

Supporting Information

Supporting Information 1: Detailed synthesis conditions

Table SI1.1. Quantities of reagents and reaction time for the synthesis of different PLA-*b*-PEG copolymers.

Sample ID	Copolymer Denomination	Lactide	HO-PEG ₅₀₀₀ -COOH	HO-PEG ₅₀₀₀ -OCH ₃	Sn(Oct) ₂	Toluene	Reaction time
1	PLA ₁₃ - <i>b</i> -PEG ₅ -COOH	7500 mg	1886 mg	0 mg	101.9 mg	10 mL	55 min
2	PLA ₄₇ - <i>b</i> -PEG ₅ -COOH	7500 mg	550 mg	0 mg	30,0 mg	10 mL	55 min
3	PLA ₈₁ - <i>b</i> -PEG ₅ -COOH	8832 mg	452 mg	0 mg	25.6 mg	12 mL	107 min
4	PLA ₁₃ - <i>b</i> -PEG ₅ -OCH ₃	7500 mg	0 mg	1886 mg	101.9 mg	10 mL	55 min

Supporting Information 2: Cell viability of RAW 264.7 macrophages in the presence of NPs

The RAW 264.7 murine macrophages cell line was supplied by ATCC. There were put in culture in DMEM growth medium, supplemented with 50 U/mL of penicillin, 50 U/mL of streptomycin, and 10% of fetal bovine serum (Lonza), and maintained at 37 °C in a 5% CO₂ atmosphere. Cells were divided twice a week at a ratio between 1/6 and 1/8 after detachment by scrapping. After thawing, cells were used for experiments from 6 to 15. The in vitro cytotoxicity of the two types of particles PLA₈₁-*b*-PEG₅-COOH spherical NPs or PLA₁₃-*b*-PEG₅-COOH worm-like NPs ranging from 0.005 to 1 mg/mL, prepared by dilution into the cell culture medium, was assessed by measuring the mitochondrial activity through the 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyltetrazolium bromide (MTT) test. 8000 cells in 100µL medium were seeded in 96-well plates. 24h after plating, 100 µL of particles suspension in medium were added, giving a particle concentration range per well of 0.005 mg - 1 mg/mL. After 24h of incubation with particles, 20 µL of a solution of tetrazolium salt in PBS was added to each well (5 mg/mL). The plates were then incubated for 3 hours, medium was discarded, and 200 µL of DMSO (dimethylsulfoxide) was added to each well to dissolve crystals of formazan in mitochondria. The absorbance was measured in a plate-reader at 540 nm. The percentage of viable cells was estimated with the ratio of the absorbance of the particles –treated cells and the absorbance of untreated cells (control absorbance). Mean values (± SD) of cell viability were plotted against the NP concentration.

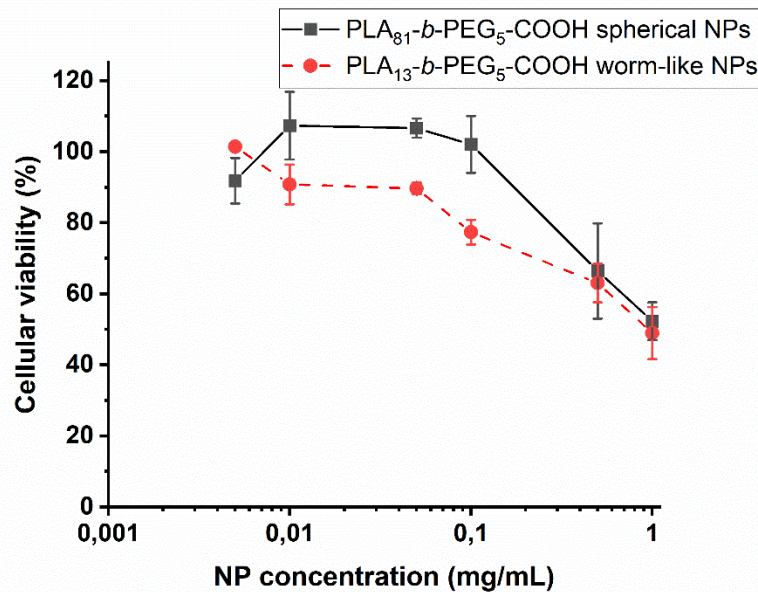


Figure S12.1. Cell viability of RAW 264.7 macrophages in the presence of PLA₈₁-*b*-PEG₅-COOH spherical NPs or PLA₁₃-*b*-PEG₅-COOH worm-like NPs, assessed by MTT assay.

