Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Temperature-dependent Reentrant Phase Transition of RNA-polycation Mixtures

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Supplementary Information

Materials and Methods

Materials: polyuridylic acid (poly(rU), MW 600-1000 kDa) was purchased from Sigma-Aldrich. Spermine tetrahydrochloride (MW 348.18 Da), Spermidine trihydrochloride (MW 254.63 Da), and $Lysine_4$ (Lys-Lys-Lys-Lys, MW 530.7 Da) were purchased from Sigma-Aldrich. $Lysine_{10}$ (PLKC10, MW 1600 Da) was purchased from Alamanda polymers. All polycations were dissolved in Nuclease-free water (Santa Cruz Biotechnology) and used without further purification. Concentrated stock solutions were split into aliquots and stored at -20 ^{o}C. Poly(rU) RNA was reconstituted in nuclease-free water and checked under the microscope to ensure the absence of any aggregates. The final poly(rU) stock concentration was calculated from the absorbance at 260 nm which was measured with a UV-Vis spectrophotometer (NanoDrop One^{C}). The stock solution was split into several aliquots and stored at -20 ^{o}C for later use.

RNA-polycation sample preparation: RNA-polycation condensates were prepared by mixing RNA [poly(rU)] and the polycation (Sp+3/Sp+4/K_4/K_{10}) in a buffer containing 25 mM Tris HCl (pH 7.5). The final concentrations of the RNA and the polycation are indicated in the corresponding figure legends. The order of addition for all the samples was Tris-HCl, salts, RNA, and polycation. Samples were thoroughly mixed and then placed on an 18 mm x 18 mm x 0.1 mm square coverslip. Next, the sample was sandwiched between the coverslip and a Tween20-coated (20% v/v) 75 mm x 25 mm x 1 mm glass slide with two strips of two-layer double-sided tape. Mineral oil was injected in the imaging chamber such that it surrounds the sample from all directions to prevent evaporation during cooling-heating cycles. A similar preparation procedure is performed for RNA-divalent cation samples with the same buffer containing 25 mM Tris-HCl (pH 7.5).

Temperature-composition phase diagram measurements: The samples were placed in a custom-built thermal stage (Instec; T_{range} = 4-95 ^{o}C) on a Zeiss primovert inverted microscope equipped with a Zeiss Axiocam 503 monochrome camera and a 40x objective. The temperature was measured using a thermocouple. To obtain the temperatures of formation (T_{phase}) and dissolution (T_{clear}), samples were heated from room temperature in steps of 10 degrees and an equilibration time of 3-5 minutes until a phase transition is observed (formation/dissolution of droplets). Next, the phase transition point is approached in steps of 5 degrees and subsequently in steps of 1 degree with a 5-minute equilibration time. During the heating and cooling cycles, several bright-field images were collected for visualization purposes. After locating the phase transition temperature, the temperature was cycled to observe the reversible formation and dissolution of the condensates. The T_{phase} was taken as the temperature at which condensates form when approached from the one-phase region in the phase diagram. The T_{clear} is the temperature at which condensates dissolve when approached from the two-phase region in the phase diagram. All phase diagram measurements were replicated three times over independent sample preparations. Phase separation temperatures are then measured for a series of polycation concentrations and plotted as a phase diagram using OriginPro software (v2021). For the variable temperature imaging series shown in Figure 4 of the main text, the samples containing Spermidine or Lys_{10} were heated and cooled across the entire experimental temperature range (4-90 ^{o}C) at different temperatures with an equilibration time of at least 3 minutes and imaged.

