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# Effect of stereocomplex formation between enantiomeric poly(L,L-lactide) and poly(D,D-lactide) blocks on self-organization of amphiphilic poly(lactide)-*block*-poly(ethylene oxide) copolymers in dilute aqueous solution

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**Table S1** Number average ($M_n$) and weight average ($M_w$) molecular weights and polydispersity indexes (PDI) of synthesized amphiphilic block copolymers of lactide and ethylene oxide.

| Sample | $M_n$/Da[a] | $\mathrm{M_n}$/Da[b] | $M_w$/Da[b] | PDI[b] |
|---|---|---|---|---|
| P(L,L)LA$_{46}$-*b*-PEO$_{113}$ | 8300 | 10100 | 11900 | 1.18 |
| P(D,D)LA$_{56}$-*b*-PEO$_{113}$ | 9000 | 10400 | 12000 | 1.16 |
| P(D,L)LA$_{66}$-*b*-PEO$_{113}$ | 9750 | 12000 | 13800 | 1.15 |

[a] Determined by $^1$H NMR. [b] Determined by GPC.

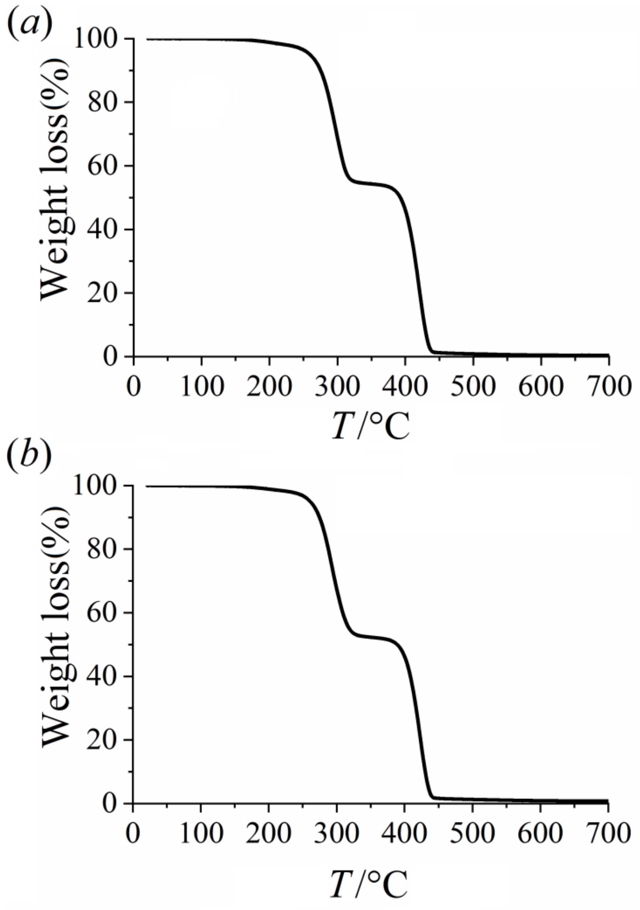

**Figure S1** TGA curves of semicrystalline copolymers (*a*) P(L,L)LA$_{46}$-*b*-PEO$_{113}$ and (*b*) P(D,D)LA$_{56}$-*b*-PEO$_{113}$.

The weight loss of both semicrystalline block copolymers was less than 1% in the temperature ($T$) range from 20 to 150 °C.

