ELECTRONIC SUPPLEMENTARY INFORMATION

Complex coacervation of natural sophorolipid bolaamphiphile micelles with cationic polyelectrolytes

Ghazi Ben Messaoud,[a] Lyndsay Promeneur,[a] Martha Brennich,[b] Sophie Roelants,[c,d] Patrick Le Griel,[a] Niki Baccile[a,*]

[a] Sorbonne Université, Centre National de la Recherche Scientifique, Laboratoire de Chimie de la Matière Condensée de Paris, LCMCP, F-75005 Paris, France
[b] European Molecular Biology Laboratory, Synchrotron Crystallography Group, 71 Avenue des Martyrs, 38042 Grenoble, France.
[c] Ghent University, Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Coupure Links 653, Ghent, Oost-Vlaanderen, BE 9000
[d] Bio Base Europe Pilot Plant, Rodenhuizekaai 1, Ghent, Oost-Vlaanderen, BE 9000

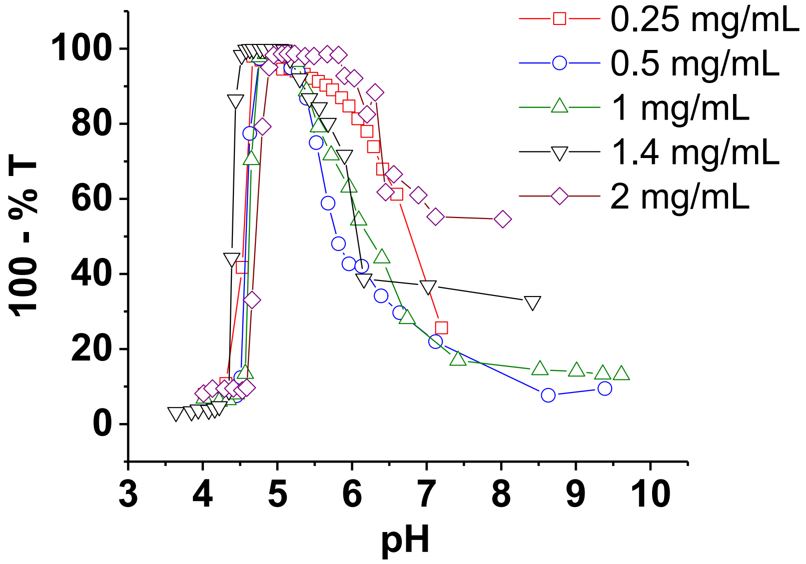

Figure S 1 - Evolution of turbidity during coacervation of SL micelles ([SL]= 10 mg/mL) with CHL ([CHL]= 0.25 - 2 mg/mL) as function of pH.

