Reversible multilayered vesicle-like structures with fluid hydrophobic and interpolyelectrolyte layers

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Abstract

Hydrophobic blocks of amphiphilic block copolymers usually form glassy micellar

cores with a rigid structure that limits their applications as nanocapsules for targeted delivery. We report here on the core/shell micelles with a soft core formed by self-assembly of block copolymer composed of hydrophobic and polycation blocks, poly(lauryl acrylate)-*block*-poly(trimethylammonioethyl acrylate) (PLA-QPDMAEA), in aqueous solution. Using scattering, microscopy and spectroscopy techniques, we showed that such copolymer forms spherical and cylindrical core/shell micelles with a fluid-like PLA core and a positively charged shell, and that these micelles can encapsulate and release hydrophobic solutes. Moreover, we discovered novel vesicle-like multicompartment structures containing both soft hydrophobic and interpolyelectrolyte (IPEC) layers formed by co-assembly of PLA-QPDMAEA core/shell micelles with another diblock copolymer composed of a hydrophilic block and polyanion block poly(ethylene oxide)-*block*-poly(methacrylic acid) (PEO-PMAA). These complex structures were characterized by small-angle neutron scattering, supported by self-consistent field modeling that confirmed the formation of vesicle-like structures with dense PEO core, IPEC inner layer, PLA soft layer, IPEC outer layer and loose PEO corona. Due to their unique tunable properties, such multicompartment micelles with fluid and IPEC layers and hydrophilic corona can be used as nanocapsules with controllable thickness of each layer, charge and stability.

Introduction

Nanomaterials for targeted delivery and controlled release of low-molar mass compounds should be biocompatible, biodegradable and stimuli-responsive.¹ Specifically the stimuli-responsiveness ensures that the nanomaterial can not only encapsulate its low-molecular cargo, but also release it in a specific location, or under specific conditions. Rational design of such nanomaterials requires understanding of the basic principles of the complex relation between their morphology, structure and loading or release properties. Polymeric nanocarriers formed by homopolymers, block copolymers or surfactants can be dynamically

formed and disassembled, self- or co-assembled in aqueous solutions into a variety of structures (core/shell²⁻⁸ or worm-like micelles,⁹⁻¹¹ lamellae,¹²⁻¹⁴ vesicles,¹⁵⁻¹⁷ etc.), and, therefore, efficiently encapsulate low-molar mass species.¹⁸

One approach for designing nanocapsules consists in self-assembly of amphiphilic block copolymers composed of hydrophobic and hydrophilic blocks¹⁹⁻²¹ in aqueous solution into core/shell micelles with a core formed by the hydrophobic blocks and a shell composed of the hydrophilic blocks.²² The morphology of such micelles depends on the ratio of the hydrophobic to hydrophilic block length; hydrophobic block aggregation is the driving force of the self-assembly process, while steric repulsion among hydrophilic blocks or electrostatic repulsion among polyelectrolyte blocks in the outer shell stops the aggregation, resulting in aggregates of a well-defined size.²³⁻²⁶ However, hydrophobic blocks of the most common amphiphilic block copolymers form core/shell micelles with a compact solid-like core, that prevents re-structuring of these micelles upon the action of an external stimulus, thereby limiting their prospective application. In contrast, soft or fluid core allows for efficient encapsulation of hydrophobic solutes into dynamic micelles. It has been shown that some poly(n-alkyl acrylates) with a low glass transition temperature, T_g , form a soft core, density of which is determined by the self-assembly behavior.^{27,28} The brush-like structure of the poly(n-alkyl acrylate) block prevents the solidification of core and results in the formation of a fluid-like and disordered core due to the long side flexible chains.^{27,29}

Another approach to create dynamical micelles is to co-assemble a copolymer consisting of neutral hydrophilic and polyelectrolyte block with an oppositely charged polyelectrolyte³⁰⁻³³ into the core-shell nanoparticles with the core formed by the insoluble interpolyelectrolyte complex (IPEC) and the shell composed of the neutral hydrophilic block. In our previous work,³⁴ we have shown that a block copolymer composed of polycationic and neutral hydrophilic block can form core/shell micelles with IPEC core by co-assembly with a polyanion. Using isothermal titration calorimetry and fluorescence spectroscopy, we proved that the IPEC core remains dynamical, so that the chains can rearrange even after the complex

is formed.

In this work, we combined both approaches for designing dynamical micelles with a soft core and an IPEC layer in order to simultaneously encapsulate both hydrophobic and multivalent species. Firstly, we studied the solution behavior of a novel copolymer poly(lauryl acrylate)-*block*-poly(trimethylammonioethyl acrylate) PLA-QPDMAEA, shown in Fig. 1, left. Its PLA block is expected to form a fluid-like core, similar to other n-alkyl acrylates, because of its high hydrophobicity and relatively low T_g of 255 K.³⁵ A combination of PLA and polyelectrolyte blocks allows PLA-QDMAEA to self-assemble into core/shell micelles, encapsulate hydrophobic species and co-assemble with oppositely charged polyelectrolytes. Secondly, we investigated the complexation of PLA-QPDMAEA with an oppositely charged diblock composed of a polyanion and a neutral hydrophilic block, and poly(ethylene oxide)-*block*-poly(methacrylic acid) PEO-PMAA, shown in Fig. 1, right. We expected that PMAA and QPDAMEA would form an interpolyelectrolyte complex while the PEO block would stabilize the resulting complex in the solution. Furthermore, two PEO-PMAA samples with different block lengths allowed us to investigate how the chain length affects the complexation and morphology of resultant structures. Recent investigations of a similar system composed of PLA-QPDMAEA and PAA-PEO using both experimental and simulation techniques suggested formation of onion-type micelles with an IPEC middle-layer between the hydrophobic core and the neutral hydrophilic corona.³⁶ In the current study, we used scattering techniques, microscopy, calorimetry and nuclear magnetic resonance in combination with self-consistent field modeling for a detailed study of the morphology of the IPEC complex of amphiphilic block PLA-QPDMAEA with double hydrophilic block copolymer PEO-PMAA in basic aqueous solution (pH 9.2). The ultimate goal of this work is fundamental understanding of the physico-chemical mechanisms controlling the assembly of multicompartment nanoparticles.

Figure 1: Chemical structures and schematic representation of PLA-QPDMAEA (left) and 'long' (top right) and 'short' (bottom right) PEO-PMAA.

Results and Discussion

Self-assembly of amphiphilic PLA-QPDMAEA copolymer

In order to follow the self-assembly behavior of PLA-QPDMAEA diblock copolymer in 50 mM sodium tetraborate buffer (pH $\approx$ 9.2), we used light scattering techniques. Dynamic light scattering (DLS) measurements showed that in aqueous solution the copolymer forms particles with hydrodynamic radius measured at scattering angle 90° , R_H , of 50 nm (see Fig. 2a insert). Highly positive zeta-potential, ζ of the particles (+26 mV), obtained using electrophoretic light scattering (ELS), confirms that positively charged QDMAEA groups are located on the surface of the particles.