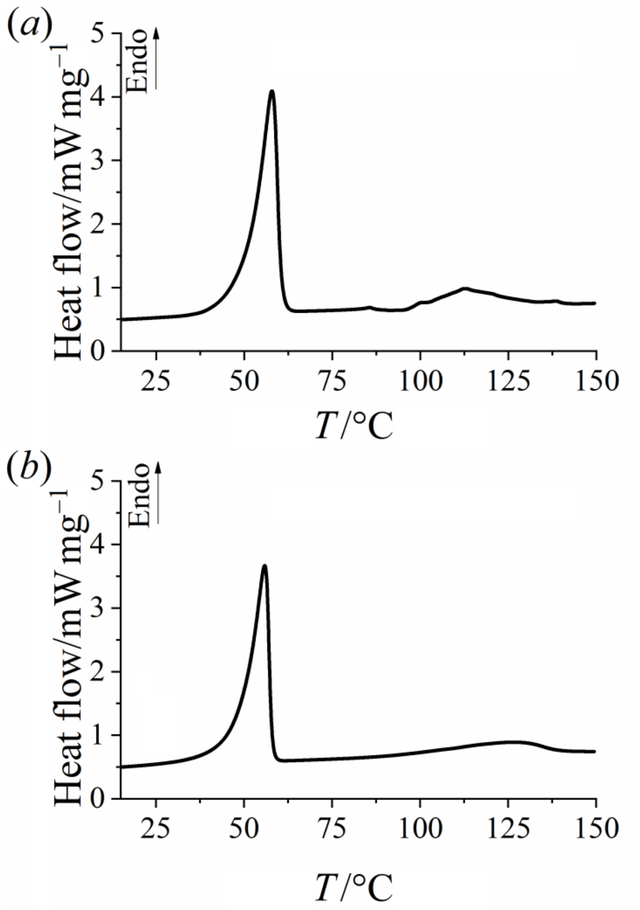

**Figure S2** DSC curves for the semicrystalline copolymers (*a*) P(L,L)LA$_{46}$-*b*-PEO$_{113}$ and (*b*) P(D,D)LA$_{56}$-*b*-PEO$_{113}$.

**Table S2** Melting temperature ($T_\mathrm{m}$) and degree of crystallinity ($\alpha$) of PEO and PLA blocks in the P(L,L)LA$_{46}$-*b*-PEO$_{113}$ and P(D,D)LA$_{56}$-*b*-PEO$_{113}$ copolymers.

| Sample | $T_\mathrm{m(PEO)}$/°C | $\alpha_\mathrm{(PEO)}$ (%) | $T_\mathrm{m(PLA)}$/°C | $\alpha_\mathrm{(PLA)}$ (%) |
|---|---|---|---|---|
| P(L,L)LA$_{46}$-*b*-PEO$_{113}$ | 57 | 61 | 117 | 27 |
| P(D,D)LA$_{56}$-*b*-PEO$_{113}$ | 56 | 61 | 125 | 35 |

The degree of crystallinity of both the hydrophilic PEO blocks and the hydrophobic PLA blocks in the P(L,L)LA$_{46}$-*b*-PEO$_{113}$ and P(D,D)LA$_{56}$-*b*-PEO$_{113}$ copolymers was calculated using equation (S1):

$$\alpha = \frac{\Delta H_\mathrm{m}}{\omega \cdot \Delta H_\mathrm{m}^{0}}, \tag{S1}$$

where $\Delta H_{\mathrm{m}}$ is the melting enthalpy of the PEO or PLA block in the block copolymer [$\Delta H^{0}_{\mathrm{m(PEO)}}$ = 196.8 J g$^{-1}$ and $\Delta H^{0}_{\mathrm{m(PLA)}}$ = 106.0 J g$^{-1}$],[S1,S2] and $\omega$ is the weight fraction of the PEO or PLA block in the block copolymer.

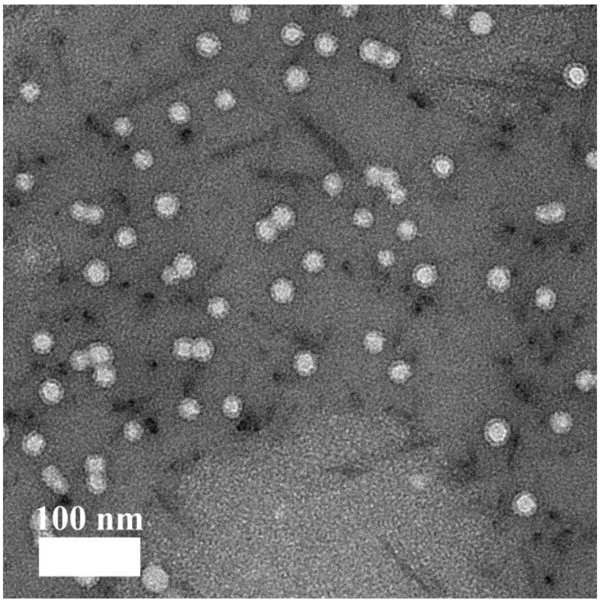

**Figure S3** Representative TEM image of the $\mathrm{P(D,L)LA_{66}}$-$b$-$\mathrm{PEO_{113}}$ micelles. Dispersion concentration $c$ = 0.5 g dm$^{-3}$.

