Supporting Information for

Size-Controlled Antimicrobial Peptide Drug Delivery Vehicles through Complex Coacervation

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1. SAXS form and structure factors for the fuzzy-surface complex coacervate model

1.1. Structure factors

In the case of micellar clusters, a structure factor is used to explain the scattering at low Q. The structure factor, $S(Q)_{cluster}$, is described by the following equations[1] (Equation S1-S6).

$$p = N_{clu} - floor(N_{clu}) \quad \text{(S1)}$$

$$D = 2 \cdot R_{tot} \cdot f_{dist} \quad \text{(S2)}$$

$$S_x = \frac{\sin(Q \cdot D)}{Q \cdot D} \quad \text{(S3)}$$

$$S_n = \frac{2}{1 - S_x} - 1 - \frac{2\left(1 - S_x^{floor(N_{clu})}\right) \cdot S_x}{floor(N_{clu}) \cdot (1 - S_x)^2} \quad \text{(S4)}$$

$$S_{n1} = \frac{2}{1 - S_x} - 1 - \frac{2\left(1 - S_x^{(floor(N_{clu}) + 1)}\right) \cdot S_x}{(floor(N_{clu}) + 1) \cdot (1 - S_x)^2} \quad \text{(S5)}$$

$$S(Q)_{cluster} = (1 - p) \cdot S_n + p \cdot S_{n1} \quad \text{(S6)}$$

In which N_{clu} is the number of cluster units, and f_{dist} is the distance correlation of the clusters. To describe the correlation peak at high Q values caused by polyelectrolyte charge correlations in the core of the C3Ms, a pseudo-structure factor is introduced ($S(Q)_{internal}$) (Equation S7).[2,3]

$$S(Q)_{internal} = \frac{|C(Q)| \cdot e^{\frac{-(Q - Q_{local})^2}{2 \cdot (W \cdot Q_{local})^2}}}{W \cdot Q_{local} \cdot \sqrt{2\pi}} \quad \text{(S7)}$$

In which $C(Q)$ is the fractal scattering, Q_{local} is the structure peak position, and W is the relative width of the local Q.

1.2. Form factors

To describe the fuzzy-surface complex coacervate micelle, a form factor for fuzzy spheres was used ($P_{Coa}(Q)$) (Equation S8-S12).[4,5]

$$F_0 = \left(\frac{R_{in}}{{\sigma_{in}}^2} + \frac{1}{\sigma_{in}}\right) \cdot \frac{\cos\left(Q \cdot \left(R_{in} + \sigma_{in}\right)\right)}{Q^4} - \frac{3 \cdot sin(Q \cdot \left(R_{in} + \sigma_{in}\right))}{Q^5 \cdot {\sigma_{in}}^2} + \left(\frac{R}{\sigma} \cdot \frac{cos(Q\left(R_{in} - \sigma_{in}\right))}{Q^4}\right.$$

(S8)

$$F_1 = \frac{-3 \cdot \sin\left(Q \cdot \left(R_{in} + \sigma_{in}\right)\right) + 6 \cdot \sin\left(Q * R_{in}\right)}{Q^5 \cdot {\sigma_{in}}^2} - \frac{2 \cdot R_{in} \cdot cos(Q \cdot R_{in})}{Q^4 \cdot {\sigma_{in}}^2}$$

(S9)

$$V_n = \frac{{R_{in}}^3}{3} + \frac{R_{in} \cdot {\sigma_{in}}^2}{6} \quad \text{(S10)}$$

$$A_{core}(Q) = \frac{F_0 + F_1}{V_n} \quad \text{(S11)}$$

$$P_{Coa}(Q) = A_{core}(Q)^2 \quad \text{(S12)}$$

The form factor includes spheres with graded interfaces described by the radius R_{in} and width of the interface, σ_{in}. $A_{core}(Q)$ is the scattering amplitude of the fuzzy core, which, to obtain the form factor, is squared. Lastly, the Debye form factor for polymers and polyelectrolytes ($P_{Debye}(Q)$) is given in Equation S13.[6]

$$P_{Debye}(Q) = \frac{2(e^{-Q^2 \cdot {R_g}^2} - 1 + Q^2 \cdot {R_g}^2)}{(Q^2 \cdot {R_g}^2)^2} \quad \text{(S13)}$$