More detailed information about morphology of the particles was obtained using cryogenic transmission electron microscopy (cryo-TEM) and small-angle neutron scattering (SANS) techniques. Cryo-TEM showed that in aqueous solution PLA-QPDMAEA forms two types of particles at high ionic strength (0.05 M sodium tetraborate): a large fraction of small spherical particles of radius $\approx$ 11 nm and a small fraction of cylindrical particles of $\approx$ 50 nm length and of $\approx$ 7 nm radius. This behavior is in agreement with previously reported ten-

Figure 2: (a) Cryo-TEM micrograph for PLA-QPDMAEA in 50 mM sodium tetraborate. Inset: Distribution of hydrodynamic radii, R_H , at scattering angle $\theta = 90^\circ$. (b) SANS curve for PLA-QPDMAEA solution; red line is a fit of curve with a combination of models for random oriented cylindrical shell with circular cross-section and for sphere with a shell.

dency of amphiphilic polyelectrolyte diblock copolymers to assemble into cylindrical particles at high ionic strength due to the strong electrostatic screening of charged blocks.^{37–40}

Further insight into the structure of PLA-QPDMAEA micelles was revealed by fitting the SANS curves, presented in Fig. 2b. In the fitting, we used a linear combination of models of a randomly oriented cylindrical shell with circular cross-section and a spherical core surrounded with a shell (see description of the models in ESI, Section 2.5.1). The fit revealed a core/shell structure of both cylindrical and spherical particles formed by PLA-QPDMAEA in aqueous solution, presumably consisting of a hydrophobic PLA core and a QPDMAEA outer shell. The dimensions of the micelles obtained by fitting the SANS curve match those obtained from cryo-TEM: spherical particles have a core of 3 nm radius and 1.5 nm shell thickness, while cylindrical particles have a core of 56 nm length and of 7 nm radius, and a shell of 2 nm thickness, see also Fig. 2b.

Having established the presence of the core/shell structure, we focused on the ability of such micelles with the PLA soft core to encapsulate hydrophobic low-molar-mass compounds which we proved by time-resolved fluorescence spectroscopy (for emission intensity decays

Figure 3: The quenching rate constant, K_q , and the emission lifetime, τ_1 , obtained by fitting the fluorescence emission intensity decays of PLA-QPDMAEA solutions with pyrene to eq.1, as function of PLA-QPDMAEA concentration, c .

see Fig. 1 in Section 1, ESI). The measurements were performed for the mixture of PLA-QPDMAEA with hydrophobic fluorophore pyrene which served as a model of a hydrophobic drug at different copolymer concentrations. The distribution of pyrene between the micelles and the aqueous phase was followed using the ability of QPDMAEA to dynamically quench the pyrene fluorescence, thus decreasing the emission lifetime of the probe loaded in the PLA core. In order to obtain information about the quenching of pyrene fluorescence inside the micelles, the emission decays (see ESI) were fitted with the following equation:

$$F(t) = A_1 \exp\left(-t/\tau_1 - C[1 - \exp(-K_q t)]\right) + (1 - A_1) \exp(-t/\tau_2) \quad (1)$$

where τ_1 is a lifetime of pyrene encapsulated into micelles, K_q is a micellar quenching constant, τ_2 is a lifetime of a free pyrene in the solution, A_1 , A_2 and C are constants. The first exponential term in eq. 1 describes the emission decay by micellar quenching mechanism,⁴¹ while the second term corresponds to the decay of free pyrene in the solution. The value of $\tau_2 = 130.6$ ns was obtained from the emission decay for pure pyrene. Fitting the emission decays of PLA-QPDMAEA micelles loaded with pyrene using a fixed value of τ_2 allowed for obtaining the dependence of K_q and τ_1 on the copolymer concentration, c , shown in Fig. 3

and thus the information about the distribution of pyrene in the micelles. Upon dilution of the copolymer solution, both K_q and τ_1 first slightly increase (down to $c = 0.1$ g/l) that is most probably caused by disruption of large clusters in aqueous solution and more effective encapsulation of pyrene into the hydrophobic PLA core. Further dilution leads to a sharp decrease in both K_q and τ_1 due to dissociation of core/shell micelles into individual copolymer chains and release of pyrene into solution. Such trend allows us not only to determine the critical micelle concentration, but it also proves that such micelles with the PLA soft core can be dynamically formed and disassembled and thus used for efficient encapsulation of low-molar mass compounds.

Co-assembly of PLA-QPDMAEA and PEO-PMAA copolymers

Our observations confirmed that the PLA-QPDMAEA copolymer formed reversible micelles, and, therefore, we expect that it would form also reversible complexes with an oppositely charged diblock PEO-PMAA composed of a neutral hydrophilic PEO block and anionic PMAA block. The PMAA block should form an interpolyelectrolyte complex (IPEC) with the QPDMAEA block, whereas the hydrophilic PEO block should form an outer shell stabilizing the complexes in solution, resulting in multilayered structures with a soft PLA layer and an IPEC layer.

To study the assembly of PLA-QPDMAEA micelles with PEO-PMAA, we prepared aqueous solutions in 50 mM sodium tetraborate (pH 9.2) to ensure the full ionization of PMAA block and to keep ionic strength constant to eliminate its effect on IPEC formation. We varied the molar ratio $[MAA]/[QDMAEA]$ to control the excess charge in the formed IPEC layer. We used two PEO-PMAA copolymers with different block lengths: 'short' PEO₃₇₅ – PMAA₃₈ and 'long' PEO₆₉₇ – PMAA₄₇₆, schematically shown in Fig. 1, in order to reveal the effect of block length of the copolymer on its ability to form IPEC and on the stability of the formed complexes in aqueous solution.

Light scattering (LS) techniques were used to study the interaction of these two copoly-

Figure 4: (a) DLS distribution of hydrodynamic radii, R_H , of PLA-QPDMAEA/PEO-PMAA complexes. The charge ratios $[\text{MAA}]/[\text{QDMAEA}]$ of complexes are indicated above each curve. (b) Mean hydrodynamic radius, R_H and (c) Zeta-potential, ζ , as functions of $[\text{MAA}]/[\text{QDMAEA}]$.

mers at various charge ratios, i.e., the ratios of negatively charged monomeric units to positively charged ones $[\text{MAA}]/[\text{QDMAEA}]$. Fig. 4a shows the distribution of hydrodynamic radii for the mixture of PLA-QPDMAEA and long PEO-PMAA at the scattering angle $\theta = 90^\circ$, R_H (the distributions for complexes with short PEO-PMAA are shown in Fig.2 in Section 2.1.1, ESI). DLS showed that pure PLA-QPDMAEA copolymer formed particles with unimodal size distribution in aqueous solutions. Upon the addition of oppositely charged PEO-PMAA the distribution of R_H remained unimodal, but it was shifted towards larger R_H values at charge ratio $[\text{MAA}]/[\text{QDMAEA}] > 0.5$.

