

Supplementary Information

Transient formation of multi-phase droplets caused by
the addition of a folded protein into complex coacervates with
an oppositely charged surface relative to the protein

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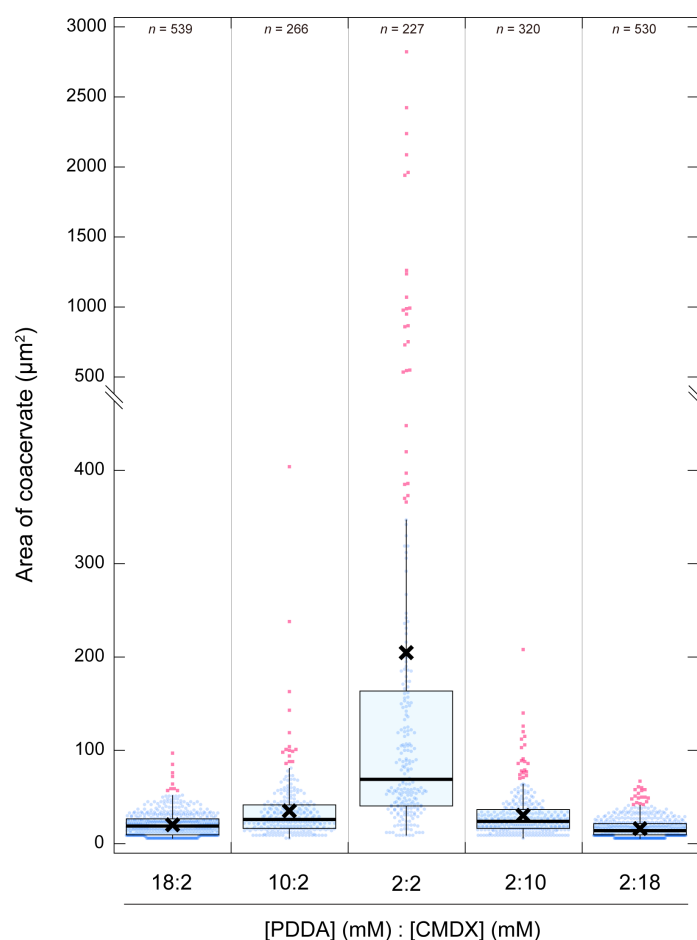


Fig. S1 Box plot representing the area of the coacervates with the mean (\times), median (horizontal bar), and outliers (dots) indicated. The areas of the PDDA/CMDX coacervates are calculated based on the microscopic images displayed in Fig. 2b. For Fig. 2b, the BF images were taken 1 h after all components were mixed. Although there were several large sized droplet outliers, as a rough trend, the droplet size tended to decrease as the PDDA:CMDX ratio deviated from stoichiometric conditions, i.e., [PDDA] (mM):[CMDX] (mM) = 2:2.