In which the R_g is the radius of gyration of the polymer/polyelectrolyte. From the fits, we can calculate some of the features of the C3Ms. We start with the average molecular weight of the complex coacervate micelle (M_w) in Equation S14.

$$M_w = P \cdot \left(f_{mix0poly1} \cdot \left(M_{PMAA_1} + M_{PEO_1}\right) + f_{mix0poly2} \cdot \left(M_{PMAA_2} + M_{PEO_2}\right) + (1 -$$

(S14)

Where P is the aggregation number (number of chains per micelle), $f_{mix0poly1}$ is the molar fraction of polymer 1 in the micelles, M_{PMAA1} is the molecular weight of the PMAA block of polymer

1, M_{PEO1} is the molecular weight of the PEO block of polymer 1, $f_{mix0poly2}$ is the molar fraction of polymer 2 in the micelles, M_{PMAA2} is the molecular weight of the PMAA block of polymer 2, M_{PEO2} is the molecular weight of the PEO block of polymer 2, f_{mix0} is the total molar fraction of polymer in the micelles, M_{Col0} is the molecular weight of colistin without the sulfate salt. Furthermore, we can also calculate the fraction of water inside the core with the following equations (S15-S17). We start with the dry volumes of the PMAA and colistin core (V_{cp}), PEO (V_{sp}) and the total dry volume (V_{tot}):

$$V_{cp} = \frac{\left(1 - f_{mix0}\right) \cdot \frac{M_{Col0}}{d_{Col}} + f_{mix0poly1} \cdot \frac{M_{PMAA_1}}{d_{PMAA}} + f_{mix0poly2} \cdot \frac{M_{PMAA_2}}{d_{PMAA}}}{N_A} \quad \text{(S15)}$$

$$V_{sp} = \frac{f_{mix0poly1} \cdot \frac{M_{PEO_1}}{d_{PEO}} + f_{mix0poly2} \cdot \frac{M_{PEO_2}}{d_{PEO}}}{N_A} \quad \text{(S16)}$$

$$V_{tot} = V_{cp} + V_{sp} \quad \text{(S17)}$$

Where d_{Col} is the density of colistin, d_{PMAA} is the density of the PMAA block, d_{PEO} is the density of the PEO block, and N_A is Avogadro's number. Now we can calculate the wet volume of the micelle and determine the water fraction in the micelle through Equations S18 and S19.

$$V_c = 4\pi \cdot \left(\frac{R_{in}}{3} + \frac{R_{in} \cdot \sigma_{in}{}^2}{6}\right) \quad \text{(S18)}$$

$$f_w = 1 - \left(\frac{V_{tot}}{V_c}\right) \quad \text{(S19)}$$

Where R_{in} is the core radius of the micelle and σ_{in} is its density distribution, both obtained from the fits. For a more detailed explanation of the model features and mass balance calculations, we refer to a previous publication.[3] A summary of the parameters used and their categorisation (fixed, calculated, experimental, or fitting parameters) is given in Table S1.

Table S1. Summary of parameters used in the fitting model.

Model parameters / f_+	**Category**	**Values for fixed parameters**	**Description**
M_{Col} (Da)	Fixed	1405.65	Molecular weight colistin sulfate
$\Delta\rho_{Col}$ (cm^{-2})	Fixed	$1.24 \cdot 10^{11}$	SLD colistin
$\Delta\rho_{PMAA}$ (cm^{-2})	Fixed	$1.09 \cdot 10^{11}$	SLD colistin
$\Delta\rho_{PEO}$ (cm^{-2})	Fixed	$1.11 \cdot 10^{11}$	SLD PEO block
$\Delta\rho_{Core}$ (cm^{-2})	Calculated		SLD average in core
$\Delta\rho_{Solvent}$ (cm^{-2})	Fixed	$9.43 \cdot 10^{10}$	SLD solvent buffer

