Electronic Supplementary Information

Block copolymers as bile salt sequestrants: Intriguing structures formed in a mixture of an oppositely charged amphiphilic block copolymer and bile salt

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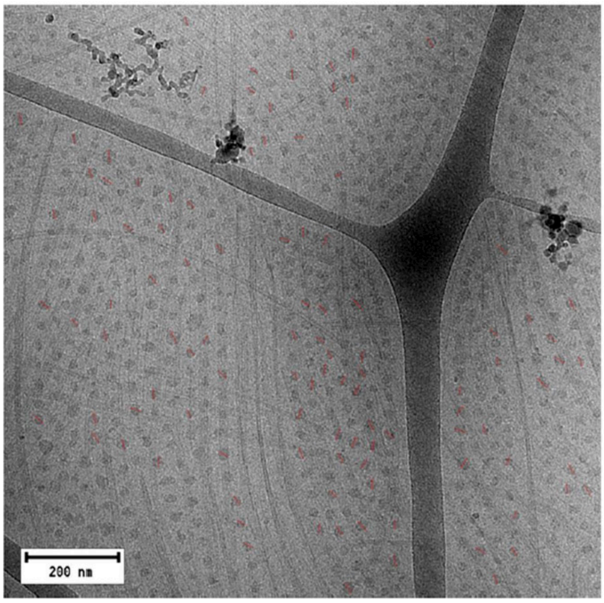
1. Structural analysis of spherical PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$-NaDC complexes by cryo-TEM

In order to calculate whether the PNIPAM corona chains are stretched in a brush-like structure at the core/corona interface of globular mixed complex, we first had to determine the aggregation number of PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$ copolymer in the complex. The value presented in the article is $N_{\text{agg}} = 174$ was estimated from $N_{\text{agg}} = V_{\text{core}}/V_{\text{pair}}$, where V_{core} is the core volume (5220 nm^3) estimated from the D_{core} (see image analysis in Figure S1), and V_{pair} is the volume of the "electrostatically neutralized pair" in the core (27.93 nm^3). V_{pair} is calculated from: $V_{pair} = V_{PAMPTMA} + V_{DC,tot} + V_{methyl}$, where $V_{PAMPTMA}$ is the volume of one PAMPTMA(+)$_{30}$ block (estimated from the partial specific volume of the AMPTMA(+) monomer, 0.256 nm^3), $V_{DC,tot}$ is the volume of 30 associated DC$^-$ anions (estimated from the partial specific volume of the DC$^-$ anion, 0.674 nm^3), and V_{methyl} is the volume of the ending methyl group of the PAMPTMA(+) block (0.03188 nm^3).[1]



Estimate of the diameter of globular particles

D_{core}/nm	21
std	3
PD	0.1

Figure S1. CryoTEM micrograph of the PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$-NaDC solution with X = 0.5 together with the dimensional parameters of the globular mixed complexes analyzed as depicted according to the red marks: mean core diameter (D_{core}), standard deviation and polydispersity ($PD = std/D_{core}$). The dark objects are crystalline ice. The scale bar corresponds to 200 nm. The copolymer concentration was 0.25 wt% and the temperature was 25 °C.

As a control, V_{AMPTMA} was also inferred from the density value (1.11 g/cm^3) given by the provider (Sigma-Aldrich, now Merck, product number 448281) for a solution of AMPTMA(+)Cl- aqueous solution of known concentration (75 wt%). From this information, a partial specific volume of 0.868 cm^3/g for the AMPTMA(+)Cl$^-$ salt ($V_{s,AMPTMA(+)Cl}$) was calculated using the assumption that the partial specific volume of water ($V_{s,water}$) is 1.00 cm^3/g at this composition using the expression: $V_{s,solution} = 1/\rho = 0.75 \cdot V_{s,AMPTMA(+)Cl} + 0.25 \cdot V_{s,water}$. The partial specific volume of 0.2611 nm^3 for an AMPTMA(+) monomer was thereafter obtained from: $V_{s,AMPTMA(+)Cl} \cdot \frac{M_{AMPTMA(+)Cl}}{N_{AV}} - V_{Cl}$, where $M_{AMPTMA(+)Cl}$, V_{Cl} and N_{AV} is the molar mass of the AMPTMA(+)Cl$^-$ salt, the theoretical partial specific volume of a Cl$^-$ ion (0.03587 nm^3)[1] and Avogadro's constant, respectively. The value of $V_{AMPTMA(+)}$ is not far from the one estimated by additive method and hence leading to the same final result of N_{agg} as quoted above.