For complexes with long PEO-PMAA, the mean value of R_H (Fig. 4b) slightly decreased from 50 nm to 35 nm up to charge ratio $[\text{MAA}]/[\text{QDMAEA}] = 0.5$, while the ζ -potential (Fig. 4c) drops from +25 mV to +15 mV. These observations confirm the complexation

of two oppositely charged blocks by a collapse of the particles due to the formation of a compact IPEC layer and by reducing the charge on the surface of the particles. Further addition of oppositely charged copolymer leads to a sharp increase of particle sizes up to 120 nm and decrease of ζ -potential down to -22 mV, indicating a morphological transition of the complexes and formation of a new negatively charged outer layer. The negative sign of ζ -potential probably originates from the excess of PMAA in the outer shell.

For the complexes formed by short PEO-PMAA, R_H of particles increased from 50 to 80 nm after each addition of PEO-PMAA up to charge ratio $[MAA]/[QDMAEA] = 0.5$, while ζ -potential decreased down to 0 mV, indicating that the mechanism of complex formation was different from that for the long PEO-PMAA. Further increase of charge ratio lead to precipitation of the formed complexes. Therefore, DLS and ELS measurements of these samples were performed after removing the precipitate by centrifugation. These measurements showed that the particles in the solution have the same R_H as the complexes with long PEO-PMAA (ca. 120 nm) with negative ζ -potential at the surface of the formed particles. Thus, the increase of particle size and simultaneous decrease of their surface charge confirmed the formation of complexes between PLA-QPDMAEA and PEO-PMAA.

To reveal the role of the fluid PLA core, we compared the complexation of PLA-QPDMAEA micelles with the complexation of micelles of polystyrene-*block*-poly(2-methylvinylpyridinium iodide) (PS₂₂₈ – QPVP₅₃) by 'long' and 'short' PEO-PMAA. Amphiphilic PS-QPVP copolymer contains PS block with a high $T_g = 325$ K and forms core/shell micelles with the solid PS core and positively charged QPVP shell. Since such copolymer cannot self-assemble into core/shell micelle in aqueous solution without special treatment, the core/shell micelles were prepared by nanoprecipitation of PS-QPVP diblock copolymer in aqueous solution from methanol. Scattering intensity of the mixture of PS-QPVP and PEO-PMAA (see Fig. 3 in Section 2.1.2, ESI) increased even at a low charge ratio $[MAA]/[QVP]$ and the mixture starts precipitating at charge ratios $[MAA]/[QVP]=0.02$ for complexes with the short PEO-PMAA and at $[MAA]/[QVP]=0.03$ for complexes with the long PEO-PMAA. These results

Figure 5: (a) Cryo-TEM micrograph of PLA-QPDMAEA/PEO-PMAA complexes at $[MAA]/[QDMAEA] = 1.0$. (b) AFM scan of aggregates at charge ratio 1.5 deposited on mica surface. Inset: section analysis.

indicated that micelles with a kinetically frozen core are not able to form stable IPEC complexes similar to those of micelles with the soft PLA core. The formation of such IPECs in solution requires the reorganization of the chains inside core/shell micelle which becomes possible only for the micelles with a soft core.

The size and shape of the PLA-QPDMAEA/PEO-PMAA particles were further confirmed by both cryo-TEM and AFM experiments. The TEM micrograph (Fig. 5a) shows that particles formed by PLA-QDMAEA with long PEO-PMAA have spherical-like shape with average radius around 90 ± 25 nm for complex with $[MAA]/[QDMAEA] = 1.0$, that is smaller than the value of R_H obtained from DLS because of the low contrast for polymeric micelles in TEM, in which the loose outer shell may not be visible by such a method. Because of the tendency of positively charged species to form secondary aggregates on the negatively charged mica, AFM was measured for the complexes at $[MAA]/[QDMAEA] = 1.5$ to ensure negative surface charge of the nanoparticles. AFM scan shows (Fig. 5b) that particles absorbed on freshly cleaved-off mica surface also have spherical-like shape, but they are strongly flattened with the diameter in the range 300 – 500 nm and height in the range

Figure 6: (a) AFM phase scan and (b) section analysis of PLA-QPDMAEA/PEO-PMAA aggregates at $[MAA]/[QPDMAEA]=1.5$.

20 – 40 nm.

At the same time, the AFM phase scan of these complexes at charge ratio 1.5 (Fig. 6) revealed a multilayered structure of the particles that became visible due to the difference in mechanical properties of the layers. The section profile of the phase scan (Fig. 6b) shows that the particles are composed of at least 3 layers: a soft core of 165 nm diameter, a stiff shell of 45 nm thickness and an outer soft shell of 25 nm thickness.

In order to obtain more detailed information about the inner structure of the PLA-QPDMAEA/PEO-PMAA, we measured ^1H NMR spectra of the PLA-QPDMAEA/PEO-PMAA complexes at various charge ratios, shown in Fig. 7. The addition of oppositely charged copolymer leads to broadening of the peak corresponding to the positively charged $\text{N}(\text{CH}_3)_3^+$ group of the QPDMAEA block (at chemical shift, $\delta = 3.2$ ppm), which does not overlap with the proton spectrum of PEO-PMAA. Such an observation indicates that the formation of complex particles is accompanied by changing the chemical environment of the positively charged group and thus supports the formation of interpolyelectrolyte complexes. Upon the addition of PEO-PMAA, the signal corresponding to PEO appear in the NMR spectrum, while the signals of PMAA protons do not appear, which confirms that the PMAA

Figure 7: ^1H NMR spectra of PLA-QPDMAEA/PEO-PMAA complexes at various charge ratios.

block is involved in the formation of a new layer and the PEO block is localized in the outer shell. Because of the fluidity of PLA core, signals of PLA protons appear in ^1H spectra even in aqueous solution in contrast to micelles with the solid core. The signal from protons of the PLA block at 1 – 2 ppm disappears at high charge ratios that is caused by 'hiding' of the PLA core under newly formed layer that is more rigid than the QPDMAEA block in the outer shell alone. Thus, the ^1H NMR experiment proved the formation of IPEC between QPDMAEA and PMAA blocks of the two copolymers and shows that the particles have a core consisting of the PLA, an inner layer composed of the IPEC and an outer layer composed of the PEO.