f_{mix0} (-)	Fixed	0.09296 or 0.05076	Total molar polymer fraction in C3M
$f_{mix0poly1}$ (-)	Calculated		Molar polymer 1 fraction in C3M
$f_{mix0poly2}$ (-)	Calculated		Molar polymer 1 fraction in C3M
$c_{Poly1,2}$ (mg/mL)	Experimental		Concentrations of polymer 1 and 2
c_{Col} (mg/mL)	Experimental		Concentration of colistin
φ (-)	Calculated		Volume fraction (from concentrations)
P (-)	Fitting parameter		Aggregation number
σ_{in} (nm)	Fitting parameter		Density distribution of radius
R_{in} (nm)	Fitting parameter		Core radius of micelle
d_{Col} (g cm^{-3})	Fixed	1.35	Density colistin
d_{PEO} (g cm^{-3})	Fixed	1.20	Density PEO block
d_{PMAA} (g cm^{-3})	Fixed	1.20	Density PMAA block
$M_{PEO1,2}$ (Da)	Fixed	Depending on polymer	Molecular weight PEO blocks
$M_{PMAA1,2}$ (Da)	Fixed	Depending on polymer	Molecular weight PMAA blocks
R_{tot} (nm)	Calculated		Total radius of micelle
f_{coa} (-)	Calculated		Fraction of material forming coacervates
$R_{g,Col}$ (nm)	Fixed	0.64	Radius of free colistin molecules
$R_{g,Poly}$ (nm)	Fixed	Polymer sizes from Table 1	Radius of free polymer molecules
f_{clu} (-)	Fitting parameter if necessary (cluster)		Fraction of clusters
N_{clu} (-)	Fitting parameter if necessary (cluster)		Number of micelles per cluster
f_{dist} (-)	Fitting parameter if necessary (cluster)		Distance between clusters
$C(Q)$ (-)	Fitting parameter		Fractal scattering of the internal structure
f_{blob} (-)	Fitting parameter		Fraction of blob scattering
ξ (-)	Fitting parameter		Blob correlation length
W (-)	Fitting parameter		Width of the internal structure peak
Q_{local} (Å^{-1})	Fitting parameter		Q position of the internal structure peak
M_{Col0} (Da)	Fixed	1165.5	Salt-free molecular weight colistin
f_{Col} (-)	Fitting parameter		Fraction of colistin in aqueous phase
f_{Poly} (-)	Calculated		Fraction of polymers in aqueous phase
f_{mix} (-)	Calculated		Molar fraction of polymers in aqueous phase
M_w (Da)	Calculated		Total molecular weight average of one complex coacervate micelle
c_{ColC3M} (mg/mL)	Calculated		Encapsulation concentration of colistin
f_w (-)	Calculated		Water fraction in the micelle
PDI (%)	Fitting parameter		Polydispersity of the radius

2. Results and Discussion

2.1. SAXS fitting parameters of the binary polymer-colistin C3Ms

In Table S2, S3, and S4, the final states of the three different PEO-b-PMAA-colistin C3Ms are compared at 0.50 wt%, 0.25 wt% and 0.125 wt%, respectively.

Table S2. The most important fit parameters for PEO_{45}-b-$PMAA_{41}$-colistin C3Ms at 0.50 wt%, 0.25 wt% and 0.125 wt% were obtained from the fuzzy-surface complex coacervate model.