2. Structural analysis of tape-like PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$-NaDC mixed complexes by cryo-TEM

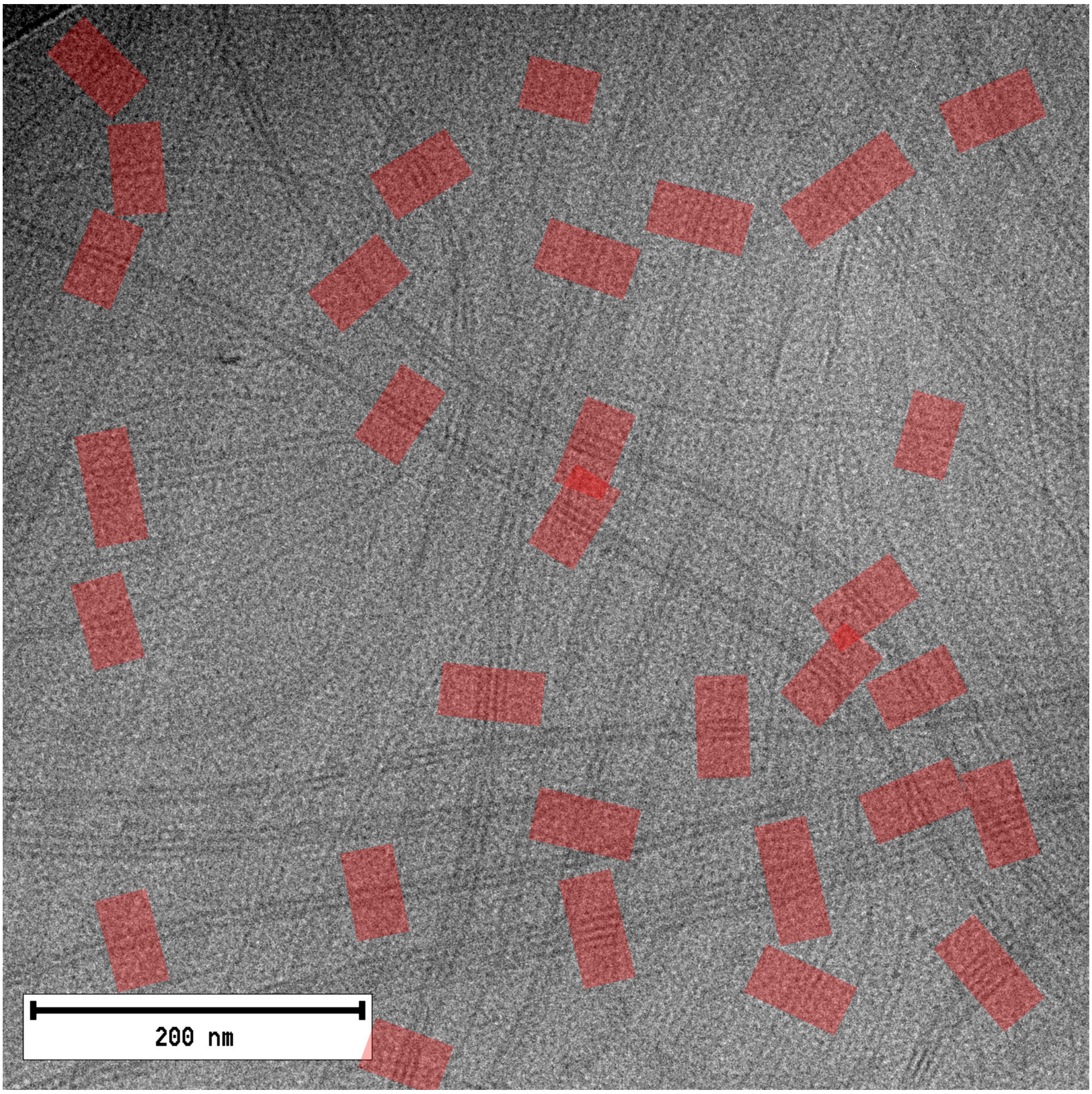


Figure S2. Cryo-TEM micrograph presenting the selected areas (in red) where the intensity profile of the tape-like PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$-NaDC mixed complexes at X = 0.50 were performed, see Figure 4c in the article. The scale bar corresponds to 200 nm. The copolymer concentration was 0.25 wt% and the temperature was 25 °C.