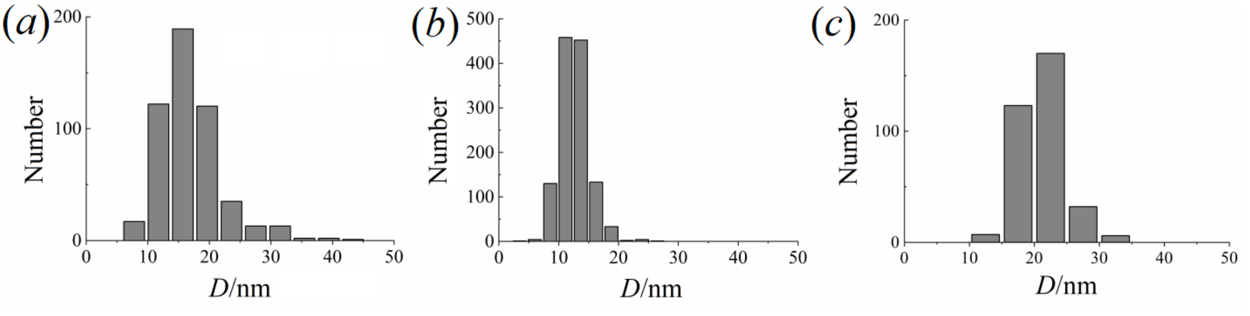

**Figure S4** Size distribution histograms of individual micelles estimated from TEM images of micelles formed by ($a$) the $\mathrm{P(L,L)LA_{46}}$-$b$-$\mathrm{PEO_{113}}$ copolymer, ($b$) an equimolar mixture of the $\mathrm{P(L,L)LA_{46}}$-$b$-$\mathrm{PEO_{113}}$ and $\mathrm{P(D,D)LA_{56}}$-$b$-$\mathrm{PEO_{113}}$ copolymers and ($c$) the $\mathrm{P(D,L)LA_{66}}$-$b$-$\mathrm{PEO_{113}}$ copolymer.

**Table S3** Estimated values for the thickness of the PLA crystalline lamella ($h_{\text{PLA}}$), the number of folds per PLA chain ($n_{\text{f}}$) and the tethering density of PEO chains ($\sigma$).

| Sample | $h_{\text{PLA}}$/nm | $n_{\text{f}}$ | $\sigma$/nm$^{-2}$ |
|---|---|---|---|
| P(L,L)LA$_{46}$-*b*-PEO$_{113}$ | 12.8 | 0 | 1.57 |
|  | 6.4 | 1 | 0.79 |
| P(L,L)LA$_{46}$/P(D,D)LA$_{56}$-*b*-PEO$_{113}$ | 7.4 | 1 | 0.60 |

The $h_{\text{PLA}}$ values of the P(L,L)LA and P(L,L)LA/P(D,D)LA crystalline lamellae were estimated from the lattice parameters of the α-form of P(L,L)LA ($a$ = 1.07 nm, $b$ = 0.595 nm, $c$ = 2.78 nm, two $10_3$ helices per unit cell) and the P(L,L)LA/P(D,D)LA stereocomplex [$a$ = 0.916 nm, $b$ = 0.916 nm, $c$ = 0.87 nm, parallel packed P(L,L)LA and P(D,D)LA chains in the $3_1$ helical conformation in the unit cell],[S3] respectively. The degree of crystallinity of the PLA lamella was taken as 100%.

The value of $\sigma$ was calculated using equation (S2):

$$\sigma = \frac{1}{a \cdot b \cdot (n_{\text{f}}+1)}, \tag{S2}$$

where $a$ and $b$ are the lattice parameters of the α-form of P(L,L)LA or the stereocomplex P(L,L)LA/P(D,D)LA.