Additional details about the inner structure of these multicompartiment particles formed by PLA-QPDMAEA and PEO-PMAA were obtained using SANS experiment for complexes at various charge ratios MAA/QDMAEA, as indicated in the legend of Fig. 8. The addition of the oppositely charged 'long' PEO-PMAA to the core/shell micelles (mixture of spherical

Figure 8: SANS curves for PLA-QPDMAEA/PEO-PMAA solutions at various charge ratios indicated at each curve. Red lines are fits of curves with block copolymer micelle model for [MAA]/[QDMAEA] up to 0.5 and with 4 layer micelle model for charge ratios above 0.5.

and cylindrical particles) did not significantly affect the shape of scattering curves up charge ratio of 0.5, while simultaneously both forward scattering, I , and radius of gyration, R_g , slightly increased (Fig. 8a, Fig. 9a). It indicates that the addition of a small amount of PEO-PMAA did not lead to the formation of a closely packed layer with a clear interface, but rather to the formation of a complex with a loose outer shell. Further increase of PEO-PMAA concentration leads to a morphology transition and the appearance of a correlation peak in SANS curves. The change of the slope of curves from -1 to -4 confirms the transition from cylindrical to compact spherical particles, which is in agreement with TEM images. At the same time, R_g and the forward scattering intensity increase ca. 5 times, indicating the formation of large structures.

The SANS curves up to charge ratio 0.5 were fitted with the same linear combination

of models as for pure PLA-QPDMAEA (a randomly oriented cylindrical shell with circular cross-section and a spherical core surrounded with a shell, see description of the models in ESI, Section 2.4.1). The shells of both spherical and cylindrical micelles become thicker upon addition of PEO-PMAA copolymer (Fig. Fig. 9b), thus, confirming IPEC formation. The thickness of shell of spherical micelles, t_{sph} increases from 2 to 4 nm, while for the shell of cylindrical micelles t_{cyl} increases from 2 to 9 nm indicating that morphological transition is more favorable for cylindrical micelles.

The curves for the complexes with charge ratio more than 0.5 were fitted with the model of a micelle with the spherical core surrounded by four layers.

Figure 9: (a) The gyration radius, R_g , and the forward scattering intensity, I , and (b) the thicknesses of shells of cylindrical and spherical micelles, t_{cyl} and t_{sph} , for [MAA]/[QDMAEA] from 0 to 0.5, the radius of the core, r , and the thicknesses of first, second and third shells, t_1 , t_2 , and t_3 , for [MAA]/[QDMAEA] from 0.8 to 2.0 obtained from the fitting of SANS curves.

The fits, shown in Fig. 8, revealed that at MAA/QDMAEA = 0.8 the particles are composed of a core with 110 nm radius, first, an inner shell of 11 nm, a second shell of 13 nm and a third shell of 5 nm thickness. At higher ratios the radius of the core and thickness of the inner layers (first and second) decreased, indicating that the layers became more compact. Simultaneously, the thickness of the outermost layer slightly increased due to further complexation. Such a large core of more than 100 nm diameter cannot be formed

by the short PLA block alone, consisting only of 24 monomeric units. Only the PEO block is long enough to form the core, suggesting a transition from core/shell micelles with fluid PLA core and cationic QPDMAEA shell to vesicle-like multilayered micelles with dense water-swollen PEO core, first IPEC layer followed by a PLA layer, then another IPEC layer and finally a loose PEO outer shell. The micelles have a vesicle-like structure with a dense Unfortunately, the values of scattering length density (SLD) could not reveal the composition of these layers because of the unknown degree of swelling of each layer. Therefore, the SLD values obtained from fitting the experimental curves (see Table 1 in Section 2.4.3, ESI) differ from the ones that could be calculated based on the assumed composition of the layers. Similar results were obtained for short PEO-PMAA copolymer (see ESI), for which the form factor was similar to pure PLA-QPDMAEA for the complexes up to charge ratio 0.3 and the morphology transition takes place at charge ratio 0.5.

Figure 10: Schematic representation of the SCF model for the system containing PLA-QPDMAEA and PEO-PMAA block copolymers.

To help us understand the composition of layers within the multicompartment particles, we used the Scheutjens-Fleer Self-Consistent Field (SF-SCF)^{42,43} modeling to predict equilibrium structure of the nanoparticles. In this model, we represented the copolymers as schematically shown in Fig. 10. The model is semi-quantitative, accounting for the hy-

Figure 11: Density profiles obtained from the SCF calculations (a) for pure PLA-QPDMAEA, (b) and (c) for PLA-QPDMAEA/PEO-PMAA at charge ratios $[MAA]/[QDMAEA] = 1.0$.

drophobic character of PLA, for charges of the PMAA and QPDMAEA segments, and for the approximate size of the segments, determined by the lattice size a . The system is represented by a lattice composed of concentric spherical shells (layers) separated by a fixed distance a . By applying the spherical symmetry approximation, we explicitly consider radial variation of the properties, while simultaneously assuming that properties within each layer are homogeneous. The spherical symmetry approximation enables fast and efficient calculation but it also sets the fundamental limitation of the model.

In Fig. 11a we show the density profiles of the structures formed by self-assembly of pure PLA-QPDMAEA, obtained from the SF-SCF calculation. These profiles correspond to a micelle with a core composed of PLA, surrounded by a shell composed of QPDMAEA. Volume fraction of PLA in the core is close to 1.0, and it decays quickly beyond the distance $R \approx 8$ nm, indicating the core-shell interface. Volume fraction of QPDMAEA in the shell is much lower and decays gradually up to the distance $R \approx 15$ nm, indicating the thickness of the shell $t_1 \approx 8$ nm. Because the SCF model is only qualitative, these sizes should not be directly compared with the experimentally determined values. Instead, they should be compared with the results of SCF calculations at other compositions.

In Fig. 11b and Fig. 11c we show the density profiles of the structures formed by a mixture of PLA-QPDMAEA + PEO-PMAA, at charge ratio $[\text{MAA}]/[\text{QPDMAEA}] = 1.0$ (analogous figures at other values of charge ratio are provided in the Section 3, ESI). The profile in Fig. 11b corresponds to a three-layer micelle with a core which consists of PLA with a radius $R \approx 8$ nm, similar to the pure PLA-QPDMAEA micelles. The middle layer consists of an interpolyelectrolyte complex of QPDMAEA and PMAA with much higher polymer volume fraction and thickness $t_1 \approx 5$ nm which is lower than the shell of pure PLA-QPDMAEA micelles. Finally, the outer shell consists of PEO with a gradually decaying density up to $R \approx 25$ nm, indicating the shell thickness $t_2 \approx 15$ nm. The profile in Fig. 11c corresponds to a four-layer micelle with the core composed of hydrated PEO at volume fraction $\phi \approx 35$ mol/l with radius $R \approx 3$ nm. The first layer with thickness $t_1 \approx 5$ nm consists

of PEO and an interpolyelectrolyte complex of QPDMAEA and PMAA. The second layer with thickness $t_2 \approx 12$ nm consists of almost pure PLA, followed by a third layer which has a similar composition and thickness as the second layer, except that it contains less PEO. The remaining PEO extends beyond the third layer and forms an outer shell of the micelle with thickness $t_4 \approx 30$ nm. Notably, the profiles in Fig. 11b and Fig. 11c were obtained under the same conditions, where the calculations differed only in the overall system size. It turns out that the SCF calculations converge either to profiles of three-layered micelles or four-layer micelles, depending on the system size. Both types of profiles yield similar values of free energy per chain, creating an oscillating pattern as a function of system size (see Section 3 in ESI for additional details). We anticipate that the preference of one or the other profile is an artifact of the discretization to layers, in combination with the assumption of spherical symmetry. The former is an inherent feature of the SF-SCF model and cannot be avoided. The latter is most likely not fulfilled in the case of four-layered micelles with PEO in the core. Therefore, we can conclude that our SCF calculations suggest that either three-layer micelles or four-layered micelles should be formed but they cannot reliably discern which of these structures is the preferred one.