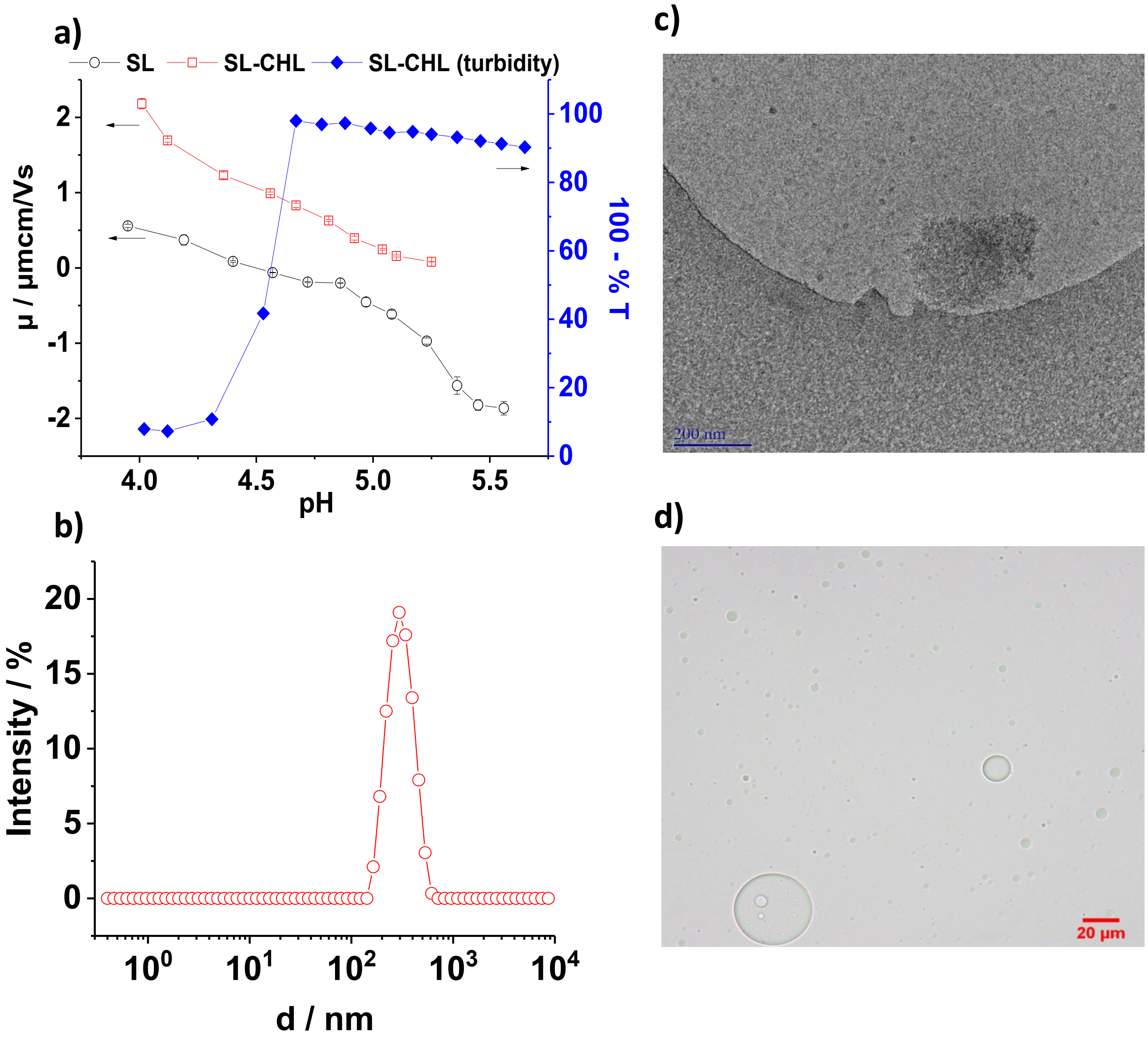

Figure S 2 – **SL-CHL ([SL]= 0.25; [CHL]= 10 mg/mL) sample: (a) evolution of the electrophoretic mobility and turbidity (100-%T) as function of pH; (b) size distribution at pH 4.56; (c) cryo-TEM image at pH 4.56 and (d) light microscopy large droplets at pH 5.2.**

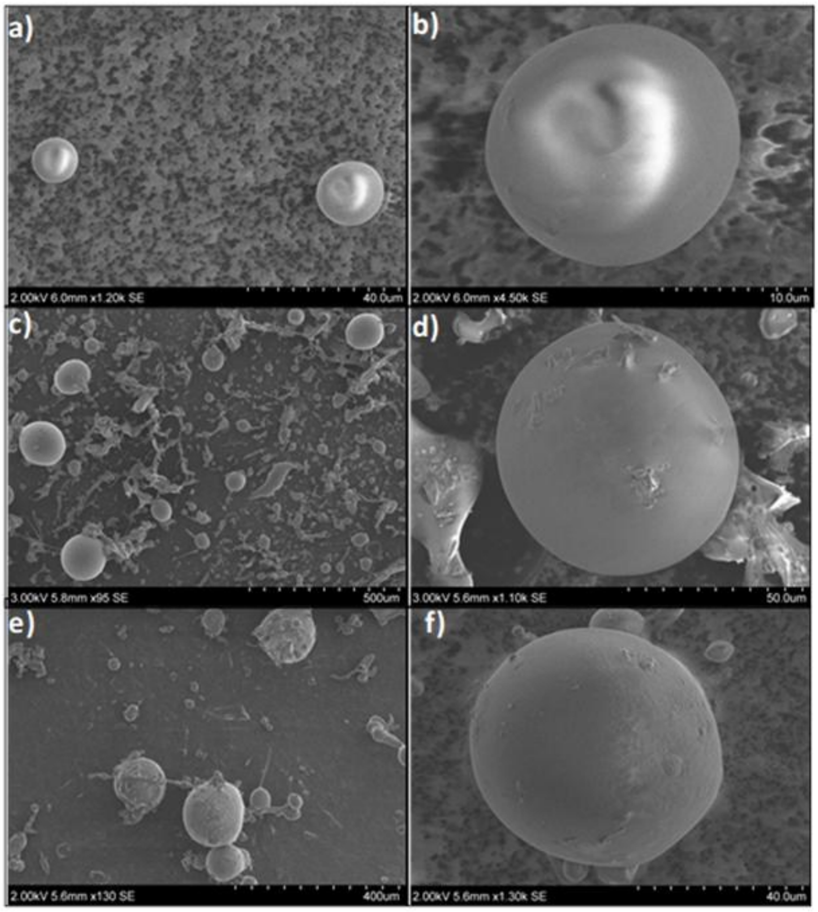

Figure S 3 - SEM images of (a,b) SL-CHL ([CHL]= 1.4 mg/mL, pH 6.2), (c,d) SL-PLL ([PLL]= 2 mg/mL, pH 7) and (e,f) SL-PAA ([PAA]= 0.75 mg/mL, pH 8) complex coacervates prepared with [SL]=5 mg/mL.