Model parameters / f_+	**0.50 wt%**	**0.25 wt%**	**0.125 wt%**
M_{Col} (Da)	1405.65	1405.65	1405.65
$\Delta\rho_{Col}$ (cm^{-2})	$1.24{\cdot}10^{11}$	$1.24{\cdot}10^{11}$	$1.24{\cdot}10^{11}$
$\Delta\rho_{PMAA}$ (cm^{-2})	$1.09{\cdot}10^{11}$	$1.09{\cdot}10^{11}$	$1.09{\cdot}10^{11}$
$\Delta\rho_{PEO}$ (cm^{-2})	$1.11{\cdot}10^{11}$	$1.11{\cdot}10^{11}$	$1.11{\cdot}10^{11}$
$\Delta\rho_{Core}$ (cm^{-2})	$1.24{\cdot}10^{11}$	$1.24{\cdot}10^{11}$	$1.24{\cdot}10^{11}$
$\Delta\rho_{Solvent}$ (cm^{-2})	$9.43{\cdot}10^{10}$	$9.43{\cdot}10^{10}$	$9.43{\cdot}10^{10}$
f_{mix0} (-)	0.09296	0.09296	0.09296
c_{Poly} (mg/mL)	1.62	0.81	0.41
c_{Col} (mg/mL)	3.38	1.69	0.84
P (-)	$1.4{\cdot}10^{3}$	$1.6{\cdot}10^{3}$	$1.2{\cdot}10^{4}$
σ_{in} (nm)	1.8	1.6	1.7
R_{in} (nm)	13.6	14.3	14.0

R_{tot} (nm)	15.4	16.0	15.8
f_{coa} (-)	0.84	0.84	0.84
$R_{g,Col}$ (nm)	0.64	0.64	0.64
$R_{g,Poly}$ (nm)	2.4	2.4	2.4
f_{clu} (-)	0	0	0
f_{dist} (-)	1	1	1
$C(Q)$ (-)	$5.6 \cdot 10^{-5}$	$3.0 \cdot 10^{-5}$	$1.1 \cdot 10^{-5}$
f_{blob} (-)	0.38	0.36	0.44
ξ (-)	3.8	3.3	3.2
W (-)	0.09	0.10	0.10
Q_{local} (Å^{-1})	0.22	0.22	0.22
M_{Col0} (Da)	1165.5	1165.5	1165.5
f_{Col} (-)	0.16	0.16	0.16
f_{Poly} (-)	0.16	0.16	0.16
f_{mix} (-)	0.093	0.093	0.093
M_w (Da)	$2.3 \cdot 10^{6}$	$2.7 \cdot 10^{6}$	$2.0 \cdot 10^{6}$
c_{ColC3M} (mg/mL)	2.8	1.4	0.71
f_w (-)	0.76	0.76	0.81
PDI (%)	18	17	17

Table S3. The most important fit parameters for PEO_{45}-b-$PMAA_{81}$-colistin C3Ms at 0.50 wt%, 0.25 wt% and 0.125 wt% were obtained from the fuzzy-surface complex coacervate model.

Model parameters / f_+	**0.50 wt%**	**0.25 wt%**	**0.125 wt%**
M_{Col} (Da)	1405.65	1405.65	1405.65
$\Delta\rho_{Col}$ (cm^{-2})	$1.24 \cdot 10^{11}$	$1.24 \cdot 10^{11}$	$1.24 \cdot 10^{11}$
$\Delta\rho_{PMAA}$ (cm^{-2})	$1.09 \cdot 10^{11}$	$1.09 \cdot 10^{11}$	$1.09 \cdot 10^{11}$
$\Delta\rho_{PEO}$ (cm^{-2})	$1.11 \cdot 10^{11}$	$1.11 \cdot 10^{11}$	$1.11 \cdot 10^{11}$
$\Delta\rho_{Core}$ (cm^{-2})	$1.24 \cdot 10^{11}$	$1.24 \cdot 10^{11}$	$1.24 \cdot 10^{11}$
$\Delta\rho_{Solvent}$ (cm^{-2})	$9.43 \cdot 10^{10}$	$9.43 \cdot 10^{10}$	$9.43 \cdot 10^{10}$
f_{mix0} (-)	0.05076	0.05076	0.05076
c_{Poly} (mg/mL)	1.41	0.71	0.35
c_{Col} (mg/mL)	3.59	1.79	0.90
P (-)	$1.4 \cdot 10^{4}$	$4.8 \cdot 10^{3}$	$5.4 \cdot 10^{3}$
σ_{in} (nm)[a]	0.0	0.0	0.0
R_{in} (nm)[a]	35.0	30.2	35.3
R_{tot} (nm)	35.0	30.2	35.3
f_{coa} (-)	0.84	0.86	0.84
$R_{g,Col}$ (nm)	0.64	0.64	0.64
$R_{g,Poly}$ (nm)	3.5	3.5	3.5
f_{clu} (-)	0.20	0.30	0.55
f_{dist} (-)	0.2	0.07	0.04
$C(Q)$ (-)	$7.2 \cdot 10^{-5}$	$3.2 \cdot 10^{-5}$	$1.3 \cdot 10^{-5}$
f_{blob} (-)	0.24	0.28	0.32
ξ (-)	2.7	2.8	3.0
W (-)	0.08	0.07	0.07
Q_{local} (Å^{-1})	0.23	0.23	0.23
M_{Col0} (Da)	1165.5	1165.5	1165.5
f_{Col} (-)	0.15	0.14	0.16
f_{Poly} (-)	0.15	0.14	0.16
f_{mix} (-)	0.051	0.051	0.051
M_w (Da)	$2.2 \cdot 10^{7}$	$7.8 \cdot 10^{6}$	$8.8 \cdot 10^{6}$
c_{ColC3M} (mg/mL)	3.0	1.5	0.75
f_w (-)	0.84	0.91	0.94
PDI (%)	22	22	21