To compare the SCF calculations with experimental observations, we note that the calculations confirmed the formation of micelles from pure PLA-QPDMAEA, they confirmed three-layered micelles at low charge ratio $[MAA]/[QDMAEA] = 0.5$, and they also confirmed a structural transition to multilayered micelles at a higher charge ratio $[MAA]/[QDMAEA] = 1.0$. The SCF model is not detailed enough to predict the correct size of the formed structures, however, it reveals their key qualitative features. In the case of multilayered micelles, the calculations revealed a structure in qualitative agreement with the results of fitting the SANS data. The modeling supported the hypothesis which has been formulated previously based on these fits. Specifically, the modeling has revealed four-layer micelles with PEO in the core, with first and third layer of very similar thickness but slightly different composition, consisting of the IPEC. The first and third layers are separated by the second

layer composed of pure PLA. Finally, the outermost layer predicted by the numerical model has a much lower polymer density than the other layers. Hence, it might not be clearly revealed by the scattering experiment because of insufficient contrast.

Conclusions

Figure 12: (a) Self-assembly of PLA-QPDMAEA into core/shell micelle with fluid spherical and cylindrical PLA core and positively charged QPDMAEA shell and (b) Interpolyelectrolyte complex formation of PLA-QPDMAEA core/shell micelles with PEO-MAA and formation of multilayered micelle with PEO core, IPEC inner layer, soft middle layer, IPEC outer layer and hydrophilic neutral corona.

We studied self-assembly of amphiphilic diblock copolymer PLA-QPDMAEA containing PLA block with low glass transition temperature and characterized the formed particles using DLS, ELS, TEM and SANS techniques. We showed that PLA-QPDMAEA self-assemble in aqueous solution, forming spherical and cylindrical core/shell micelles with soft PLA core and positively charged QPDMAEA shell. Due to fluidity of the core, such micelles are able to encapsulate and release hydrophobic solutes efficiently and to assemble and disassemble dynamically, that we proved by studying the release of pyrene using time-resolved fluorescence spectroscopy. Moreover, we showed that PLA-QPDMAEA core/shell micelles form complexes with oppositely charged double hydrophilic block copolymer PEO-PMAA of two different chain lengths, 'short' PEO₃₇₅ – PMAA₃₈ and 'long' PEO₆₉₇ – PMAA₄₇₆. Using DLS, ELS TEM and AFM we showed that they form large spherical particles with the charge on the surface which depends on the charge ratio. Their multilayered structure and the presence of a core with at least 3 layers was proven by AFM and SANS. The presence of vesicle-like structure of the PLA-QPDMAEA/PEO-PMAA complexes was also predicted by means of self-consistent field computer modeling. The modeling has shown that these structures consist of a dense PEO core, a soft PLA layer surrounded by two IPEC layers, and an outermost layer formed by a loose PEO corona, schematically shown

in Fig. 12. The ability to tune the size, charge, and stability of such multicompartment micelles, containing dynamical IPEC and fluid PLA layers, allows for their application in delivery of both hydrophobic and multivalent solutes.

Experimental Section

Materials

The poly(lauryl acrylate)-*block*-poly(trimethylammonioethyl acrylate) (PLA₂₄ – QPDMAEA₁₈, $M_W = 11.3 \text{ kgmol}^{-1}$, Fig. 1) is the quaternized analogue of poly(lauryl acrylate)-*block*-poly(dimethylammonioethyl acrylate) (PLA-PDMAEA) diblock copolymers. The PLA-PDMAEA precursor was synthesized by sequential RAFT polymerization in dioxane, using 2,2'-Azobis(2-methylpropionitrile) (AIBN) as the radical initiator and 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) as the CTA. The preparation of the PLA-QPDMAEA diblock copolymers was achieved by a simple quaternization reaction in THF, using methyl iodide (CH₃I) as the quaternizing agent. The synthetic procedure is described in detail in our previous work.³⁶

Details of the synthesis of polystyrene-*block*-poly(2-methylvinylpyridinium iodide) diblock copolymer (PS₂₂₈ – QPVP₅₃) are reported in the publication by Meristoudi et al.⁴⁴ The poly(ethylene oxide)-*block*-poly(methacrylic acid) copolymers ('short' PEO₃₇₅ – PMAA₃₈ and 'long' PEO₆₉₇ – PMAA₄₇₆) were purchased from Polymer Source, Inc.

Solutions of PLA-QPDMAEA and PEO-PMAA in 0.05M aqueous sodium tetraborate (Na₂B₄O₇) were mixed under stirring and were left for 1 h for equilibration prior to measurements. For all measurements PEO-PMAA was added to PLA-QPDMAEA with the concentration of PLA-QPDMAEA in mixed solutions of 1 gL⁻¹.

Methods

Dynamic Light Scattering (DLS). The dynamic light scattering setup (ALV, Langen, Ger-

many) consisted of a 22 mW He–Ne laser ($\lambda = 632.8$ nm), an ALV CGS/8F goniometer, an ALV High QE APD detector, and an ALV 5004 multi-bit, multi-tau autocorrelator. The DLS measurements were carried out at 25°C at the scattering angle, $\theta = 90^\circ$. DLS measurements were analyzed by fitting the measured normalized time autocorrelation function of the scattered light intensity using the constrained regularization algorithm (CONTIN).⁴⁵ The copolymer mass concentration in the solution was $c_{\text{pol}} = 1.0$ g L⁻¹. The samples were filtered using 0.45 μm Acrodisc PVDF membrane filters prior to measurements.

Electrophoretic Light Scattering (ELS). Zeta-potential measurements were carried out with a Nano-ZS Zetasizer (Malvern Instruments, U.K.) at 25°C. The value of electrophoretic mobility, μ , was averaged over three subsequent measurements, each of which consisted of 15 runs, and used to calculate zeta-potential values, ζ , from the Henry equation in the Smoluchowski approximation, $\mu = \epsilon\zeta/\eta$, where μ is the electrophoretic mobility and ϵ is the dielectric constant of the solvent.