Supplementary Information

Supplementary Figures

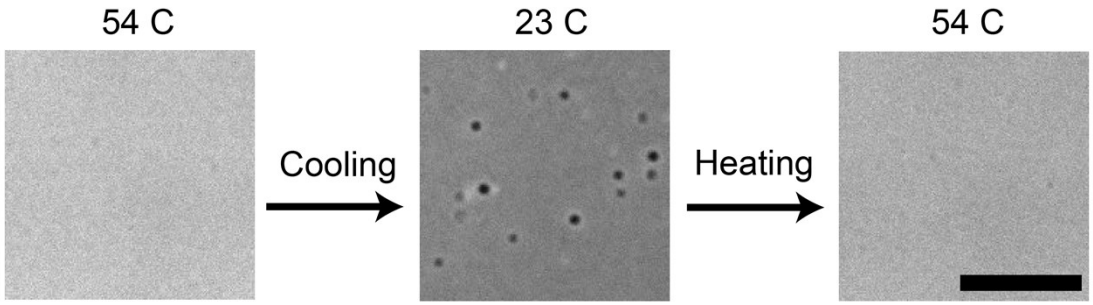

Figure S1. Bright-field microscopy images of poly(rU)-Mg^{+2} mixtures upon a cooling-heating cycle. The mixture is homogeneous at 54 ^{o}C. Cooling the sample to 23 ^{o}C leads to the formation of condensates and subsequent heating re-dissolves the condensates, indicating that phase separation is reversible upon thermal cycling. The poly(rU) concentration was 1.5 mg/ml and Mg^{+2} concentration was 350 mM in a 25 mM Tris-HCl (pH 7.5) buffer. Scale bar represents 10 μm.