[a] It was not possible to extract a reliable width parameter for these systems, most likely due to the increased polydispersity. The size is still extracted well based on the R_{in} parameter.

Table S4. The most important fit parameters for PEO_{114}-b-$PMAA_{81}$-colistin C3Ms at 0.50 wt%, 0.25 wt% and 0.125 wt% were obtained from the fuzzy-surface complex coacervate model.

Model parameters / f_+	**0.50 wt%**	**0.25 wt%**	**0.125 wt%**
M_{Col} (Da)	1405.65	1405.65	1405.65
$\Delta\rho_{Col}$ (cm^{-2})	$1.24\cdot10^{11}$	$1.24\cdot10^{11}$	$1.24\cdot10^{11}$
$\Delta\rho_{PMAA}$ (cm^{-2})	$1.09\cdot10^{11}$	$1.09\cdot10^{11}$	$1.09\cdot10^{11}$
$\Delta\rho_{PEO}$ (cm^{-2})	$1.11\cdot10^{11}$	$1.11\cdot10^{11}$	$1.11\cdot10^{11}$
$\Delta\rho_{Core}$ (cm^{-2})	$1.24\cdot10^{11}$	$1.24\cdot10^{11}$	$1.24\cdot10^{11}$
$\Delta\rho_{Solvent}$ (cm^{-2})	$9.43\cdot10^{10}$	$9.43\cdot10^{10}$	$9.43\cdot10^{10}$
f_{mix0} (-)	0.05076	0.05076	0.05076
c_{Poly} (mg/mL)	1.73	0.86	0.43
c_{Col} (mg/mL)	3.27	1.64	0.82
P (-)	$1.5\cdot10^{4}$	$1.6\cdot10^{4}$	$1.0\cdot10^{4}$
σ_{in} (nm)[a]	0.0	0.0	0.0
R_{in} (nm)[a]	36.8	35.1	30.6
R_{tot} (nm)	36.8	35.1	30.6
f_{coa} (-)	0.87	0.86	0.84
$R_{g,Col}$ (nm)	0.64	0.64	0.64
$R_{g,Poly}$ (nm)	3.5	3.5	3.5
f_{clu} (-)	0	0	0
f_{dist} (-)	1	1	1
$C(Q)$ (-)	$9.1\cdot10^{-5}$	$3.8\cdot10^{-5}$	$1.9\cdot10^{-5}$
f_{blob} (-)	0.22	0.20	0.24
ξ (-)	2.0	1.5	1.3
W (-)	0.09	0.09	0.10
Q_{local} ($Å^{-1}$)	0.23	0.23	0.22
M_{Col0} (Da)	1165.5	1165.5	1165.5
f_{Col} (-)	0.09	0.14	0.16
f_{Poly} (-)	0.09	0.14	0.16
f_{mix} (-)	0.051	0.051	0.051
M_w (Da)	$2.7\cdot10^{7}$	$2.8\cdot10^{7}$	$1.9\cdot10^{7}$
c_{ColC3M} (mg/mL)	2.9	1.4	0.68
f_w (-)	0.89	0.80	0.78
PDI (%)	22	19	14

[a] It was not possible to extract a reliable width parameter for these systems, most likely due to the increased polydispersity. The size is still extracted well based on the R_{in} parameter.