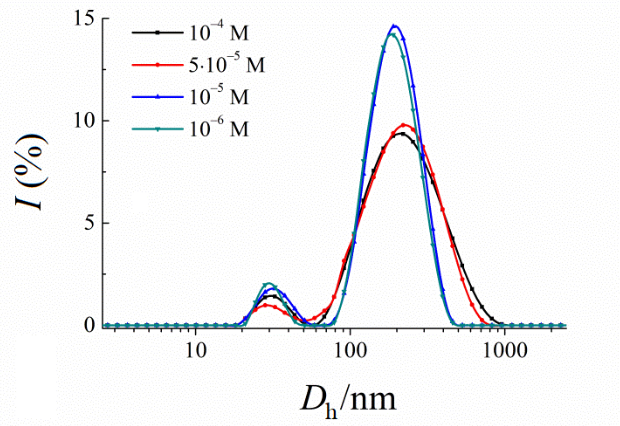

**Figure S5** DLS intensity size distribution curves for aqueous dispersions of the P(L,L)LA$_{46}$/P(D,D)LA$_{56}$-*b*-PEO$_{113}$ stereocomplex micelles with various concentrations.

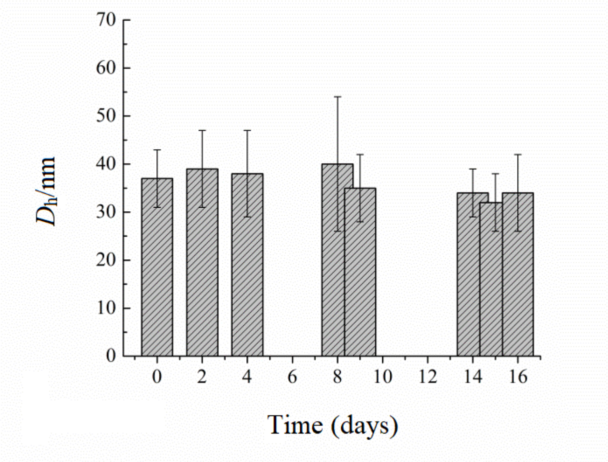

**Figure S6** Changes in the hydrodynamic diameter ($D_h$, nm) of the P(L,L)LA$_{46}$/P(D,D)LA$_{56}$-*b*-PEO$_{113}$ stereocomplex micelles with time at $T$ = 25 °C. Dispersion concentration $c$ = 1 g dm$^{-3}$.

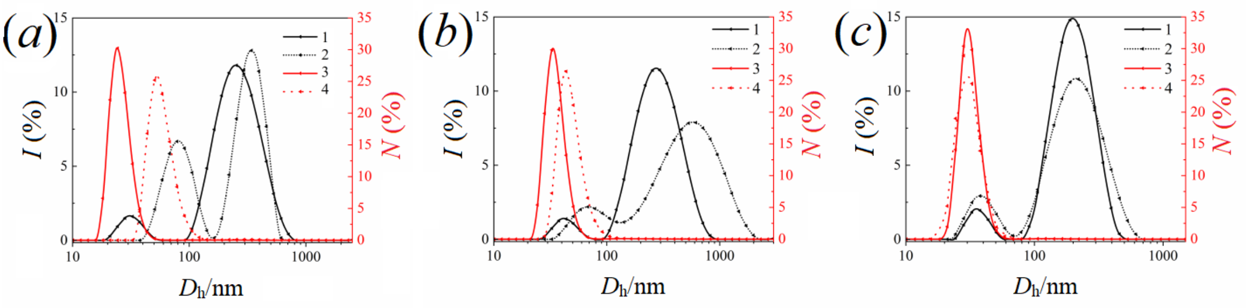

**Figure S7** Size distribution curves of (*1*),(*2*) DLS intensity ($I$, %) and (*3*),(*4*) DLS numbers ($N$, %) for micelles formed by (*a*) P(L,L)LA$_{46}$-*b*-PEO$_{113}$, (*b*) P(D,L)LA$_{66}$-*b*-PEO$_{113}$ and (*c*) the P(L,L)LA$_{46}$/P(D,D)LA$_{56}$-*b*-PEO$_{113}$ stereocomplex (*1*),(*3*) before and (*2*),(*4*) after freeze-drying followed by resuspension in bidistilled water. Dispersion concentration $c$ = 0.5 g dm$^{-3}$ and dispersion volume $V$ = 2 ml.