Small-Angle Neutron Scattering (SANS). SANS experiments were performed at FRM II, Garching, Germany, at the KWS-2 instrument.⁴⁶ Measurements were carried on a position-sensitive 2D detector consisting of an array of 128 ³He tubes (pixel size 8 mm) using a non-polarized, mono-chromatic (wavelength, λ with a distribution $\Delta\lambda = 10\%$, set by a velocity selector) incident neutron beam collimated with rectangular apertures for two sample-to-detector distances, namely 2, 8, and 20 m ($\lambda = 0.6$ nm). With this setup, the investigated q-range was 0.015–4.6 nm⁻¹. In all cases, the 2-dimensional scattering patterns were isotropic and were azimuthally averaged, resulting in the dependence of the scattered intensity $I(q)$ on the momentum transfer $q = 4\pi \sin \theta / \lambda$, where 2θ is the scattering angle. The curves were corrected for the dark current, the background scattering from the empty cell and for detector efficiency. Helma quartz cells 1 and 2 mm thick were used for experiments. SANS experiments were performed in 50 mM sodium tetraborate in D₂O. The buffer was measured separately and scattering from the buffer was subtracted.

Atomic force microscopy (AFM). Atomic force microscopy measurements were performed

in the tapping mode under ambient conditions using a NT-MDT Ntegra Prima scanning probe microscope, equipped with a Nanosensors silicon cantilever. Samples were prepared by fast dip coating of a freshly peeled out mica surface. After the evaporation of water, the samples were dried in a vacuum oven at ambient temperature for ca. 5 hours.

Cryogenic Transmission Electron Microscopy (cryo-TEM). TEM micrographs for PLA-QPDMAEA copolymer and PLA-QPDMAEA/PEO-PMAA complexes were obtained with a Techain G2 Spirit Twin 12 microscope (FEI, Czech Republic) equipped with a cryo-attachment (Gatan, CA, USA). $3\mu\text{L}$ of the sample solution were dropped to an electron microscopy grid covered with a holey carbon supporting film (Electron Microscopy Science), which was hydrophilized just before the experiment by means of glow discharge (Expanded Plasma Cleaner, Harrick Plasma, USA). The excess of the solution was removed by blotting (Whatman no. 1 filter paper) for 1 s, and the grid was plunged into liquid ethane held at 181°C . The frozen sample was immediately inserted in the cryo-holder, transferred into the microscope and observed at -173°C using bright field imaging the accelerating voltage of 120 kV.

Fluorometry . Fluorescence emission measurements were carried out in 1 cm quartz cuvettes using a Fluorolog FL 3-22 fluorometer (Horiba Jobin Yvon, France) equipped with double-grating excitation and emission monochromators, a FluoroHub time-correlated single photon counting module and a TBX single photon counting detector. Fluorescence emission decays were measured using a 378 nm NanoLed diode laser source with the pulse FWHM of ca. 200 ps, operated at the repetition frequency of 1 MHz. Pyrene was dissolved in small amount of methanol with further dilution by deionized water and added to PLA-QPDMAEA in water ($c = 1\text{ g/l}$) such that the concentration of pyrene in mixture was $\approx 10^{-6}\text{ M}$. The resulting mixture was diluted by water to achieve desired concentrations. Emission decays were measured at the emission wavelength of 450 nm in a time window of 57 ns with a resolution of 6.98 ps per channel.

Nuclear Magnetic Resonance (NMR). The ^1H NMR data for PLA-QPDMAEA and PLA-

QPDMAEA/PEO-PMAA were recorded on Bruker Avance III 600 MHz spectrometer at 25°C. The samples were prepared at concentration of PLA-QPDMAEA 15 gL⁻¹ in 50 mM borax solution in D₂O. All data processing and fitting was done using MestReNova software.

SCF model and method. We used the Scheutjens-Fleer Self-Consistent Field modeling^{42,43} to investigate the self-assembly of block copolymer studied in the experiments, representing the polymers as schematically shown in Fig. 10. For simplicity, all polymer segments except PLA were treated as hydrophilic by setting $\chi = 0$, where χ is the Flory-Huggins parameter. PLA segments were made hydrophobic by setting $\chi = 2.7$. This value was estimated based on the Hansen solubility parameters,⁴⁷ as described in the Section 3 in ESI. In addition, MAA segments were set negatively charged and QDMAEA segments were set positively charged. The length of each block was chosen to match the short PEO-PMAA block copolymers used in the experiments (Fig. 1). The calculations were performed assuming spherical symmetry of the system. This assumption reduces the three-dimensional problem to one dimensional, thereby decreasing the computational requirements by several orders of magnitude. To determine the equilibrium structures, we performed a set of SCF calculations at fixed polymer concentrations but at different sizes of the simulation box. Then, we selected the structures that correspond to the minimum of free energy as a function of system size at fixed overall composition. Additional technical details of the SCF calculations are provided in the Section 3, ESI.

Acknowledgments

This research has been supported by the Grant agency of the Charles University (GAUK), project No.1375219, by the Czech Science foundation, project No.19-10429S, by the Charles University Research Centre (Programme No. UNCE/SCI/014) and by the Ministry of Education, Youth and Sports of the Czech Republic, Operational Programme Research, Development and Education: “Excellent Research Teams”, Project No. CZ.02.1.01/0.0/0.0/15_003/0000417-

CUCAM. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 731019 (EUSMI) to access beam-time at the KWS-2 instrument operated by JCNS at Heinz Maier-Leibnitz Zentrum (MLZ), Garching, Germany. The authors also thank the European Synchrotron Radiation Facility (ESRF), Grenoble, France for granting SAXS beam time (Project No. SC-4621 at the ID02 beamline). The help of Dr. Ewa Pavlova with cryo-TEM experiments is also gratefully acknowledged.

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Graphical TOC Entry

Supporting Information. Reversible multilayered vesicle-like structures with fluid hydrophobic and interpolyelectrolyte layers

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1 Encapsulation and release of a solute

1.1 Fluorescence study of pyrene release from PLA-QPDMAEA micelles

The emission decays for pyrene in the borax solution and for pyrene encapsulated into PLA-QPDMAEA micelles are shown in Fig. 1.

Figure 1: Emission decays at 383 nm (excitation 340 nm) of pyrene in PLA-QPDMAEA aqueous solutions. Concentration of PLA-QPDMAEA copolymer are shown above the individual curves. Red curves are fits of the curves to eq. 1.

2 Characterization of IPECs

2.1 DLS study of IPECs

2.1.1 PLA-QPDMAEA/PEO-PMAA complexes

The distribution of hydrodynamic radii for the complexes of PLA-QPDMAEA copolymer with short PEO-PMAA is shown in Fig. 2. The complexes precipitate at charge ratio $[MAA]/[QDMAEA]$ above 1.0, so for the charge ratio 1.5 DLS was measured for the solution above precipitate after centrifugation.

Figure 2: Distributions of hydrodynamic radii at scattering angle 90° , R_H , of PLA-QPDMAEA complexes with short PEO-PMAA. Charge ratios $[MAA]/[QDMAEA]$ are shown above the individual curves.