Supporting Information 3: Zoomed out TEM images

These images are zoomed out images corresponding to the samples showed Figure 1. It shows that the images provided in the main text are representative of the samples. Image (a) seems to be divided in two parts with different contrast, because the camera was damaged the day of the observation. The image is still clear enough to assess the representativity of the sample.

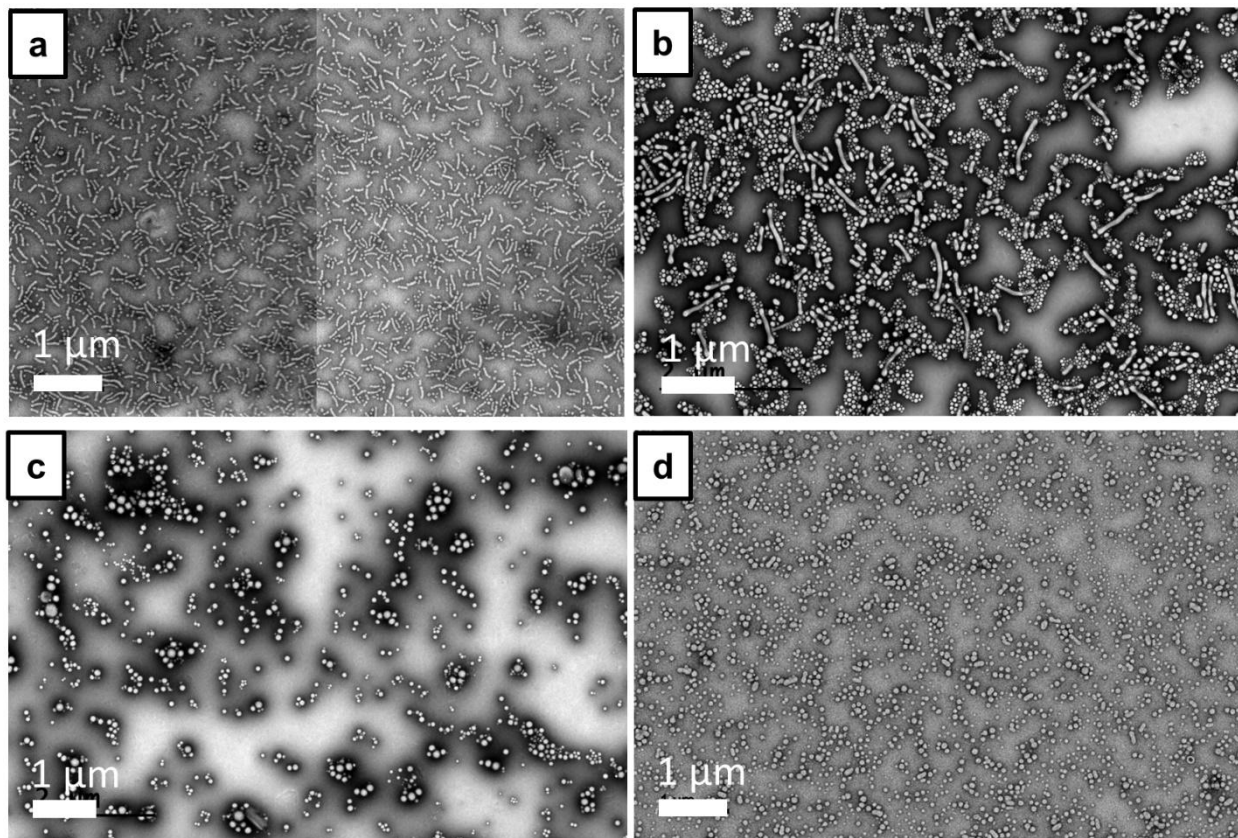


Figure S13.1. Zoom out images of NPs obtained with acid-ending copolymers of different molar mass: (a) $\text{PLA}_{13}\text{-b-PEG}_5\text{-COOH}$, (b) $\text{PLA}_{47}\text{-b-PEG}_5\text{-COOH}$, (c) $\text{PLA}_{81}\text{-b-PEG}_5\text{-COOH}$ and (d) $\text{PLA}_{13}\text{-b-PEG}_5\text{-OCH}_3$. The scale bars represent 1 μm.

Supporting Information 4: DLS analysis of the NPs (size and ζ -potential)

The mean hydrodynamic diameter of NPs was measured by Dynamic Light Scattering (DLS) using a Zetasizer (Nano ZS, Malvern Instruments, France, 633 nm He-Ne laser) operating at 25°C with a 173° scattering angle. The NP suspension was diluted, until reaching an attenuator value of 6, in MilliQ water. Each sample was analysed three times to obtain the hydrodynamic diameter (z-average, obtained by the cumulant fit analysis in the “General Purposes” mode) and the Pdl. For each condition, three sample were analysed, to calculate an average value \pm standard error (Table SI4.1).