## Materials and methods

*Materials.* L,L-, D,D- and D,L-lactide (3,6-dimethyl-1,4-dioxane-2,5-dione, 99%) were purchased from Corbion (Netherlands) and recrystallized in butyl acetate before use. Monomethoxy poly(ethylene oxide) (mPEO) with a molecular weight of 5 kDa was purchased from J&K Scientific (USA) and dried in vacuum at 100–150 °C for 1 h before use. Stannous 2-ethylhexanoate ($SnOct_2$) and pyrene fluorescence probe were purchased from Sigma-Aldrich (USA) and used as received. All organic solvents were of analytical grade and were used without further purification. All aqueous solutions were prepared using bidistilled water (Merck KGaA, Germany).

Oxaliplatin {[*trans*-(*R*,*R*)-cyclohexane-1,2-diamine]oxalatoplatinum(II)} was synthesized and kindly provided by PhD A. A. Nazarov (Department of Chemistry, M. V. Lomonosov Moscow State University).

*Synthesis of PLA-b-PEO copolymers.* Amphiphilic diblock copolymers poly(L,L-lactide)-*block*-poly(ethylene oxide) [P(L,L)$LA_{46}$-*b*-$PEO_{113}$], poly(D,D-lactide)-*block*-poly(ethylene oxide) [P(D,D)$LA_{56}$-*b*-$PEO_{113}$] and poly(D,L-lactide)-*block*-poly(ethylene oxide) [P(D,L)$LA_{66}$-*b*-$PEO_{113}$] were synthesized by ring-opening polymerization of L,L-, D,D- and D,L-lactide, respectively, in the presence of mPEO. $SnOct_2$ (1400 ppm) was used as a catalyst (Figure S8). The polymerization was carried out at 180 °C for 4 h in an argon atmosphere with constant stirring. The synthesized block copolymers were cooled to room temperature and dissolved in tetrahydrofuran (THF), then precipitated twice in *n*-hexane. Finally, they were dried under vacuum at low pressure at 80 °C for 24 h.

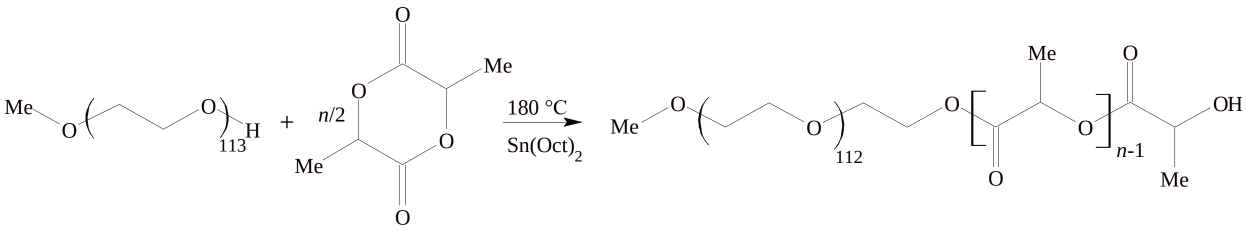

**Figure S8** Scheme for the synthesis of the PLA$_{n}$-*b*-PEO$_{113}$ copolymers, where *n* is the polymerization degree of the hydrophobic PLA block.

*Characterization of the synthesized block copolymers. $^{1}$H nuclear magnetic resonance ($^{1}$H NMR).* The number average molecular weight ($M_{\mathrm{n}}$) of the synthesized block copolymers and the polymerization degree (*n*) of the hydrophobic PLA block were determined by $^{1}$H NMR. The spectra were recorded on a 600 MHz Agilent (Varian) VNMRS 700 spectrometer at room temperature. For measurements, the block copolymer (30 mg) was dissolved in deuterated chloroform (CDCl$_3$, 1 ml). A typical $^{1}$H NMR spectrum of the PLA-*b*-PEO copolymer is presented in Figure S9. Integrals of the peaks corresponding to the PEO methylene protons (–CH$_2$–) at 3.64 ppm and the PLA methine protons (–CH) at 5.20 ppm were used to calculate the *n* values of the PLA block and the $M_{\mathrm{n}}$ values of the block copolymer.