2.1.2 PS-QPVP/PEO-PMAA complexes

DLS measurements for polystyrene-*block*-poly(2-methylvinylpyridinium iodide) copolymer (PS₂₂₈ – QPVP₅₃) that forms micelles with solid core and its complexes with both long and short PEO-PMAA were performed (Fig. 3) to show the difference in co-assembly in comparison to micelles with soft core. The complexes of PS-QPVP with PEO-PMAA precipitated at very low charge ratios [MAA]/[QVP].

Figure 3: (a) Correlation functions and (c) distributions of hydrodynamic radii at scattering angle 90° , R_H , of PS-QPVP with 'short' PEO-PMAA. (b) Correlation functions and (d) distributions of R_H of PS-QPVP with 'long' PEO-PMAA. The charge ratios [MAA]/[QVP] are indicated at each individual curve. Dash line indicates correlation functions for complexes which precipitated. (e, f) Mean R_H and scattering intensities of PS-QPVP with 'short' and 'long' PEO-PMAA, respectively, normalized to intensity of pure PS-QPVP solution, I/I_0 . Grey points indicate the scattering intensities of the solutions after precipitation.

2.2 NMR spectra of PLA-QPDMAEA/PEO-PMAA complexes

Figure 4: ^1H NMR spectra of PLA-QPDMAEA, 'short' PEO-PMAA and their complexes at various charge ratios, indicated above individual spectra.

2.3 AFM scans of PLA-QPDMAEA/PEO-PMAA aggregates

Figure 5: AFM scan and cross-section (a,b) and phase scan and its cross-section (c,d) for complexes of PLA-QPDMAEA with 'long' PEO-PMAA at $[\text{MAA}]/[\text{QDMAEA}]=1.5$.

Figure 6: AFM scan and cross-section (a,b) and phase scan and its cross-section (c,d) for complexes PLA-QPDMAEA/'long' PEO-PMAA at $[\text{MAA}]/[\text{QDMAEA}]=1.5$.

2.4 Fitting SANS curves of PLA-QPDMAEA/PEO-PMAA solutions

2.4.1 Description of fitting models

Sphere with three shells model. The scattering intensity for sphere with 3 shells, $P_{sph}(q)$, is calculated based on the core-shell model and is given by:

$$P_{sph}(q) = (K(q, r, \eta - \eta_1) + K(q, r + t_1, \eta_2 - \eta_1) + K(q, r + t_1 + t_2, \eta_3 - \eta_2) + K(q, r + t_1 + t_2 + t_3, \eta_3 - \eta_0))^2 \quad (1)$$

$$K(q, r, \Delta\eta) = \frac{4}{3}\pi r^3 \Delta\eta^3 \frac{\sin qr - qr \cos qr}{(qr)^3} \quad (2)$$

where r is the radius of core, t_1 , t_2 , t_3 are thicknesses of the first, second and third shell; η , η_1 , η_2 , η_3 and η_0 are SLDs of core, shells, and solvent respectively. To account for polydispersity, a Schulz-Zimm distribution of r was included.

The background contribution was taken as

$$c_0 + c_1 q^{-n} \quad (3)$$

Random oriented cylinders with circular cross-section model.

$$P_{cyl} = \int_0^1 (K_{cyl}(q, \eta_{core} - \eta_{shell}, r_{cyl}, L, x) + K_{cyl}(q, \eta_{shell} - \eta_{solv}, r_{cyl} + t_{cyl}, L, x))^2 dx \quad (4)$$

$$K_{cyl}(q, \Delta\eta, r_{cyl}, L, x) = 2\pi \Delta\eta r_{cyl}^2 L \frac{J_1(qr\sqrt{1-x^2})}{qr\sqrt{1-x^2}} \frac{\sin(qLx/2)}{qLx/2} \quad (5)$$

where r_{cyl} is the core radius, t_{cyl} is the shell thickness, L is the cylinder length, η_{cyl}^{core} , η_{cyl}^{shell} , η_{solv} are SLDs of core, shells and solvent respectively.

The systems with low charge ratios were fitted by a combination of these two form factors with thicknesses of shells 2 and 3 for the model of the “sphere with three shells” were set to 0.

The Gaussian distribution over t_{shell} was included for both contributions.

2.4.2 SANS study of complexes with 'short' PEO-PMAA

Figure 7: SANS curves for PLA-QPDMAEA/PEO-PMAA solutions at various charge ratios indicated at each curve. Red lines are fits of curves by sphere with 3 shells model for charge ratios above 0.5.

2.4.3 Results of the fitting SANS curves

Figure 8: Radii of cores of spherical and cylindrical micelles, r_{sph} and r_{cyl} , thicknesses of shells, t_{sph} and t_{cyl} , and length of cylindrical micelle, L_{cyl} formed by PLA-QPDMAEA with 'long' PEO-PMAA.

Table 1: Parameters obtained from SANS curves for complexes of PLA-QPDMAEA with 'short' PEO-PMAA: the gyration radius, R_g , the forward scattering intensity, I , the radius of the core, r , and the thicknesses of first, second and third shells, t_1 , t_2 , and t_3 .

[MAA]/[QDMAEA]	I , cm^{-1}	R_g , nm	r , nm	t_1 , nm	t_2 , nm	t_3 , nm
0.5	50	97	121	8	13	9
1.0	512	59	59	8	13	9

Table 2: Parameters obtained by fitting SANS curves with the model of four-layered micelles.

Sample	[MAA]/[QDMAEA]	η^{core} , 10^{-6}	η_1^{shell} , 10^{-6}	η_2^{shell} , 10^{-6}	η_3^{shell} , 10^{-6}
'long'	0.8	6.32	6.33	6.32	6.34
	1.0	6.30	6.32	6.30	6.35
	2.0	6.30	6.32	6.30	6.35
'short'	0.5	6.32	6.33	6.32	6.34
	1.0	6.32	6.33	6.32	6.34

2.5 Fitting SAXS curves of PEO-PMAA solution

2.5.1 Description of fitting model

'Long' PEO-PMAA copolymer was fitted with model for generalized Gaussian coil. The scattering intensity $I_{coil}(q)$ is given by:

$$I_{coil}(q) = I_0 \left(\frac{\gamma(\frac{1}{2\nu}, U)}{\nu U^{1/2\nu}} - \frac{\gamma(\frac{1}{\nu}, U)}{\nu U^{1/\nu}} \right) \quad (6)$$

with modified variable, U determined as

$$U = \frac{1}{6}(2\nu + 1)(2\nu + 2)q^2 R_g^2 \quad (7)$$

and the lower incomplete Gamma Function

$$\gamma(a, x) = \int_0^x t^{a-1} dt \quad (8)$$

R_g is the radius of gyration, ν is the excluded volume parameter from the Flory mean field theory and I_0 is forward scattering.