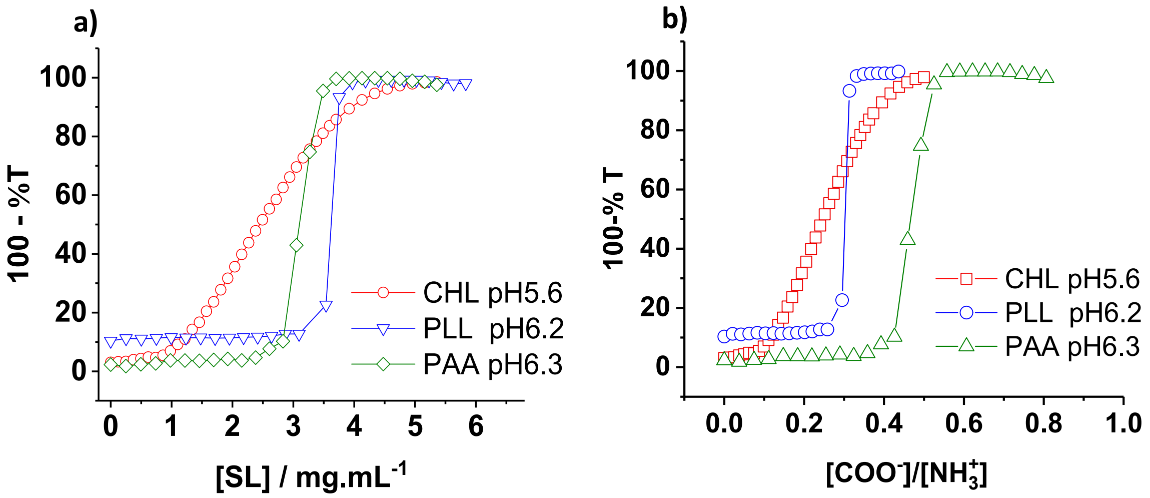

Figure S 4 - Evolution of the turbidity of PEC solutions during stepwise addition of SL solution as function of (a) SL concentration and (b) charge ratio. [CHL]= 1.4 mg/mL, [PLL]= 2 mg/mL, and [PAA]= 0.75 mg/mL

The difference of the critical aggregation concentration (cac) among the three systems (Figure S *4*b) could be related to the molecular structure of each PEC. In theory, cac is usually lower than the cmc, however we are not able to explain the high cac compared to the cmc (0.1 mg/mL) due to the complex behavior of SL. Indeed, during gradual addition of SL, free molecules can exist in solution or can even preferentially adsorb to the air-water interface to expose the intermediate aliphatic chain to the hydrophobic air phase. Furthermore, for bolaform surfactants, the relationship between the cmc and the free energy of micellization is complex and it requires taking into account the contribution of counterions.[1] The analysis becomes even more complex due to the pH-sensitive nature of SL. In fact, the degree of ionization, also called the apparent degree of counterions dissociation,[2] will vary strongly with pH. In addition, the counterions distribution (condensation and release during electrostatic interaction) will also be affected by the binding process of SL micelles to each PEC.

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[1] R. Zana, Critical Micellization Concentration of Surfactants in Aqueous Solution and Free Energy of Micellization. *Langmuir*, 1996, 12, 1208–1211.

[2] B. L. Bales, A Definition of the Degree of Ionization of a Micelle Based on Its Aggregation Number. *J. Phys. Chem. B*, 2001, 105, 6798–6804

Determination of the ionization degrees

The evolution as function of the pH of the theoretical degrees of ionization values α for (SL, pKa 5.8) and β for (CHL, pKa 6.5), (PLL, pka 10) and (PAA, pKa 9.5) were calculated as function of pH from the modified Henderson–Hasselbalch equation:

For SL:

$$pKa = pH + log\frac{(1-\alpha)}{\alpha}$$

Where $$\alpha = \frac{[COO^-]}{[COOH]+[COO^-]}$$

For PEC:

$$pKa = pH + log\frac{\beta}{(1-\beta)}$$

Where $$\beta = \frac{[NH_3^+]}{[NH_2]+[NH_3^+]}$$

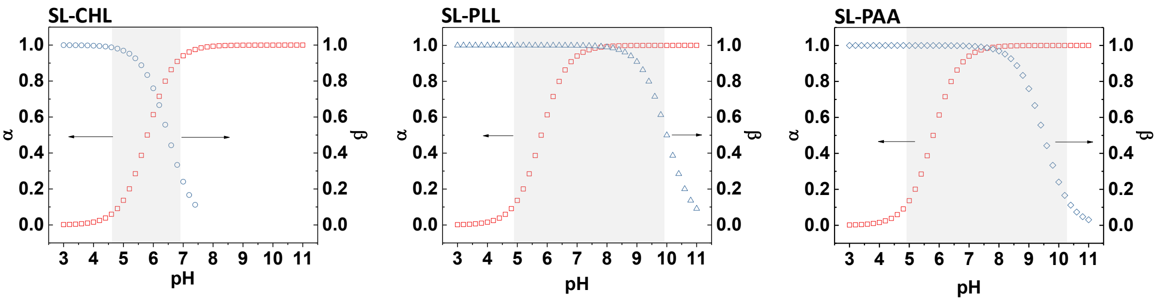

Figure S 5 - Evolution of the theoretical degrees of ionization α and β as function of pH for SL and polymers

Determination of the charge ratio: $[COO^-]:[NH_3^+]$

The stoichiometric ratio for chargeable groups (-/+) and defined = [S] /(n×[P][3,4,5] where [S] and [P] are the molar concentrations for the SL and for the PEC, respectively and n is the number of PEC monomers or binding sites. If we consider that the average molecular weight of SL, CHL, PLL and PAA are respectively 633, 4000, 5000 and 17,500 g/mol. The CHL, PLL and PAA are therefore made from average monomers of 15, 23 and 128, respectively. However, for CHL the number of binding sites is assumed to be (15 x 2) because each monomer contains two amino groups. By including the theoretical ionization degrees (α and

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[3] Hervé, P., et al. "Novel core-shell structure for colloids made of neutral/polyelectrolyte diblock copolymers and oppositely charged surfactants." *EPL (Europhysics Letters)* 58.6 (2002): 912

[4] Berret, Jean-Francois, et al. "Colloidal complexes obtained from charged block copolymers and surfactants: A comparison between small-angle neutron scattering, Cryo-TEM, and simulations." *The Journal of Physical Chemistry B* 107.32 (2003): 8111-8118

[5] Berret, Jean-François, et al. "Electrostatic self-assembly of oppositely charged copolymers and surfactants: A light, neutron, and X-ray scattering study." *Macromolecules* 37.13 (2004): 4922-4930

β) is therefore possible to estimate the charge ratio Z (-/+) or [COO-]:[NH3+] which will be equal to: $Z= (\alpha x [S]) / (n x \beta x [P])$.