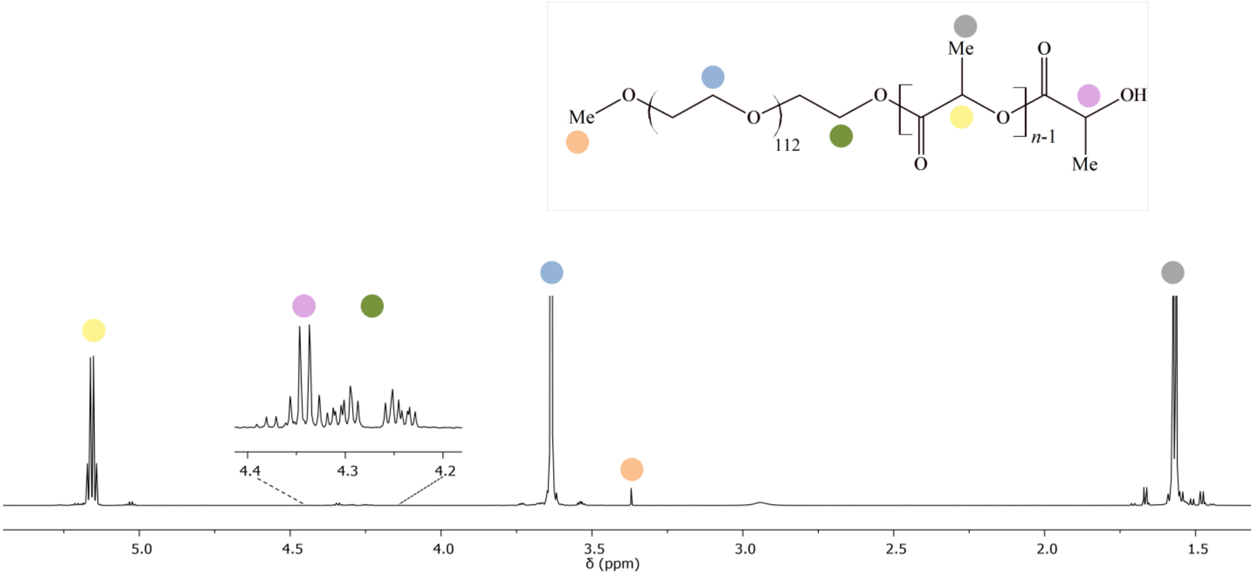

**Figure S9** $^{1}$H NMR spectrum (600 MHz, CDCl$_3$) of the P(L,L)LA$_{46}$-*b*-PEO$_{113}$ copolymer, $\delta$: 5.07–5.28 (q, H-1), 4.34 (q, H-2), 4.28 (t, H-3), 3.64 (s, H-4), 3.36 (s, H-5), 1.50–1.64 (d, H-6), 1.47 (d, H-7).

*Gel permeation chromatography (GPC).* The molecular weights and polydispersity indices of the synthesized block copolymers were determined by GPC. GPC measurements were carried out on a Knauer analytical chromatograph (Germany) equipped with a refractometric detector and a Phenogel 5 μm $10^3$ Å column. Column calibration was performed using polystyrene standards (Polymer Laboratories). THF was used as the mobile phase at a flow rate of 1 ml min$^{-1}$ at 40 °C. For measurements, the block copolymer (5 mg) was dissolved in THF (1 ml).

*Investigation of thermophysical properties of semicrystalline block copolymers. Thermogravimetric analysis (TGA).* The TGA method was used to determine the onset temperature of degradation of the synthesized block copolymers. The measurements were carried out on a PerkinElmer Pyris 1 TGA thermogravimetric analyzer (USA) in the temperature range from 30 to 700 °C at a heating rate of 10 °C min$^{-1}$ in a nitrogen flow of 100 ml min$^{-1}$, using a standard 60 μl platinum crucible.

