938–1944). The homopolymer samples were placed under an inert atmosphere at 4 °C.

Subsequently, the previously synthesized and isolated TBD–PHB–crotonate sample (850 mg, 0.27 mmol, $M_{n,NMR} = 3100$ g mol⁻¹) was placed in a Schlenk tube and dissolved in dry toluene (0.3 mL; [PHB]₀ = 0.9 mol L⁻¹), prior to the addition of MLABe (1.6 g, 8 mmol, 32 equiv.). The mixture was then stirred at 60 °C for the appropriate reaction time (not systematically optimized). The polymerization was quenched with an excess of acetone (ca. 2 mL). The resulting mixture was concentrated to dryness under vacuum and the conversion of MLA^{Be} was determined from ¹H NMR analysis of the residue in CDCl₃. The crude TBD–PMLA^{Be}-*b*-PHB–crotonate copolymer sample was purified by dialysis following the procedure described above for the purification of PHB. The final copolymer was then isolated (up to ca. 2 g), dried under vacuum overnight and analyzed by NMR and SEC. ¹H and ¹³C{¹H} NMR data agreed with previously reported data (refer to C. G. Jaffredo, J.-F. Carpentier and S. M. Guillaume, *Macromolecules*, **2013**, 46, 6765–6776). The copolymer samples were then placed under an inert atmosphere at 4 °C.

Typical hydrogenolysis of PMLA^{Be}-*b***-PHB into PMLA**-*b***-PHB according to the reported procedure.** G. Barouti, K. Jarnouen, S. Cammas-Marion, P. Loyer, and S. M. Guillaume, *Polym. Chem.*, **2015**, *6*, 5414-5429.

In a typical hydrogenolysis reaction, the isolated TBD–PMLA^{Be}-*b*-PHB–crotonate (2.0 g, 0.21 mmol, $M_{n,NMR}$ = 9600 g.mol⁻¹) was dissolved in acetone (10 mL) at room temperature in a stainless-steel autoclave prior to addition of Pd/C (300 mg, 10 wt%). The reactor was sealed, flushed several times with H₂, and finally placed under H₂ pressure (25 bar). The reaction mixture was then allowed to stir at room temperature for 48 h. The reactor was next vented to atmospheric pressure. The crude reaction mixture was then filtered twice through Celite using acetone as the eluent to remove the Pd/C catalyst, thereby providing a clear filtrate from which the solvent was evaporated under vacuum overnight (0.9–1.1 g, 69–92 wt% typical isolated yield). Dialysis of the copolymer was, although not systematically, carried out as described for the PMLA^{Be}-*b*-PHB copolymers. The final colorless copolymer PMLA-b-PHB was then analyzed by NMR, TGA, DSC, and contact angle analyses. The theoretical molar mass of the PMLA-b-PHB copolymers (PMLA-PHB $M_{n,theo}$; not including either the -OH or the -C(0)CH₂CH₂CH₃ chain end groups) can be evaluated from the initial molar mass of the PMLA^{Be}-*b*-PHB polymers determined by NMR analysis (PMLA^{Be}-PHB $M_{n,NMR}$) based on the loss of the benzyl units $(M_{CH2Ph} = 91 \text{ g mol}^{-1})$. Since this depletion amounts to roughly half the molar mass of the MLA^{Be} units ($M_{MLABe} = 206 \text{ g mol}^{-1}$), the molar mass of (only) the PMLA segment is simply divided by two. Since the ¹H NMR spectra of the PMLA-*b*-PHB samples showed the same intensity ratio for all the signals of the PMLA^{Be}/PMLA and PHB units before and after deprotection, respectively, these PMLA-PHB $M_{n,theo}$ values end-up being the same as those of PMLA-PHB M_{n,NMR}.

Typical procedure for the ROP of MLA^{Be} using PHB-diol as a macroinitiator, according to the previously reported synthesis of PMLA^{Be}-*b***-PHB-***b***-PMLA^{Be}.** G. Barouti, and S. M. Guillaume, *Polym. Chem.*, **2016**, *7*, 4603-4608.

In a typical experiment, HO-PHB-OH (500 mg, 0.1 mmol, 1 equiv., $M_{n,NMR}$ = 4900 g.mol⁻¹; [PHB-diol]₀ = 0.1 M) was charged in a Schenk flask in the glovebox and dissolved in dry toluene (1 mL) prior to the addition of Nd(OTf)₃ (60 mg, 0.1mmol, 1 equiv.). The