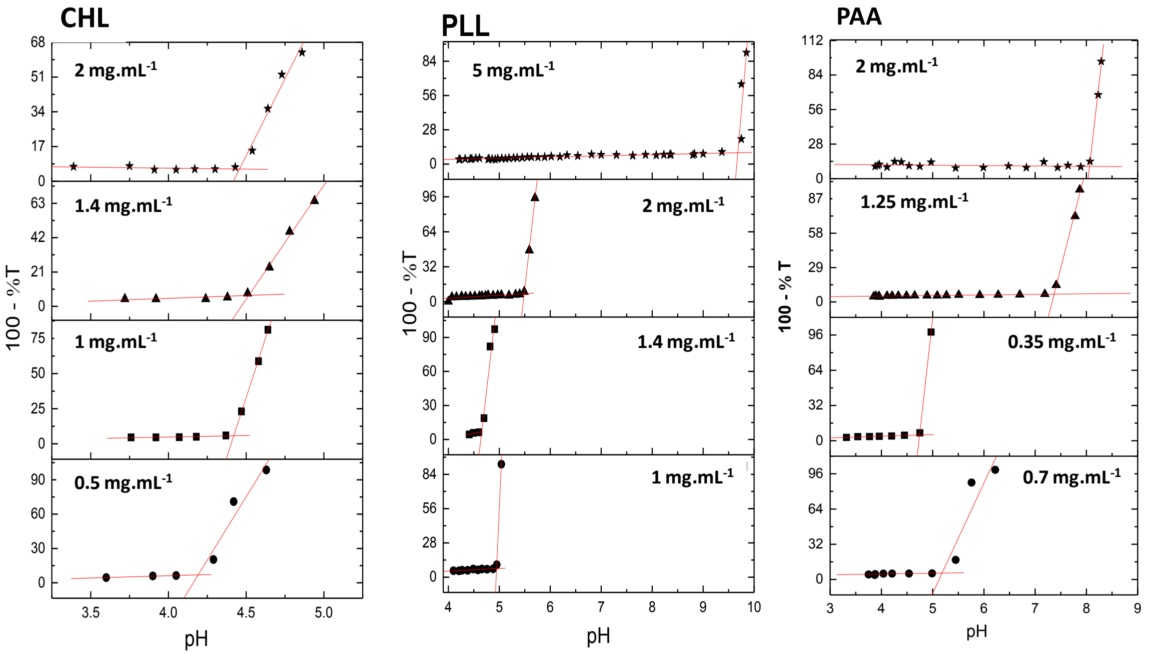


Figure S 6 - Determination of pHϕ as points as the intercept of the initial linear portion of the curve with the tangent to the rapidly increasing portion of the curve. [SL]= 5 mg/mL.