*Differential scanning calorimetry (DCS).* The thermophysical properties of the block copolymers were studied on a PerkinElmer DSC8500 differential scanning calorimeter (USA) in the temperature range from 0 to 150 °C at heating/cooling rates of 20 °C min$^{-1}$ in a nitrogen flow of 20 ml min$^{-1}$. Standard 40 μl aluminum pans were used. The samples were weighed on a Sartorius CPA225D electronic balance with an accuracy of 0.01 mg.

*Preparation of block copolymer micelles.* Block copolymer micelles were prepared by dialysis. Briefly, the copolymer P(L,L)LA$_{46}$-*b*-PEO$_{113}$ or P(D,L)LA$_{66}$-*b*-PEO$_{113}$ (10 mg) was first dissolved in the non-selective solvent THF (5 ml). The resulting solutions were transferred to dialysis tubes (Zellu Trans Dialysis Tube T2, Scienova, MWCO = 3.5 kDa) and dialyzed against bidistilled water (1000 ml) at room temperature for one week to remove the organic solvent. External water was renewed every day. As a result, an opalescent aqueous dispersion with a concentration of 1 g dm$^{-3}$ was obtained. All experiments were carried out with freshly prepared dispersions.

To prepare micelles of the P(L,L)LA$_{46}$/P(D,D)LA$_{56}$-*b*-PEO$_{113}$ stereocomplex, an equimolar mixture of copolymers P(L,L)LA$_{46}$-*b*-PEO$_{113}$ (5 mg) and P(D,D)LA$_{56}$-*b*-PEO$_{113}$ (5 mg) was dissolved in THF (5 ml). After that, micelles of the stereocomplex were prepared similarly to P(L,L)LA$_{46}$-*b*-PEO$_{113}$ and P(D,L)LA$_{66}$-*b*-PEO$_{113}$ micelles.

To prepare micelles of the P(L,L)LA$_{46}$/P(D,D)LA$_{56}$-*b*-PEO$_{113}$ stereocomplex loaded with oxaliplatin, the latter was preliminary dissolved in dimethylformamide (DMF) ($c$ = 5 g dm$^{-3}$). Then the resulting solution (100 μl) was added to a THF solution (4.9 ml) of an equimolar mixture of copolymers P(L,L)LA$_{46}$-*b*-PEO$_{113}$ (5 mg) and P(D,D)LA$_{56}$-*b*-PEO$_{113}$ (5 mg). Thus, the drug content in the organic phase ($\omega_0$) was 5 wt% with respect to the total amount of the copolymers. After that, the organic solution (DMF/THF) was transferred into a dialysis tube and dialyzed against bidistilled water (1000 ml) at room temperature for 4 h. External water was renewed every 30 min. To evaluate the content of loaded oxaliplatin, the resulting aqueous dispersion was frozen at −18 °C and freeze-dried (Alpha 2–4 LSC, Martin Christ, Germany) for 48 h at a vacuum of 0.001 mbar. Freeze-dried micelles were stored at −4 °C.

*Fluorescence spectroscopy.* The critical micelle concentration (CMC) of the synthesized block copolymers was determined by fluorescence spectroscopy using pyrene as a fluorescent probe.[S4,S5] Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (USA) at room temperature in the wavelength range from 300 to 360 nm with an emission wavelength of 392 nm.

*Dynamic light scattering (DLS).* The DLS measurements were performed on a Zetasizer Nano ZSP instrument (Malvern Instruments Ltd, USA) equipped with a He–Ne laser with a wavelength of 633 nm and a scattering angle of 173°. Aqueous dispersions of block copolymer micelles with different concentrations were placed in polystyrene cuvettes with an optical path length of 2 mm. The measurements were carried out at 25 °C. Analysis of autocorrelation functions was performed using the Zetasizer software.

*Electrophoretic light scattering (ELS).* The electrokinetic potential of the block copolymer micelles was determined by ELS. The measurements were conducted on a Zetasizer Nano ZSP instrument (Malvern Instruments Ltd, USA). Aqueous dispersions with a concentration of 1 g $dm^{-3}$ were placed in a U-shaped capillary cuvette. The measurements were carried out at 25 °C.