With the same apparatus, the ζ -potential of NPs was assessed by Electrophoretic Light Scattering operating at 25°C (using Smoluchowski equation). The NP suspension was diluted in a 1 mM NaCl aqueous solution. Each sample was analysed three times to the ζ -potential of the NPs. For each condition, three sample were analysed, to calculate an average value \pm standard error (Table SI3.1).

Table SI4.1. Size and ζ -potential of NPs characterized in DLS. The influence of molar mass of PLA block on NP characteristics was studied.

Polymer composing the NPs	z-average (nm)	pdl	ζ -potential (mV)
PLA ₁₃ - <i>b</i> -PEG ₅ -COOH (n = 3)	132.1 \pm 9.2	0.208 \pm 0.020	-23.3 \pm 3.0
PLA ₈₁ - <i>b</i> -PEG ₅ -COOH (n = 2)	154.9 \pm 3.8	0.127 \pm 0.016	-35.8 \pm 2.5
PLA ₁₃ - <i>b</i> -PEG ₅ -OCH ₃ (n = 3)	137.1 \pm 10.7	0.104 \pm 0.002	-15.5 \pm 1.3

Supporting Information 5: Degradation of the PLA block during the division of worm-like NPs

When stored at 37°C and above after their preparation and centrifugation, worm-like NPs tended to divide into smaller spheres as described in the main article. To prove this behaviour was not due to the hydrolysis of the PLA block, we performed some measurements of the PLA₁₃-*b*-PEG₅-COOH molar mass overtime. The number-averaged molar masses M_w were determined by SEC relative to PMMA standards (Figure SI5.1).

First, the initial molar mass of raw PLA₁₃-*b*-PEG₅-COOH determined by SEC was 21,000 g/mol instead of 18,000 g/mol. This is not surprising, as this measurement is relative to PMMA standards, and not absolute as the measurement made by ¹H NMR. After the formation of NPs, we analysed the molar mass of the polymer again (initial point of the curve), and found 23,000 g.mol⁻¹, showing the NP preparation process had no influence on the polymer molar mass. Then, we observed the great stability of PLA at both 4 and 37°C over 4 days. This means that the division observed on worm-like NPs after 24 h at 37°C was not due the hydrolysis of the PLA block.

The measurements performed on the sample stored at 60°C showed a fast degradation of the copolymer, as its molar mass decreased to 18,000 g/mol in 24 h. It even divided in two oligomers of 12,000 and 3,000 g/mol, after 4 days. This degradation could be related to the macroscopic behaviour of the NP suspension stored at 60°C, that seemed cloudier than the others after 7 days. This observation was in line with an absence of individual nanoparticles on TEM images, showing only irregular aggregates (Figure SI5.2).

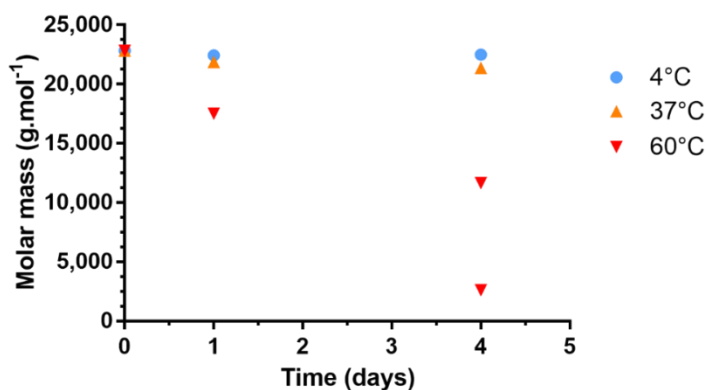


Figure SI5.1: Evolution of the molar mass of PLA₁₃-*b*-PEG₅-COOH contained in a suspension of worm-like NPs stored at different temperature, assessed by SEC relative to poly(methylmethacrylate) standards.