3. SAXS on pure PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$ and NaDC solutions

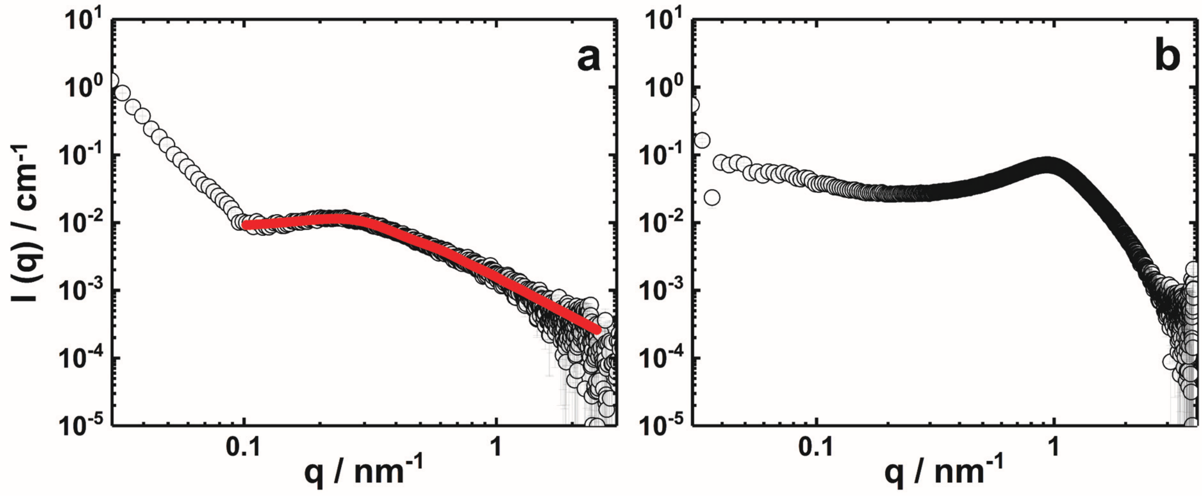


Figure S3. SAXS intensity ($I(q)$) as a function of the magnitude of the scattering vector q for **(a)** a 0.5 wt% PNIPAM$_{120}$-b-PAMPTMA(+)$_{30}$ block copolymer aqueous solution (circles). The red line is the fitted curve using Gaussian chain form factor multiplied by a hard sphere structure factor and **(b)** a 5 wt% NaDC aqueous solution. The error bars correspond to $\pm std$ in $I(q)$. The temperature was 25 °C.

4. SAXS data analysis

Table S1. Data analysis of the SAXS data of an unfiltered mixed solution of PNIPAM$_{120}$-*b*-PAMPTMA(+)$_{30}$ and NaDC with X = 0.50 and a copolymer concentration of 0.5 wt% (see Figure 6b in the article).

Model-independent analysis	
q range for Guinier fit $\left[\ln(I(q)) = \ln(I(0)) + q^2R_g^2/3\right]$ / nm^{-1}	0.053-0.089
R_g from Guinier fit / nm	14.2
$I(0)$ from Guinier fit / cm^{-1}	5.06
q range for indirect Fourier inversion of $I(q)$ / nm^{-1}	0.053-3.0
D$_{max}$ / nm	45
Model fitting	
Software used	SASfit[2]
Model	Form factor of a spherical particle with Gaussian chains attached to the surface*,[3]
Parameters	
Scaling factor = (Number density / cm^{-3}) · 10^{-14} cm^{2}nm^{-2}	$6.4\cdot10^{-4}$
N_{agg}	113
$R_{g,shell}$ / nm	5.15
d (penetration parameter, fixed)	1
η_{core} / nm^{-2}	$10.2\cdot10^{-4}$
η_{shell} / nm^{-2}	$10.0\cdot10^{-4}$
$\eta_{solvent}$ / nm^{-2} (fixed)	$9.4\cdot10^{-4}$
$x_{solvent,core}$	0.017
V_{core} / nm^{3} (fixed)	27.9
V_{brush} / nm^{3}	94.8
R_{core} / nm (calculated from N_{agg}, V_{core}, $x_{solvent,core}$)	9.15

* “SPHERE+Chains(RW)_Nagg” in the SASfit documentation (paragraph 3.2.11.2).