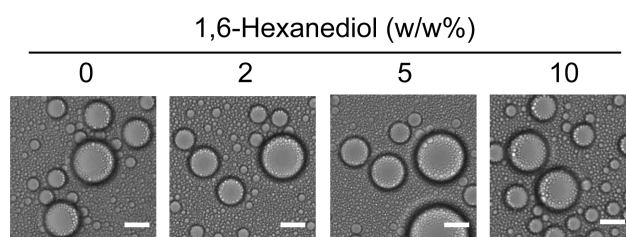
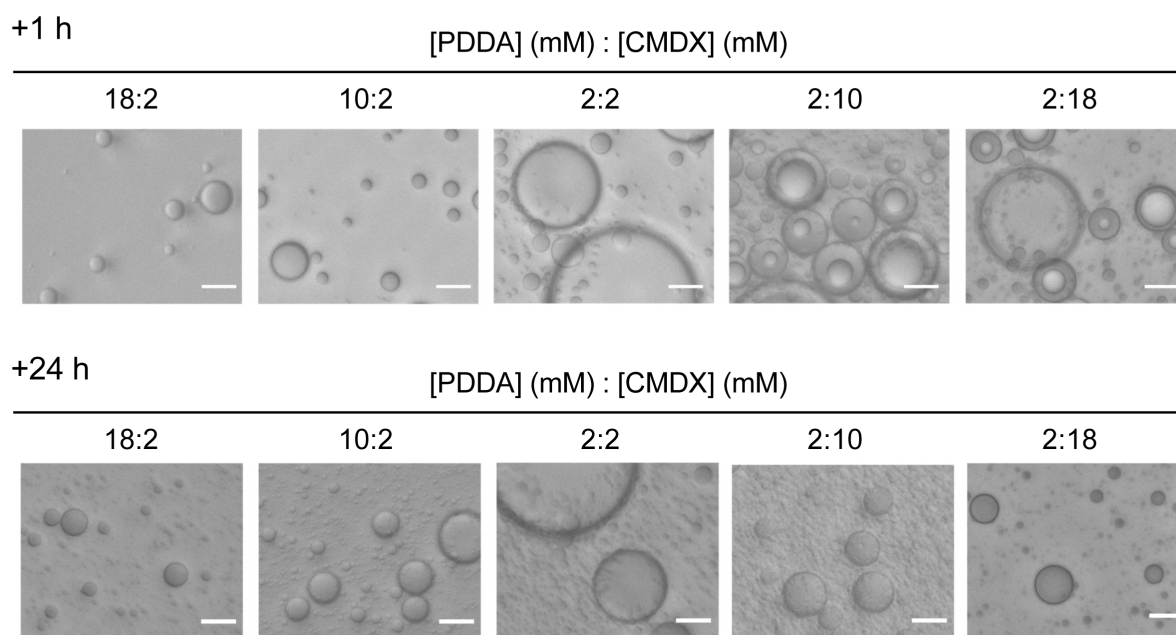


Fig. S2 Dissolution of the coacervates ([PDDA] (mM):[CMDX] (mM) = 2:2) following addition of 1,6-hexanediol (0–10 w/w%). Scale bar: 10 μ m. The BF images were taken 1 h after all components were mixed.