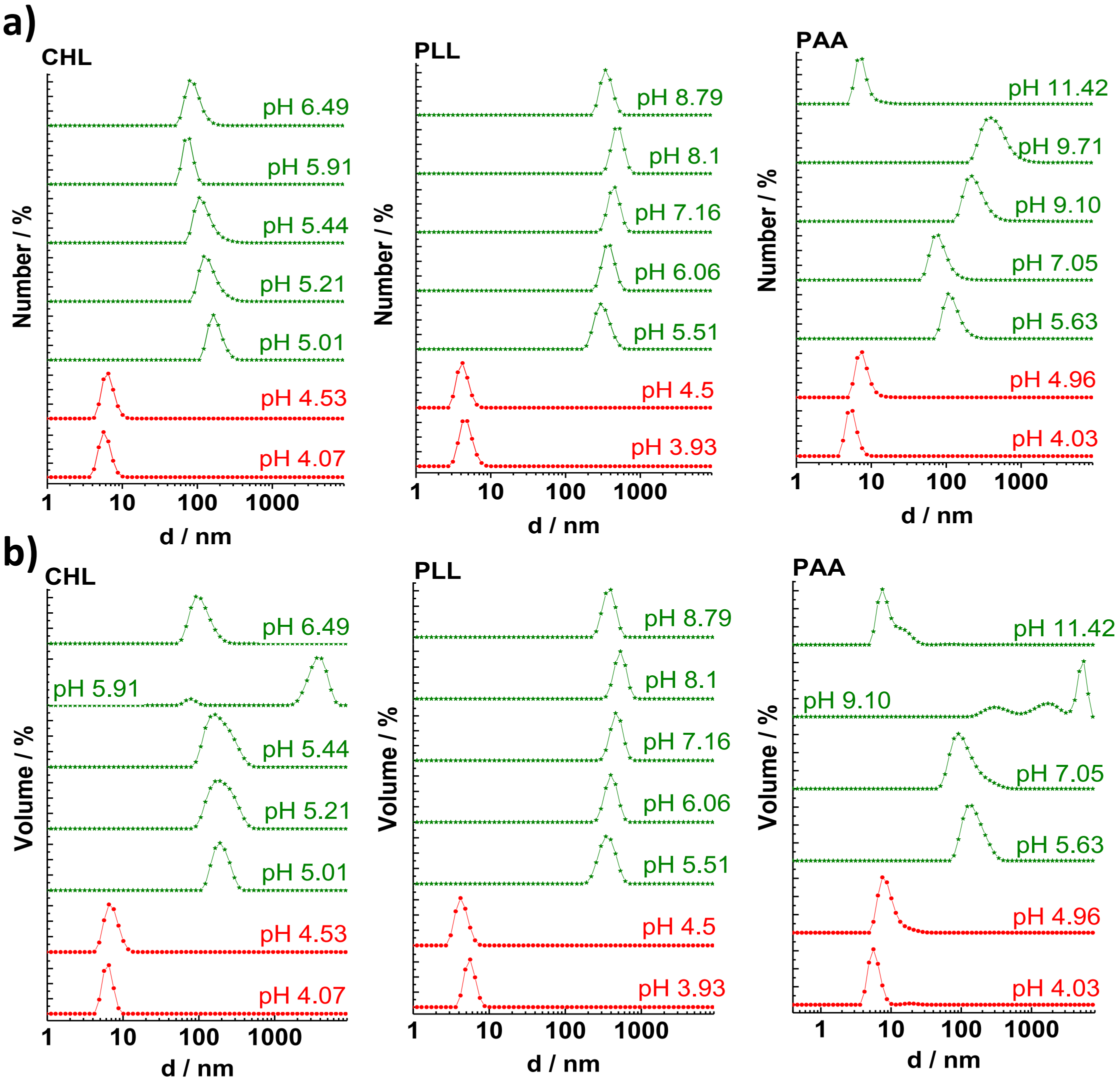


Figure S 7 - Evolution of the hydrodynamic diameter of SL- PEC complexes with pH, as function of the (a) relative number and (b) relative volume at 25 °C. The pH values are indicated above the curves. [SL]= 5 mg/mL, [CHL]= 1.4 mg/mL, [PLL]= 2 mg/mL, and [PAA]= 0.75 mg/mL

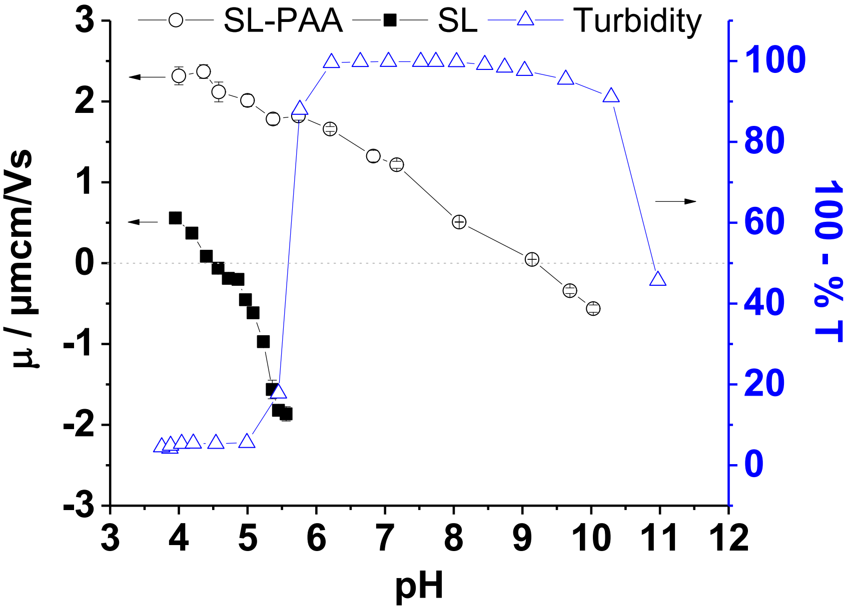


Figure S 8 - Evolution of turbidity and electrophoretic mobility as function of pH during complex coacervation of SL-PAA ([SL]= 5 mg/mL, [PAA]= 0.75 mg/mL).