Table S2. Data analysis of the SAXS data of a filtered mixed solution of PNIPAM$_{120}$-*b*-PAMPTMA(+)$_{30}$ and NaDC with X = 0.50 and a copolymer concentration of 0.5 wt% (see Figure 7b in the article).

Model-independent analysis	
q range for Guinier fit $\left[\ln(I(q)) = \ln(I(0)) + q^2R_g^2/3\right]$ / nm^{-1}	0.078-0.132
R_g from Guinier fit / nm	9.8
$I(0)$ from Guinier fit / cm^{-1}	0.0017
q range for indirect Fourier inversion of $I(q)$ / nm^{-1}	0.078-0.4
D_{max} / nm	36
Model fitting	
Software used	SASview[4]
Model	Rectangular parallelepiped with uniform scattering length density[5]
Parameters	
Scaling factor = volume fraction	$3.17 \cdot 10^{-4}$
SLD / 10^{-4} nm^{-2}	10
SLD solvent / 10^{-4} nm^{-2}	9.4
a (height) / nm	16.0
b (width) / nm	27.5
c (length) / nm	1000

5. DLS on tape-like PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$-NaDC complexes

To characterize the dynamics of the tape-like structures, dynamic light scattering (DLS) experiments at 25 °C were carried out on the same sample as in the cryo-TEM image in Figure 7a in the article. An ALV goniometer instrument described in ref [6] was used, with the exception that a Glan-Thomson polarizer was put in front of the detector unit to ensure vertical-vertical polarization geometry. The pseudo-cross correlation functions of the scattered intensity at different scattering angles (θ) in the range 25 °–145 ° are presented in Figure S4a.

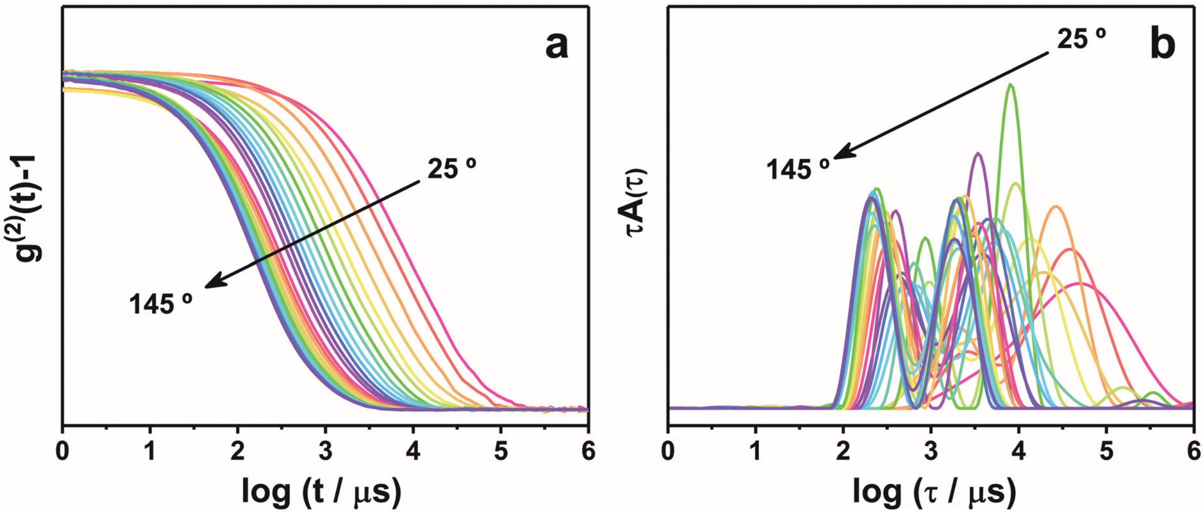


Figure S4. (a) Normalized pseudo-cross correlation functions of the scattered intensity ($g^2(t-1)$) of the PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$-NaDC mixed solution with X = 0.50 (same as in Figure 7 in the article) and **(b)** Corresponding relaxation time distributions obtained from regularized inverse Laplace transformation of intensity correlations functions in (a). The range in scattering angles with an increment of 5° is indicated (arrow). The copolymer concentration was 0.10 wt% and the temperature was 25 °C.