2.5.2 SAXS curve for 'long' PEO-PMAA

SAXS curve for PEO-PMAA in 50 mM sodium tetraborate was fitted with the generalized Gaussian coil model. The fit provided the values $I_0 = 0.093 \text{ cm}^{-1}$, $R_g = 16.7 \text{ nm}$ and $\nu = 0.66$.

Figure 9: SAXS curve for 'long' PEO-PMAA. Red line represents fit of the curve with generalized Gaussian coil model.

3 SCF modeling

The Scheutjens-Fleer self-consistent field method (SF-SCF) is a mean-field method in which the free energy is expressed as a functional of density, while accounting for the connectivity of polymer chains.^{1,2} In the SF-SCF calculation we use an iterative procedure to find the density profiles that minimize the corresponding free energy functional. Short-range polymer-solvent interactions within the SF-SCF model are treated using the Flory-Huggins approach, whereas long-ranged electrostatic interactions are treated by the Poisson-Boltzmann equation. In the current work, we employed the spherical symmetry approximation by discretizing the system into a set of concentric shells (layers) separated by a constant distance a . In this approximation, we assume that density within each layer is homogeneous, but it can vary in the radial direction. As a result of the calculation, we obtain the density profiles of all segments as a function of the radial coordinate R , and the corresponding free energy.

To represent the experimental system, we used diblock copolymers PLA₂₄ – QPDMAEA₁₈ and PMAA₃₈ – PEO₃₇₅. For all segment types except PLA we used the Flory-Huggins parameter, $\chi = 0$. For PLA segments we used $\chi = 2.7$ which we estimated using the Hansen solubility parameters

$$\chi \approx \frac{v_1}{RT} \left((D_1 - D_2)^2 + \frac{1}{4} (P_1 - P_2)^2 + \frac{1}{4} (H_1 - H_2)^2 \right), \quad (9)$$

where indices 1 and 2 refer to solvent and solute, respectively. The parameters D , P and H are Hansen solubility parameters, which represent corresponding impacts of dispersion, polar and hydrogen bonding interactions on solubility.³ To estimate χ for PLA, we used the values for water $v_1 = 18 \text{ ml/mol}$, $D_1 = 15.5$, $P_1 = 16.0$, $H_1 = 42.3$ and for lauryl methacrylate $D_2 = 14.4$, $P_2 = 2.2$, $H_2 = 5.1$. To account for the brush-like structure and higher molar volume of lauryl acrylate segments due to the dodecyl tail, we represented each PLA segment by three segments in the SCF model. To account for the electrostatic interactions, we set the relative permittivity to $\varepsilon_r = 80$ and assigned charges $+e$ to all QPDMAEA segments

and $-e$ to all PMAA segments, where e is the elementary charge. The lattice separation $a = 0.31$ nm approximately corresponds to the Kuhn length of acrylic monomers. Assuming ambient conditions, our settings yield the Bjerrum length $l_B \approx 0.7$ nm, and we obtain the expected charge density on the methacrylic acid chain $l_B/a \approx 2$. Within the SF-SCF model all segments are assumed to have equal size. Therefore, it was not possible to simultaneously match the charge density on QPDMAEA monomers. Furthermore, we added salt ions Na^+ and Cl^- as concentration $c_{\text{NaCl}} = 5 \times 10^{-2}$ mol/l. One of these two ions was treated as neutralizer, i.e. its concentration was adjusted to ensure electroneutrality of the whole system.

When performing the calculations, we first selected the size of the simulation box $R_{\text{max}} = La$, where the integer L defines the total number of layers. Next, we added a fixed amount of $\text{PLA}_{24} - \text{QPDMAEA}_{18}$, such that their concentration in the spherical box volume $V = 4\pi/3 \cdot (La)^3$ matched the polymer concentration $c = 1$ g/ml used in experiments. The we added $\text{PMAA}_{38}\text{PEO}_{375}$ chains to obtain the desired charge ratio $Q = [\text{MAA}]/[\text{QDMAEA}] \in \{0.0, 0.5, 1.0, 1.5\}$.

In order to model the formation of micelles we followed the scheme used by Lebouille et al.⁴ To obtain a non-trivial solution with an inhomogeneous density profile, we pinned the first PLA segments to the first layer. Using this density profile as initial guess, we unpinned the PLA segments and performed the SF-SCF calculation again in order to obtain the equilibrium density profiles without additional constraints.

To determine the aggregation number of micelles and their equilibrium structure, we performed the same SF-SCF calculations for various box sizes, L , and searched for the minimum of free energy $F(L)$, shown in Figs.10, 11, 12 and 13. In all cases we observed that $F(L)$ exhibited an overall decaying profile modulated by minor oscillations, generating a set of local minima and maxima which differ typically by a fraction of $k_B T$ per chain. At charge ratio $[\text{MAA}]/[\text{QDMAEA}] = 0.0$ we always obtained density profiles corresponding to a micelle with PLA core and QPDMAEA shell (Fig. 10). At higher values of $[\text{MAA}]/[\text{QDMAEA}] \in \{0.5, 1.0, 1.5\}$ we observed that the local minima correspond to den-

(a) Excess free energy per PLA-QPDMAEA chain

(b) Density profiles

Figure 10: Excess free energy per chain as a function of system size at charge ratio $[MAA]/[QDMAEA] = 0.0$ and density profiles of individual segments at system size which correspond to the free energy minimum indicated by an arrow.

sity profiles of three-layer micelles while the local maxima correspond to density profiles of four-layer micelles, as seen in Figs.11, 12 and 13. Because the free energy difference between the local maxima and minima was very low, the oscillating pattern of $F(L)$ could be attribute to artifacts of the discretization which is inherent to the SF-SCF model. Furthermore, fluctuations in shape are neglected by the spherical symmetry approximation. Therefore, we think that it is impossible to conclude based on the SF-SCF calculations whether four-layer or three-layer micelles should be more stable. Nevertheless, the calculations indicate that both these structures are feasible and can be obtained close to the minimum of free energy.

(a) Excess free energy per PLA-QPDMAEA chain

(b) Three-layer micelle.

(c) Four-layer micelle.

Figure 11: Excess free energy per chain as a function of system size at charge ratio $[MAA]/[QDMAEA] = 0.5$ and density profiles of individual segments at system size which correspond to the free energy at system sizes indicated by the arrows.

(a) Excess free energy per PLA-QPDMAEA chain

(b) Three-layer micelle.

(c) Four-layer micelle.

Figure 12: Excess free energy per chain as a function of system size at charge ratio $[MAA]/[QDMAEA] = 1.0$ and density profiles of individual segments at system size which correspond to the free energy at system sizes indicated by the arrows.

(a) Excess free energy per PLA-QPDMAEA chain

(b) Three-layer micelle.

(c) Four-layer micelle.

Figure 13: Excess free energy per chain as a function of system size at charge ratio $[MAA]/[QDMAEA] = 1.5$ and density profiles of individual segments at system size which correspond to the free energy at system sizes indicated by the arrows.

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