*Wide-angle X-ray scattering (WAXS).* The crystal structure of the core of the block copolymer micelles was studied by the WAXS method at the BIOSAX station of the Kurchatov Synchrotron Radiation Source (National Research Center ‘Kurchatov Institute’, Moscow, Russia), using an X-ray wavelength $\lambda$ of 0.1445 nm. The beam size on the sample was $0.5 \times 0.35$ $mm^2$. Diffraction patterns were recorded using a Pilatus 3 1M detector (Dectris). The measurements were carried out at 25 °C. The aqueous dispersions were freeze-dried before WAXS measurements. WAXS patterns of the block copolymers micelles are presented after integration and background subtraction.

*Small-angle X-ray scattering (SAXS).* Aqueous dispersions of the block copolymer micelles were studied by the SAXS method at the BIOSAX station of the Kurchatov Synchrotron Radiation Source (National Research Center ‘Kurchatov Institute’, Moscow, Russia). The scattering intensity $[I(s)]$ was recorded in the range of the momentum transfer $0.03 < s < 2.75$ $nm^{-1}$, where $s = 4\pi(\sin\theta)/\lambda$, $2\theta$ is the scattering angle and $\lambda = 0.1445$ nm is the X-ray wavelength. The measurements were carried out at 25 °C. Data analysis was performed using the ATSAS software suit.[S6]

*Transmission electron microscopy (TEM).* TEM imaging was performed on a Tecnai 12 G2 BioTwin Spirit microscope (FEI, USA) at an accelerating voltage of 120 kV with an Eagle 4K camera (FEI, USA) operating in the bright field mode. The negative staining procedure was used for TEM experiments. Copper grids for TEM coated with a thin carbon film were subjected to a glow discharge for 10 s in a Pelco easiGlow system at 25 mA current (Ted Pella, USA). A drop (3 μl) of an aqueous suspension with a concentration of 0.5 g dm$^{-3}$ was deposited to the carbon side of the grid and incubated for 1 min. Then the carbon side of the grid was rinsed with bidistilled water (10 μl), after which a uranyl acetate solution (10 μl) with a concentration of 0.5 wt% was applied to the grid and incubated for 30 s. After each step, the excess solution was removed by contacting the edge of the grid with filter paper. The grid was then dried for 30 min at ambient conditions.

*Inductively coupled plasma mass spectrometry (ICP-MS).* The content of oxaliplatin in micelles of the P(L,L)LA$_{46}$/P(D,D)LA$_{56}$-*b*-PEO$_{113}$ stereocomplex was evaluated using ICP-MS. Briefly, the freeze-dried micelles (5 mg) were decomposed in *aqua regia* (1 ml) at 180 °C for 1 h in a SAA-1 autoclave opening system (IMT RAS, Russia). The resulting solutions were transferred into tubes and diluted to 8 ml. Then an internal indium standard (10 μg dm$^{-3}$) was added. The measurements were carried out on an XSeries II spectrometer (Thermo Scientific, USA) with a PolyCon concentric sprayer and a quartz spray chamber cooled to 3 °C. The output power of the generator was 1400 W, the flow rate of Ar plasma was 13 dm$^3$ min$^{-1}$, the flow rate of auxiliary Ar was 0.9 dm$^3$ min$^{-1}$, the flow rate of Ar in the nebulizer was 0.89 dm$^3$ min$^{-1}$, the flow rate of the analyzed solution was 0.8 dm$^3$ min$^{-1}$ and the resolution was 0.8 M.

The value of drug load content ($\omega$, wt%) was calculated using equation (S3):

$$\omega = \frac{m_1}{m_0} \cdot 100\%, \tag{S3}$$

where $m_1$ is the mass of oxaliplatin incorporated in the micelles, and $m_0$ is the mass of the micelles.

The drug encapsulation efficiency ($\varphi$, %) was calculated by equation (S4):

$$\varphi = \frac{\omega}{\omega_0} \cdot 100\%, \tag{S4}$$

where $\omega_0$ is the initial mass of oxaliplatin.

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