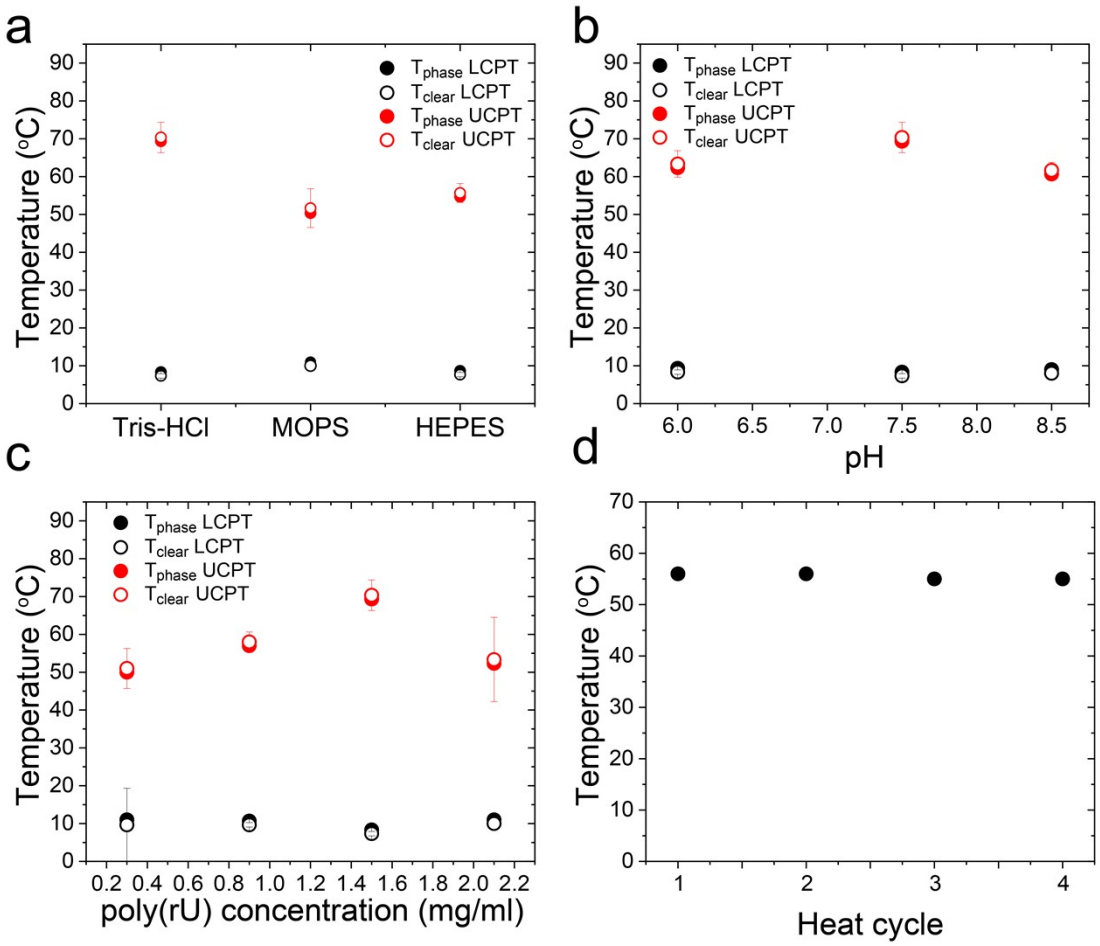

Figure S2. (a) Measurements of UCPTs and LCPTs of poly(rU)-Lys_4 mixtures with different buffers at pH 7.5. Note that both MOPS and HEPES buffer pH values are less dependent on temperature than Tris-HCl[1]. These data show that the temperature-controlled reentrant phase behavior is not dependent on the temperature-induced pH change of the buffer. The samples were prepared by mixing poly(rU) at a final concentration of 1.5 mg/ml with Lys_4 at a final concentration of 50 mM in a buffer containing 25 mM of either Tris-HCl (pH 7.5), MOPS (pH 7.5), or HEPES (pH 7.5). **(b)** A plot showing UCPT and LCPT for the same sample in (a) prepared in 25 mM Tris-HCl buffer of varied pH. **(c)** A plot showing the variation of UCPT and LCPT with total polymer concentration. The samples were prepared at a fixed mixture stoichiometry by mixing poly(rU) and Lys_4 at various total polymer concentrations in 25 mM Tris-HCl buffer (pH 7.5) containing 35 mM NaCl. Poly(rU) concentrations were set to 0.3, 0.9, 1.5, and 2.1 mg/ml. The corresponding Lys_4 concentrations were 5, 15, 25, and 35 mM, respectively. **(d)** A plot showing the upper cloud-point temperature of poly(rU)-Lys_4 mixture measured on the same sample during 4 consecutive heating and cooling cycles. The sample was heated to 75 °C, incubated for 5 minutes, and then cooled down slowly to obtain the phase separation temperature at each cycle. These data indicate that LLPS in this system is reversible without any thermal hysteresis and there are no significant effects of possible RNA degradation upon heating. The sample was prepared by mixing poly(rU) and Lys_4 at final concentrations of 1.5 mg/ml and 50 mM, respectively, in a 25 mM Tris-HCl buffer (pH 7.5).

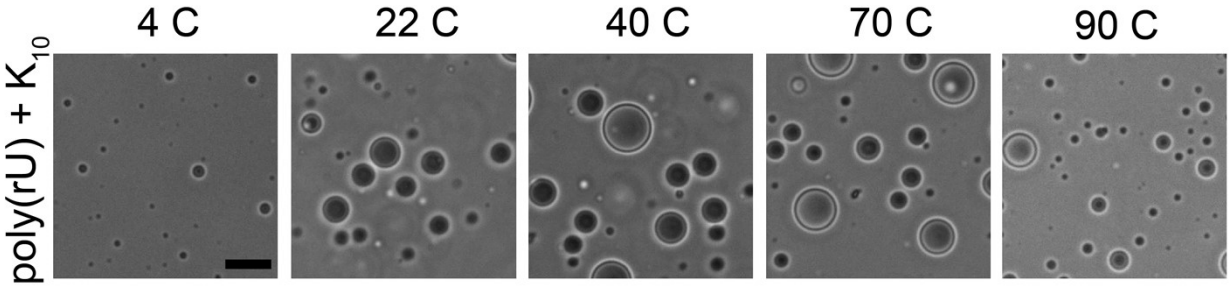

Figure S3. Bright-field images of poly(rU)-K_{10} mixtures at different temperatures. The droplet size decreases at the extreme hot and cold temperatures indicating the proximity of both LCST and UCST transitions. The poly(rU) concentration was 1.5 mg/ml and K_{10} concentration was 40 mM in a 25 mM Tris-HCl (pH 7.5) buffer. Scale bar represents 10 μm.

Supplementary Information

Supplementary Movie Legends

Movie S1. The emergence and disappearance of poly(rU)-Sp^{+4} condensates during a temperature sweep from 60 ^{o}C to 15 ^{o}C. The sample contains 1.5 mg/ml poly(rU) and 100 mM Sp^{+4} in a 25 mM Tris-HCl buffer (pH 7.5). The sample is kept at 60 ^{o}C initially. Upon cooling, the UCST is observed first at ~50 ^{o}C as evidenced by the formation of condensates. Upon further cooling, condensates initially grow and subsequently shrink and dissolve at 23 ^{o}C (LCST). The scale bar represents 10 μm.

Movie S2. The emergence and disappearance of poly(rU)-Lys_4 condensates during a temperature sweep from 50 ^{o}C to 5 ^{o}C. The sample contains 1.5 mg/ml poly(rU) and 60 mM Lys_4 in a 25 mM Tris-HCl buffer (pH 7.5). The sample is kept at 50 ^{o}C initially. Upon cooling, the UCST is observed first at ~44 ^{o}C as evidenced by the formation of condensates. Upon further cooling, condensates initially grow and subsequently shrink and dissolve at 9 ^{o}C (LCST). The scale bar represents 10 μm.

Supplementary references

1 Ellis, K. J. & Morrison, J. F. in *Methods in enzymology* Vol. 87 405-426 (Elsevier, 1982).