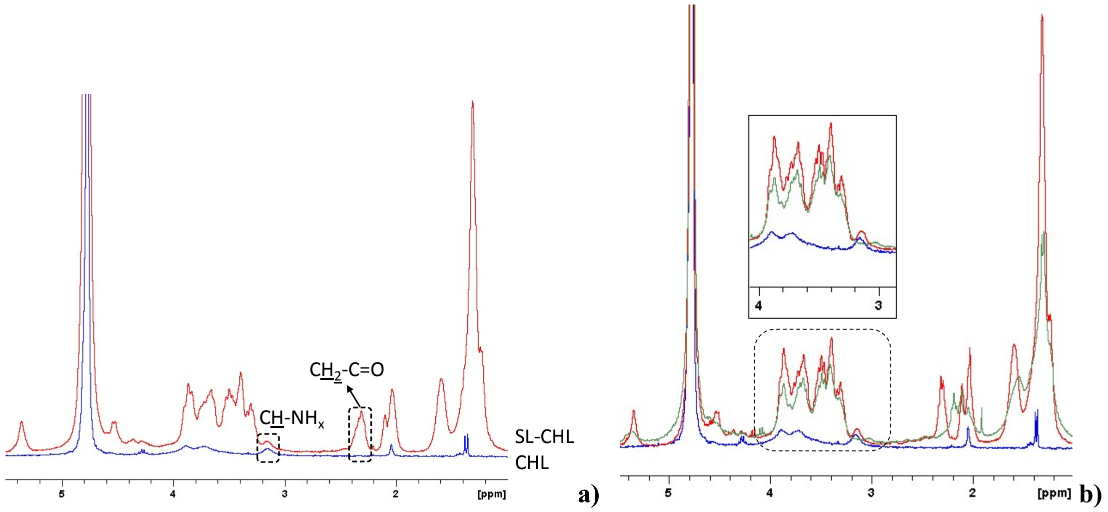


Figure S 9 – Quantification of the a) C=O/NH$_x$ molar ratio in the SL-polyelectrolyte mixture in the coacervation region and b) coacervation extent. Experiments are provided for the SL-CHL system. All experiments are carried out in D$_2$O. pH is adjusted with DCl and NaOD. The numerical data and relative discussion are provided in the main text.

a) ^{1}H NMR spectra of CHL solution (blue line, C= 1.4 mg/mL, pH 6.09). The red signal corresponds to a SL-CHL solution initially prepared in Region 3, on the coacervation plateau, (C_{SL}= 5 mg/mL, C_{CHL}= 1.4 mg/mL, pH 6.12) and eventually centrifuged (3000 rpm, 1h) to recover the coacervate phase only; the coacervate is finally dispersed in 500 µL D$_2$O, intentionally set at pD< 5, out of the coacervation plateau. The C$\underline{H}$-NH$_x$ and C$\underline{H_2}$-C=O integrals are used to quantify the C=O/NH$_x$ molar ratio. b) ^{1}H NMR spectra of SL-CHL solution (C_{SL}= 5 mg/mL, C_{CHL}= 1.4 mg/mL) before (red line, pH 4.46) and after (green line, pH 6.12) coacervation. In blue, the signal of CHL (C= 1.4 mg/mL, pH 6.09). The spectra are superimposed as such; no adjustment of the relative intensity is operated. The highlighted region between 4 ppm and 3 ppm is used to measure the intensity loss before and after coacervation.