2.2. Swelling ternary coacervate systems

In Figure S1, the SAXS patterns and fits of the ternary complex coacervate systems T5, T6, T7, and T8 are shown at 0.125 wt%, including their R_{tot} fit parameter (from the fuzzy-surface complex coacervate model) for the complete polymer molar fraction set ($0 \leq \Psi \leq 1$). In Figure S2, T3 and T4 ternary coacervate systems are shown to not swell at the complete polymer molar fraction set based on the fits from the fuzzy-surface complex coacervate model. Lastly, in Figure S3, the *PDI* behaviour (from the fuzzy-surface complex coacervate model) for the complete polymer molar fraction set is shown for T5, T6, T7, and T8 complex coacervate systems.

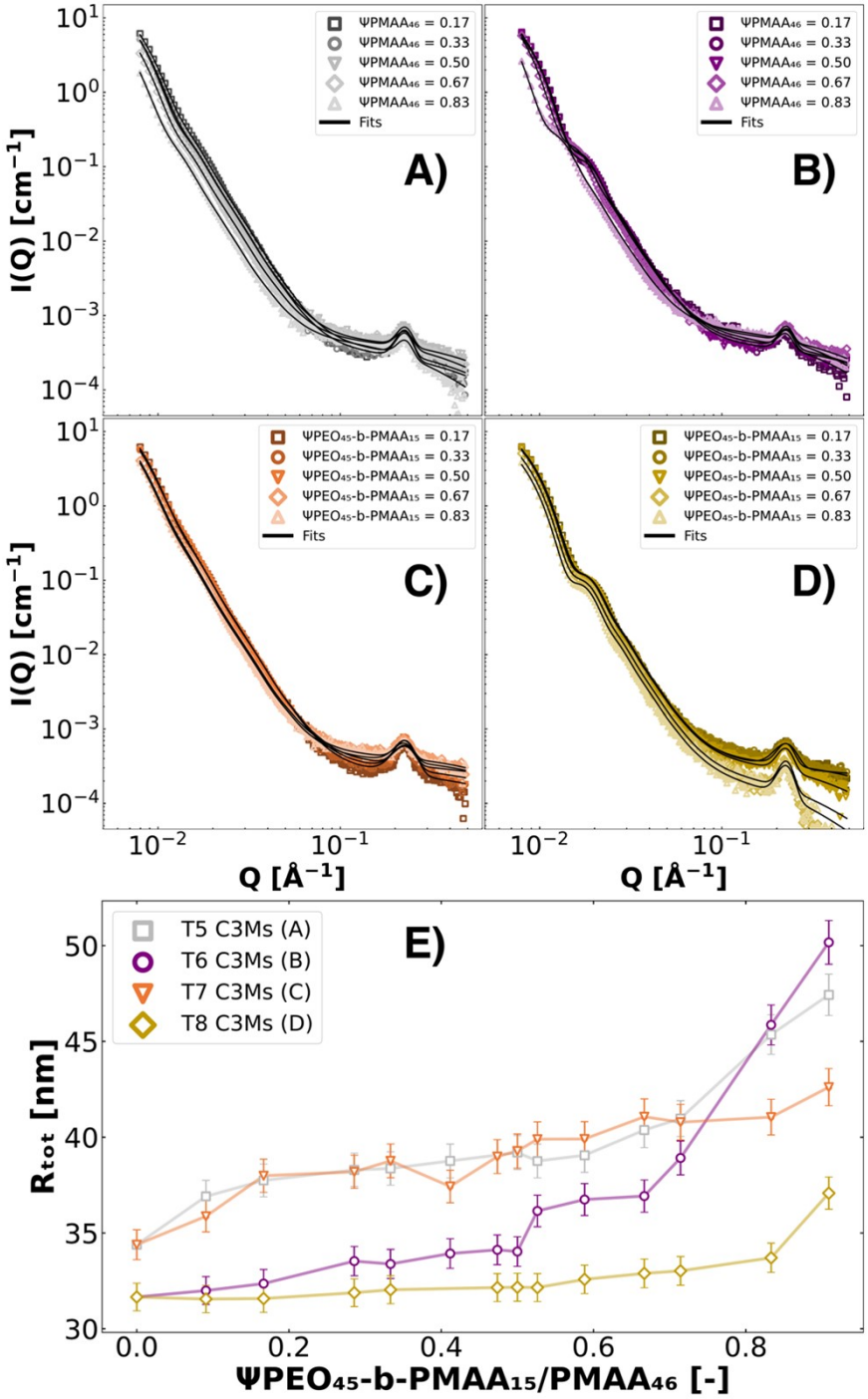