(a) LYZ



(b) OVA

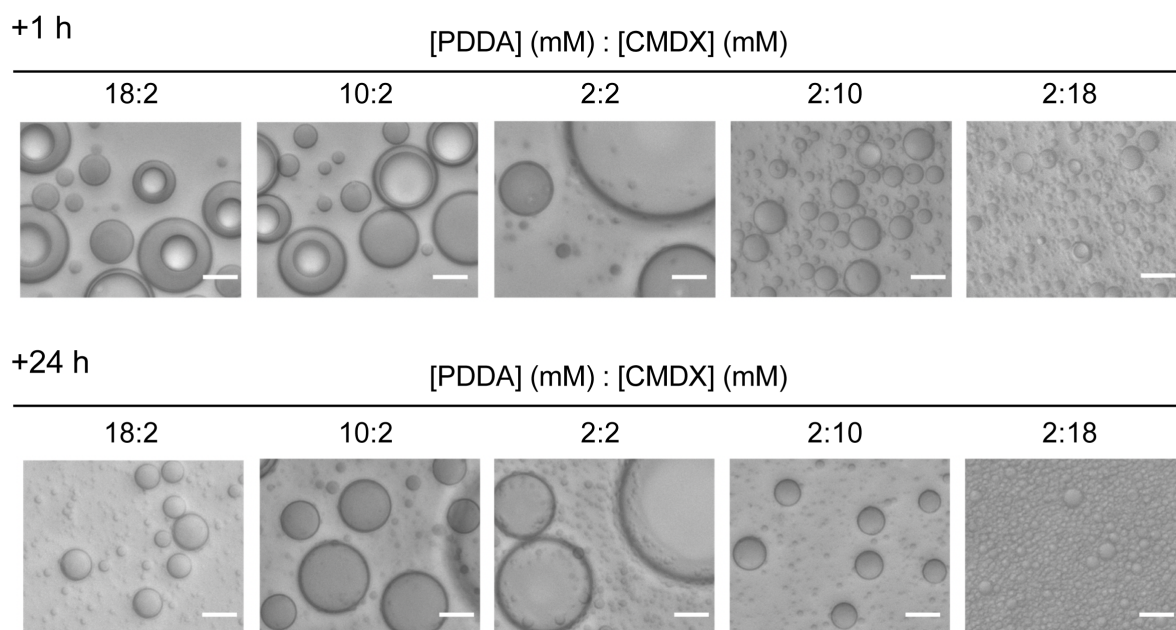


Fig. S3 Bright-field optical microscopy images taken 1 h and 24 h after addition of the protein ((a) LYZ and (b) OVA) to the PDDA/CMDX coacervates. Samples contain polyelectrolytes (PDDA and CMDX; 18, 10, or 2 mM) and protein (LYZ or OVA; 1 μ M). Scale bar: 10 μ m.

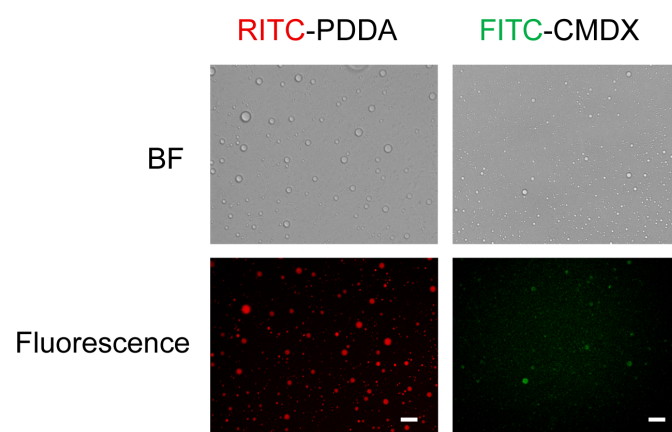


Fig. S4 Bright-field and fluorescence microscopy images of the PDDA/CMDX coacervates ($[PDDA]$ (mM): $[CMDX]$ (mM) = 10:2) with (a) 5% RITC-PDDA and (b) 5% FITC-CMDX. Scale bar: 10 μ m.

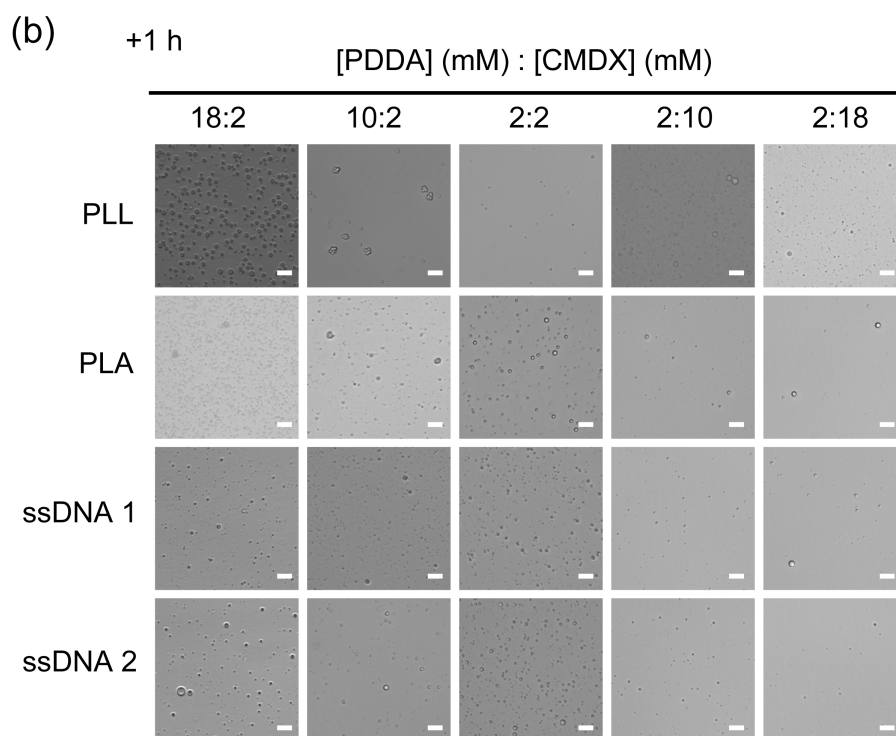
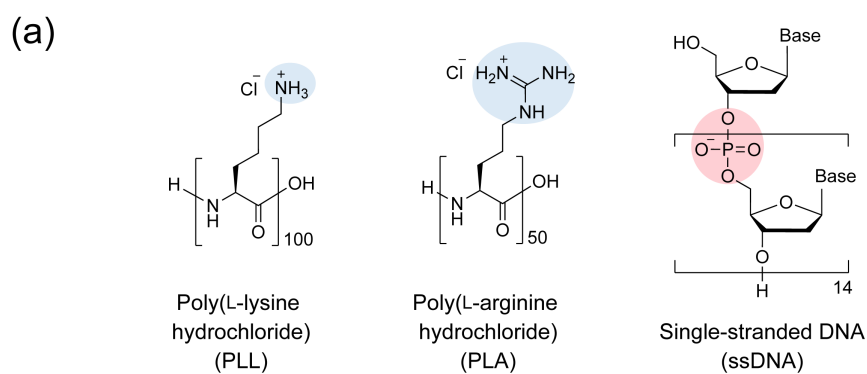