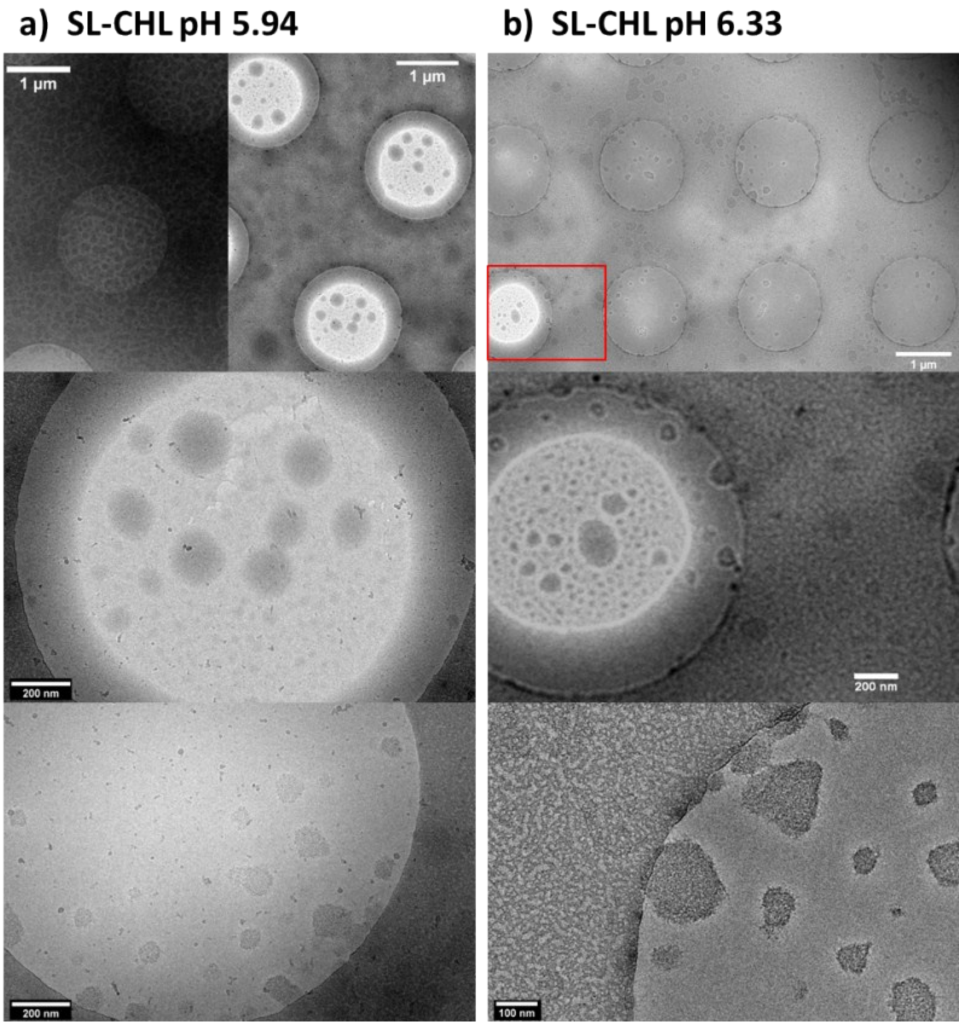
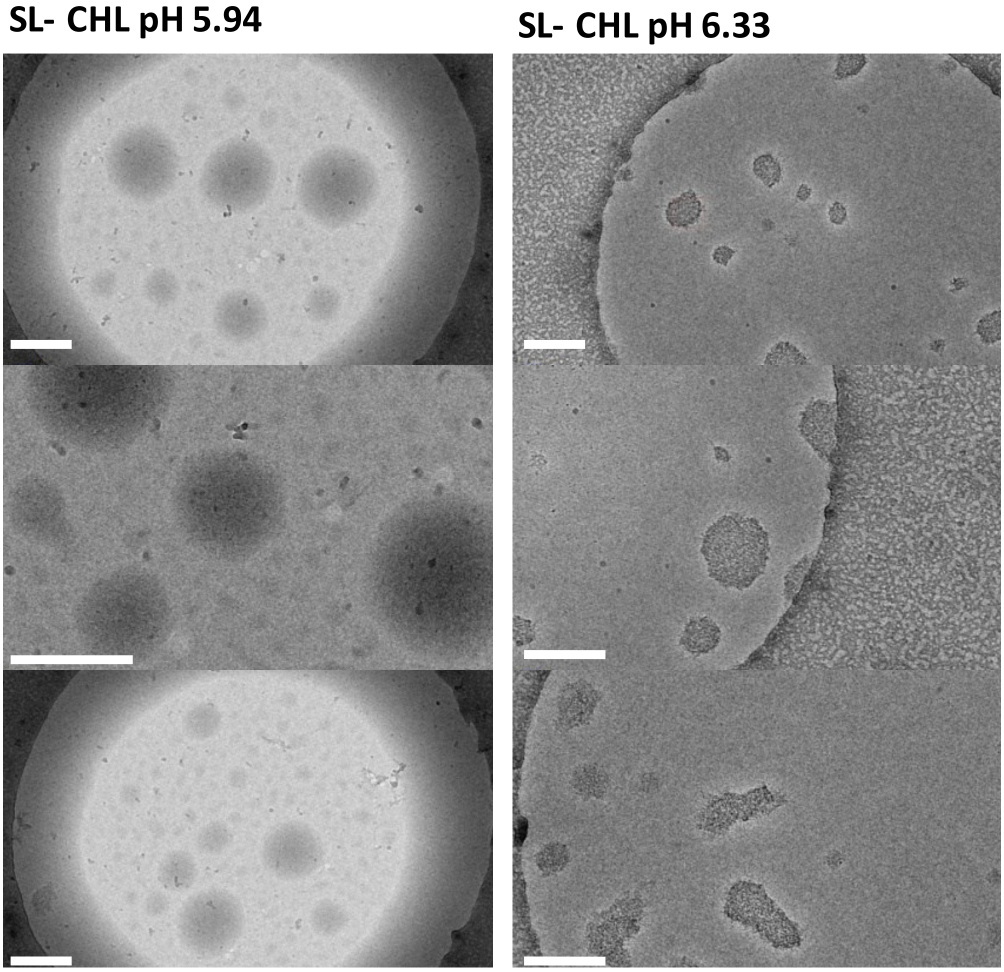


Figure S 10 - Cryo-TEM images of SL-CHL coacervates at pH 5.94 and pH 6.33 ([SL]= 5 mg/mL, [CHL]= 1.4 mg/mL).



SL- CHL pH 5.94

SL- CHL pH 6.33

Figure S 11 - Cryo-TEM images of SL-CHL coacervates ([SL]= 5 mg/mL, [CHL]= 1.4 mg/mL). Scale bar is 200 nm