Figure S1. SAXS patterns of colistin-C3Ms formed from four different ternary coacervate formulations at 0.125 wt%. C3Ms were prepared from a combination of colistin and two other polymers: $PMAA_{46}/PEO_{45}$-b-$PMAA_{81}$ (T5, grey symbols, A), $PMAA_{46}/PEO_{114}$-b-$PMAA_{81}$ (T6, purple symbols, B), PEO_{45}-b-$PMAA_{15}/PEO_{45}$-b-$PMAA_{81}$ (T7, orange symbols, C), and PEO_{45}-b-$PMAA_{15}/PEO_{114}$-b-$PMAA_{81}$ (T8, yellow symbols, D). The

complete polymer molar fraction set $0 \leq \Psi \leq 1$ was investigated and fitted with the fuzzy-surface C3M model, while for visibility reasons only a couple of SAXS patterns are shown. The fit parameter R_{tot} is shown for T5, T6, T7, and T8 along the complete polymer molar fraction set (E).

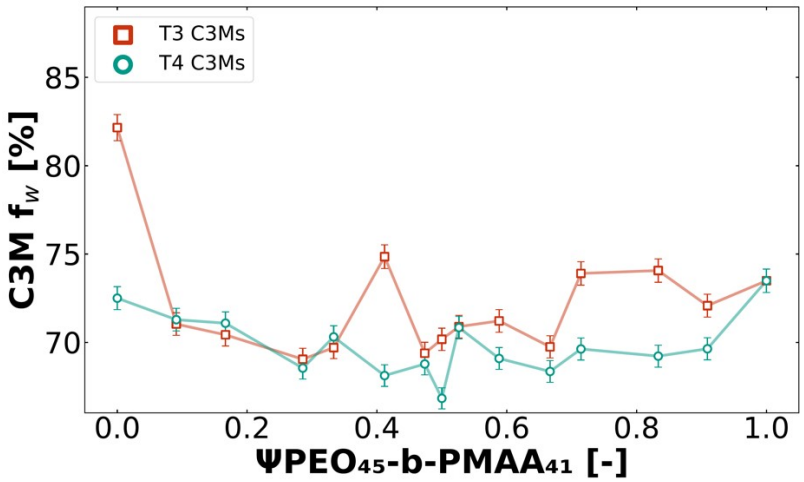

Figure S2. From the fuzzy-surface C3M model fits, the C3M water content (f_w) was plotted against the molar fraction of PEO_{45}-b-$PMAA_{41}$ (T3, red squares and T4, green circles).