Fig. S5 (a) Chemical structures of unfolded polymers used as client molecules. (b) Bright-field images taken 1 h after the addition of client molecules (PLL, PLA, and ssDNAs) into PDDA/CMDX complex coacervates. Scale bar: 10 μ m.

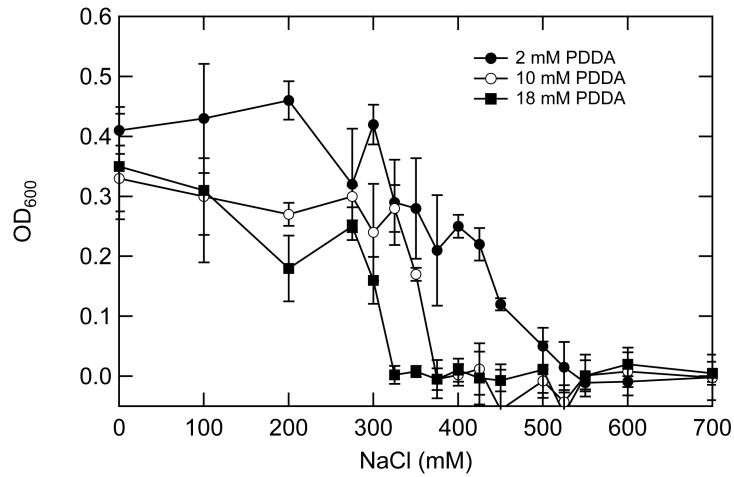
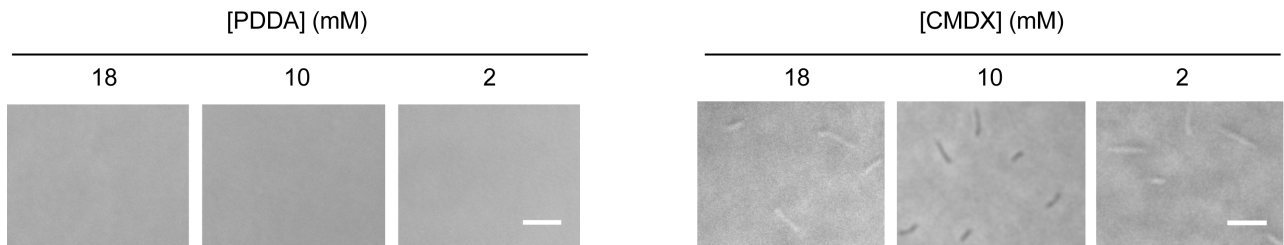


Fig. S6 Effect of adding NaCl to the PDDA/POX mixtures on the optical density at 600 nm (OD_{600}). Samples contain PDDA (18, 10, or 2 mM) and POX (1 μ M) in the presence of NaCl (0–700 mM).