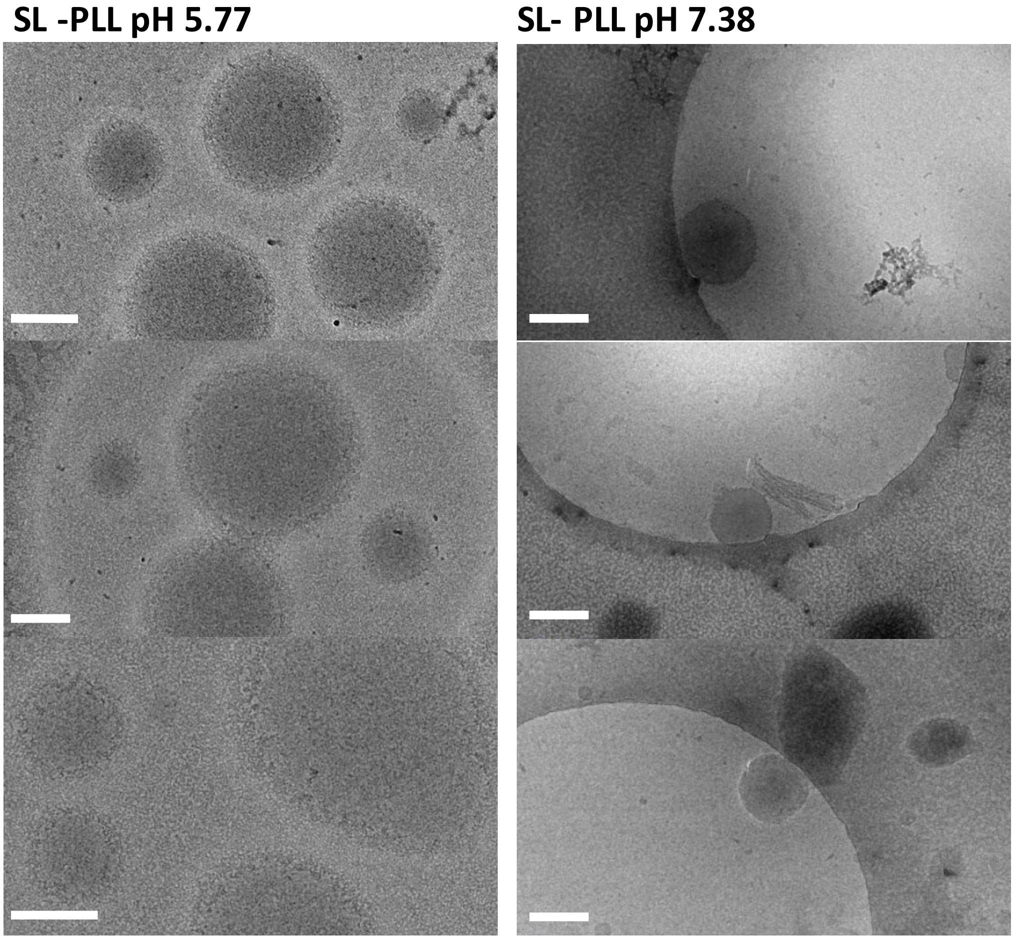


Figure S 12 - Cryo-TEM images of SL-PLL coacervates ([SL]= 5 mg/mL, [PLL]= 2 mg/mL). Scale bar is 200 nm

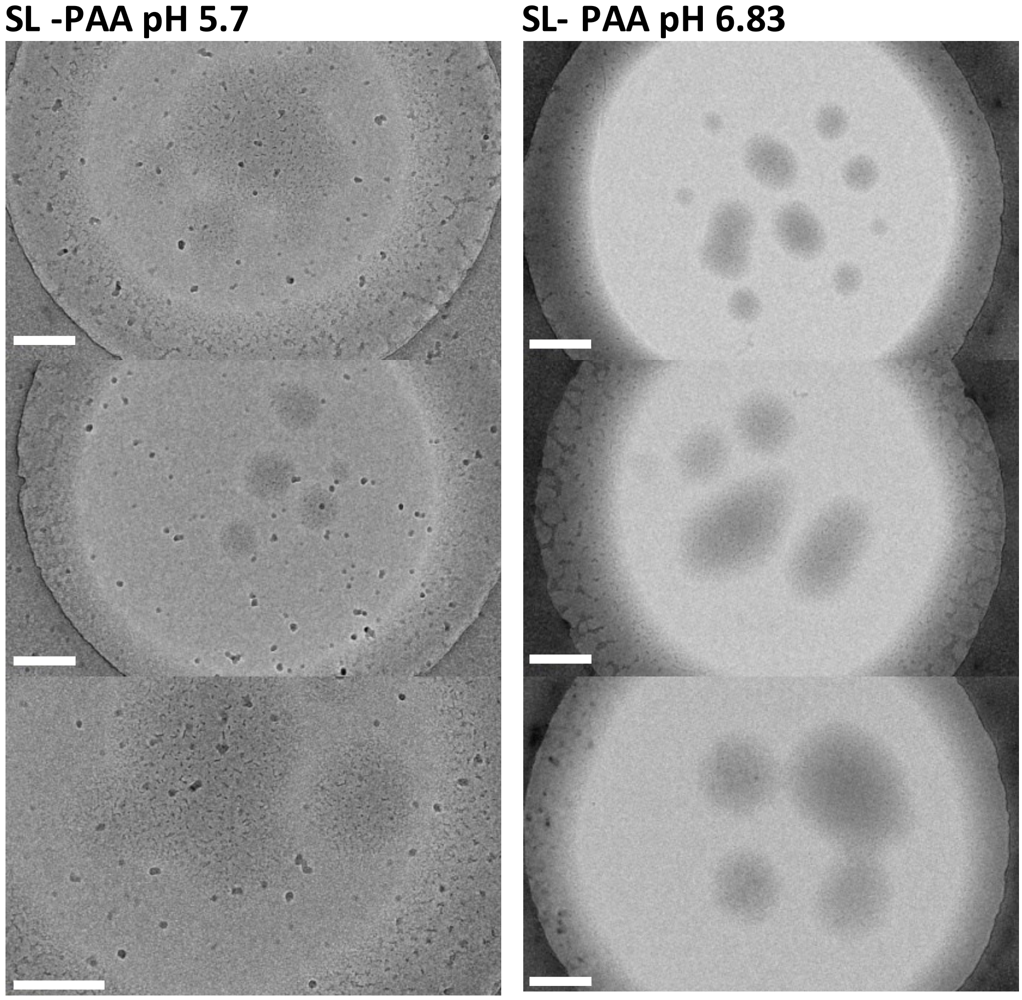


Figure S 13 - Cryo-TEM images of SL-PAA coacervates ([SL]= 5 mg/mL, [PAA]= 0.75 mg/mL). Scale bar is 200 nm