The measured intensity pseudo-cross correlation functions $G^{(2)}(t)$ were evaluated by the non-linear regularized inverse Laplace transformation algorithm, REPES, which different from CONTIN,[7, 8] performs the analysis on the normalized intensity correlation function $g^{(2)}(t)$ to extract the distribution of relaxation times $A(\tau)$.[9-12]

$$g^{(2)}(t) - 1 = \beta \left| \int_0^\infty A(\tau) \exp(-t/\tau) \mathrm{d}\tau \right|^2 \qquad (1)$$

where $g^{(2)}(t)$ is related to $g^{(1)}(t)$ by the Siegert relation: $g^{(2)}(t) - 1 = \beta \left| g^{(1)}(t) \right|^2$,[13] in which β is a factor taking into account deviations from ideal correlation and experimental geometry. τ is the relaxation time, which is equal to $1/\Gamma$, where Γ is the relaxation rate. In this work, the relaxation time distributions are presented as $\tau A(\tau)$ vs. $\log(\tau)$ for equal area representation. The "probability-to-reject" term was set to 0.5.

The data analysis provided bimodal the relaxation time distributions, see Figure S4b (third low-amplitude peak sometimes found is an artifact peak of the Laplace transformation procedure). The slow relaxation mode is diffusive, i.e., dependent on the magnitude of the scattering vector squared q^2 ($q = 4\pi n \sin(\theta/2)/\lambda$, where n and λ is the refractive index of the solvent (here water) and the laser wavelength (here 632.8 nm), respectively). The fast mode could be linked to the some kind of thermal bending fluctuations similar to semiflexible wormlike polymers.[14, 15] However, to the best of the authors' knowledge, at present there is no theory on such internal motions to be applied on tape-like structures.

The tape-like mixed complexes are remarkably stable with time. Figure S5 presents the relaxation time distributions of the filtered aqueous solution of PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$ and NaDC at X = 0.5 with more than 9 months difference in time.

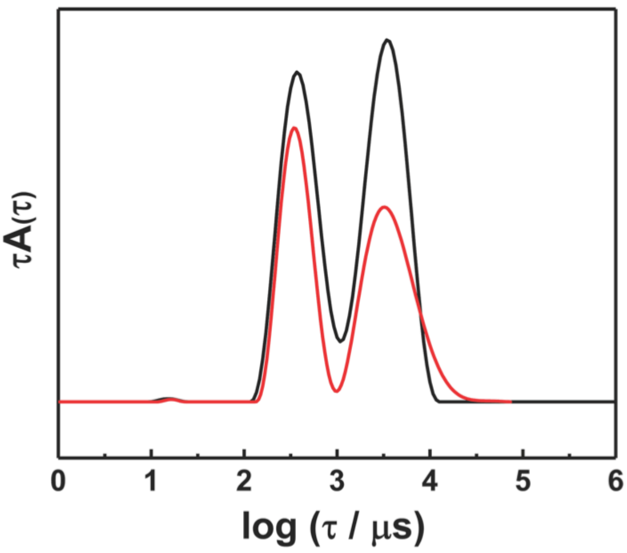


Figure S5. A comparison of the relaxation time distributions obtained from regularized inverse Laplace transformation of the corresponding intensity correlation functions measured at θ = 90° one day after filtration (black, data from Figure S4) of the PNIPAM$_{120}$-*b*-PAMPMTA(+)$_{30}$-NaDC mixed solution with X = 0.50 (same as in Figure 7 in the article) and after about 9 months (red). The copolymer concentration was 0.1 wt% and the temperature was 25 °C.

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7. References

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