(a) LYZ



(b) OVA

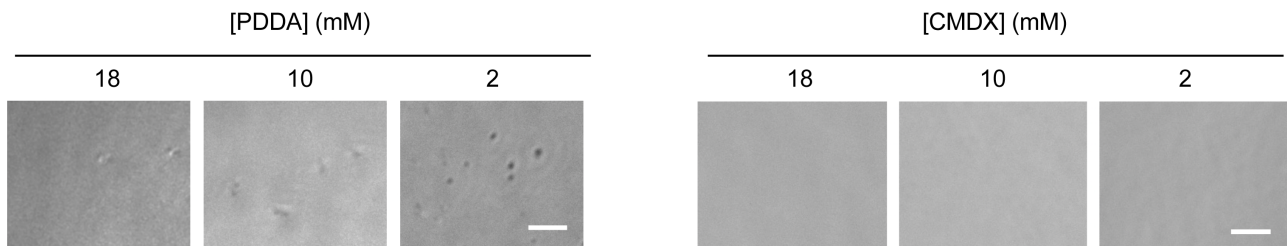


Fig. S7 Bright-field microscopy images of the mixtures of proteins (a) LYZ and (b) OVA with the polyelectrolytes PDDA and CMDX. Samples contain polyelectrolyte (PDDA or CMDX ; 18, 10, or 2 mM) and protein (LYZ or OVA; 1 μ M). Scale bar: 10 μ m.

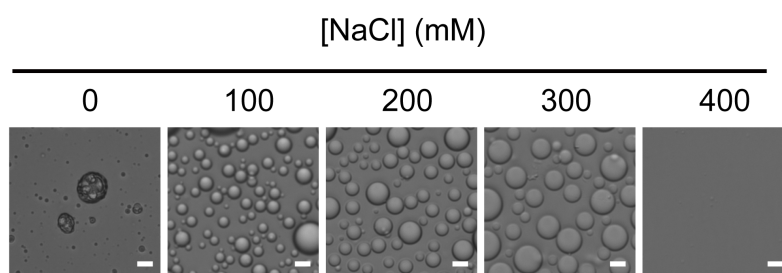


Fig. S8 Effect of ionic strength on the formation of the multi-phase droplets. Bright-field images taken 1 h after the addition of LYZ into PDDA/CMDX complex coacervates ([PDDA]:[CMDX] = 2:10) containing NaCl (0, 100, 200, 300, and 400 mM). Scale bar: 10 μ m.

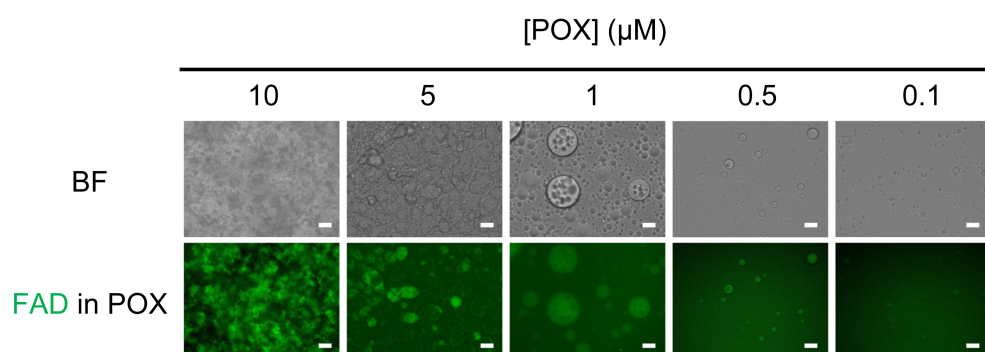


Fig. S9 Effect of the protein concentration on the formation of the multi-phase droplets. Bright-field and fluorescence images taken 1 h after the addition of POX into PDDA/CMDX complex coacervates ([PDDA]:[CMDX] = 10:2). Scale bar: 10 μ m.