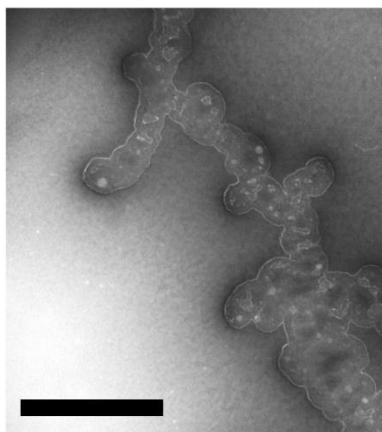
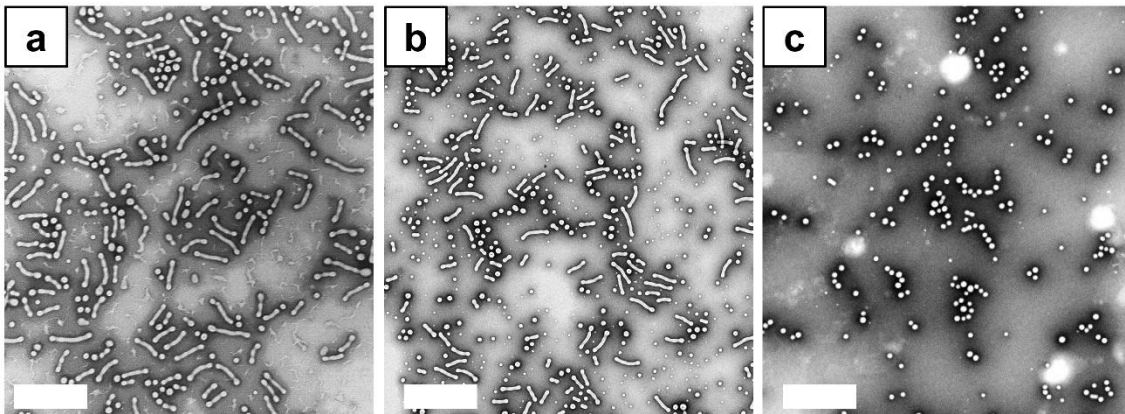


Figure S15.2: TEM image of particles obtained with PLA₁₃-*b*-PEG₅-COOH after 4 days at 60°C in water (Scale bar: 500 nm).

Supporting Information 6: Effect of the presence of sodium cholate on the division of the worm-like NPs

Worm-like NPs prepared with PLA₁₃-*b*-PEG₅-COOH were prepared as described in the main article. A half of this sample was stored in a heat chamber at 45°C for 24h. The second half of the sample was centrifuged to remove the sodium cholate prior to the storage at 45°C during 24 h. It must be noted that the efficiency of the sodium cholate removal by centrifugation has already been verified by our team, in a previous work (O. Diou, N. Tsapis, C. Giraudeau, J. Valette, C. Gueutin, F. Bourasset, S. Zanna, C. Vauthier, E. Fattal, Long-circulating perfluorooctyl bromide nanocapsules for tumor imaging by 19FMRI, *Biomaterials*. 33 (2012) 5593–5602. doi:10.1016/j.biomaterials.2012.04.037). TEM was then performed to observe the morphology of the worm-like NPs at the end of this treatment (Figure SI6.1).

The presence of sodium cholate in the NP suspension had a dramatic influence on the division phenomenon. Indeed, the presence of cholate in the worm-like NP suspension seemed to inhibit the division. Surfactant removal was a necessary condition to allow the Plateau-Rayleigh-instability-induced division at temperatures above T_g .



FigureSI6.1: TEM images of particles obtained with PLA₁₃-*b*-PEG₅-COOH (a) at the end of the preparation process, (b) after 24 h at 45°C in a solution of sodium cholate 1.5% w/v and (c) after 24 h at 45°C in water (Scale bar: 500 nm).

Supporting Information 7: Determination of the glass transition temperature of PLA₁₃-*b*-PEG₅-COOH worm-like NPs suspended in water

An example of thermogram is given in Figure SI7.1, for PLA₁₃-*b*-PEG₅-COOH worm-like NPs. The three dashed lines represent the tangent lines of the curve at the baseline (before and after the glass transition) and at the inflection point of the glass transition step. The first crossover point defines the onset of the glass transition (T_g onset), while the second one defines its end (T_g end). The midpoint is midway between these two crossover points, and the corresponding temperature is the “ T_g midpoint”.

This T_g midpoint was measured at a scan rate of 60°C/h, and was equal to 34.5°C.

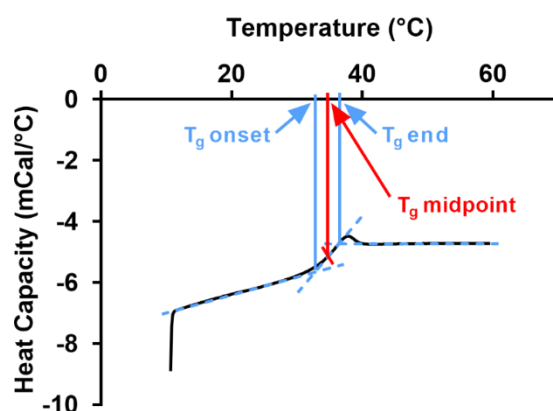


Figure SI7.1: Thermogram of worm-like NPs suspended in water (50 mg/mL), performed at a heating of 60°C/h.