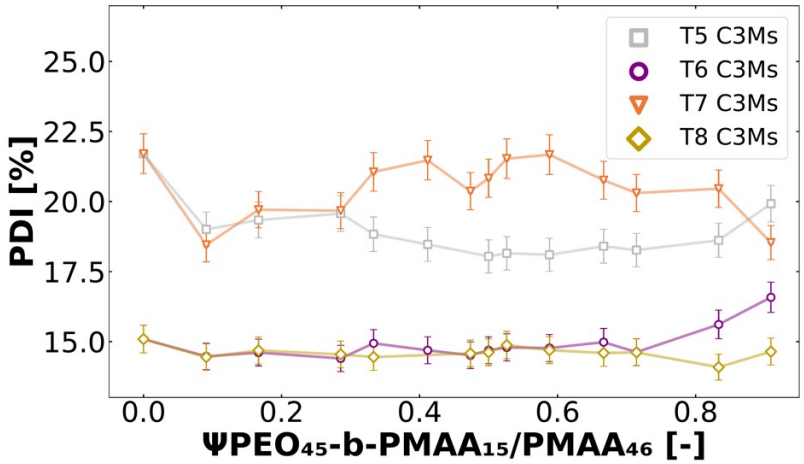

Figure S3. Polydispersity index (*PDI*) behaviour for T5 (grey squares), T6 (purple circles), T7 (orange triangles), and T8 (yellow rhombuses) ternary colistin complex coacervate systems for the complete polymer molar fraction set ($0 \leq \Psi \leq 1$). From the fuzzy-surface C3M model fits, the *PDI* values were plotted against the molar fraction of either PEO_{45}-b-$PMAA_{15}$ or $PMAA_{46}$.

2.3. Formation kinetics of ternary coacervate systems

In Figure S4, the power law behaviour of T1-T4 ternary coacervate systems for R_{tot} and P (from the fuzzy-surface complex coacervate model) is shown from the kinetic growth process over time, analysed with a TR-SAXS setup at 0.125 wt% and $\Psi = 0.50$.

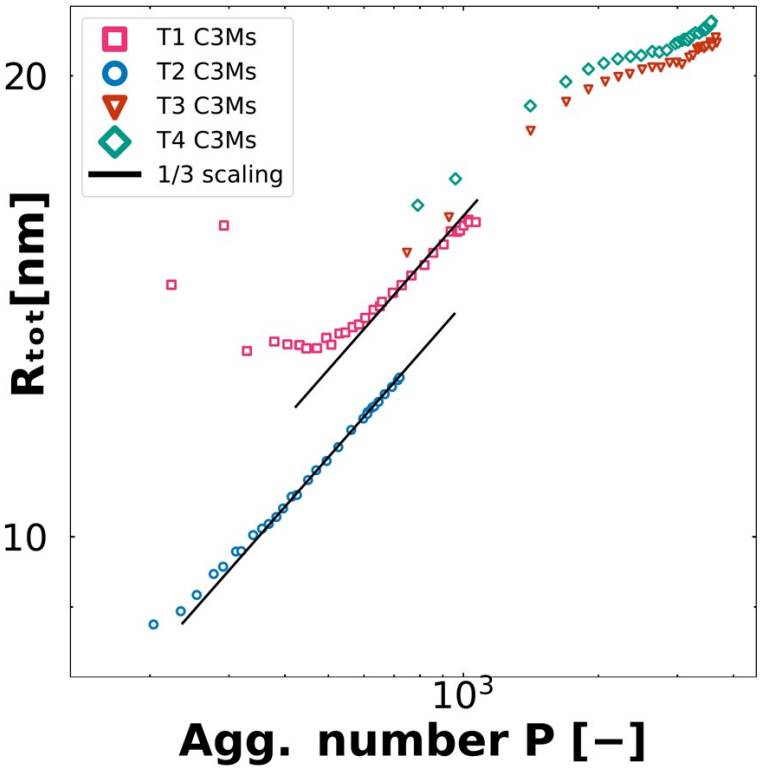

Figure S4. Power law relation of R_{tot} and P on log-log scale for colistin-C3Ms from T1 (pink squares), T2 (blue circles), T3 (red triangles) and T4 (green rhombuses) analysed through TR-SAXS, including fits from the fuzzy-surface complex coacervate model. 1/3 fits for the first two are added to visualise the power law of equal density growth. We observe some density changes in the growth process of complex coacervates.

3. References

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