polymerization was performed at 60 °C in a very small amount of toluene, yet sufficient enough to afford a suitable homogeneous reaction medium for the ROP of this highly viscous monomer to proceed. The mixture was next stirred for 10 min, and MLA^{Be} (505 mg, 0.24 mmol, 24 equiv) was then added in. The resulting mixture was then stirred at 60 °C for 40 h (the reaction time was not systematically optimized). The polymerization was then stopped upon addition of an excess of acetone (ca. 2 mL), and the resulting mixture was concentrated to dryness under vacuum. MLA^{Be} conversion was then determined from ¹H NMR analysis of this residue in acetone- d_6 . The crude polymer was next dissolved in acetone (5 mL) and dialyzed for 48 h with a dialysis membrane (MWCO of 3500 g.mol⁻¹) using acetone (800 mL) which was renewed twice. The resulting polymer solution (recovered inside the membrane) was finally purified through a silica column using acetone (10 mL) as eluent and finally dialyzed once more as described above. The recovered polymer was dried under vacuum overnight (0.90 g, 89 wt%) and then analyzed by SEC, ¹H, ¹³C{¹H} J-MOD NMR in acetone- d_6 as HO-PMLA^{Be}-*b*-PHB-*b*-PMLA^{Be}-OH. The copolymer samples were stored under inert atmosphere at 4 °C.

Typical hydrogenolysis of PMLA^{Be}-*b*-PHB-*b*-PMLA^{Be} **into PMLA**-*b*-PHB-*b*-PMLA, **according to the previously reported synthesis.** G. Barouti, and S. M. Guillaume, *Polym. Chem.*, **2016**, *7*, 4603-4608.

In a typical hydrogenolysis reaction, the isolated HO-PMLA^{Be}-*b*-PHB-*b*-PMLA^{Be}-OH copolymer (900 mg, 0.10 mmol, $M_{n.NMR}$ = 9300 g.mol⁻¹) (Table 1, entry 5) was dissolved in acetone (10 mL) at room temperature in a stainless-steel autoclave prior to the addition of Pd/C (250 mg, 28 wt%). The reactor was sealed, flushed several times with H₂, and finally placed under H₂ pressure (25 bar). The reaction mixture was then allowed to stir at room temperature for 48 h, and the reactor was next vented to atmospheric pressure. The crude reaction mixture was then filtrated through Celite using acetone (200 mL) as eluent to remove the Pd/C catalyst. Solvent evaporation from the resulting clear filtrate under vacuum then afforded a viscous colorless oil. Dialysis in acetone of the resulting copolymer was next carried out as described above for HO-PMLA^{Be}-*b*-PHB-*b*-PMLA^{Be}-OH. The final copolymer sample, recovered as a colorless oil (600 mg, 67 wt%), was then analyzed by ¹H, ¹³C{¹H}J-MOD and DOSY NMR in acetone- d_6 /TFA (98:02, v/v) and TGA. The theoretical molar mass of the PHB block (not including the central tetramethylene moiety) was evaluated from the initial molar mass value of the PHB segment in the PMLA^{Be}-*b*-PHB-*b*-PMLA^{Be} copolymers as determined by ¹H NMR analysis (PMLA^{Be} *b*-PHB*b*-PMLA^{Be}, $M_{n,NMR}$). The theoretical molar mass value of the PMLA blocks was based on the loss of the benzyl units ($M_{CH2Ph} = 91$ g.mol⁻¹). Since this depletion amounts to roughly half the molar mass of the MLA^{Be} units ($M_{MLABe} = 206 \text{ g.mol}^{-1}$), the molar mass of (only) the PMLA^{Be} segments was simply divided by two. Note that in the case of the HO-PMLA-*b*-PHB-b-PMLA-OH copolymers, the more precise theoretical molar mass was obtained upon incrementing PMLA-*b*-PHB-*b*-PMLA $M_{n,theo}$ by the molar mass of the two hydrogen chain-end and of the central tetramethylene moiety (with $M_H = 1$ g.mol⁻¹ and $M_{0(CH2)40} =$ 88 g.mol⁻¹; i.e. HO-PMLA-*b*-PHB-*b*-PMLA-OH $M_{n,theo}$ = PMLA-*b*-PHB-*b*-PMLA $M_{n,theo}$ + 90 g.mol⁻¹).



Figure S1 : Dependence of the N_{agg} on the degree of polymerization (DP_{PMLA}) of the hydrophilic block for diblock (•) and triblock (•) copolymers nanoprecipitated in PBS.



Fig S2 a : Dependence of the KC/R $_{\theta}$ with q² for diblock T82/18 at various concentration indicated in the legend of the figure.

Fig S2 b : Dependence of the D with q^2 for triblock T82/18 at various concentration indicated in the legend of the figure.



Fig S3 a : Dependence of the KC/R $_{\theta}$ with q² for diblock D50/50 at various concentration indicated in the legend of the figure.

Fig S3 b : Dependence of the D with q^2 for diblock D50/50 at various concentration indicated in the legend of the figure.



Fig S4 a : Dependence of the KC/R $_{\theta}$ with q² for diblock D76/24 at various concentration indicated in the legend of the figure

Fig S4 b : Dependence of the D with q^2 for diblock D76/24 at various concentration indicated in the legend of the figure



Fig S5 a : Dependence of the KC/R $_{\theta}$ with q² for triblock T15/85 at various concentration indicated in the legend of the figure

Fig S5 b : Dependence of the D with q^2 for for triblock T15/85 at various concentration indicated in the legend of the figure