Deciphering Electrostatic and Allosteric Determinants of Thermoresponsive Liquid–Liquid Phase Separation in Rabies Virus Phosphoprotein

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Liquid–liquid phase separation (LLPS) is the process by which membrane-less compartments form in biological systems. However, the molecular driving forces behind LLPS remain incompletely understood in these systems. The rabies virus phosphoprotein (P) is an intrinsically disordered scaffold protein of the viral factories that provides a compelling model for investigating how sequence patterning, conformational heterogeneity, electrostatics and multivalency interplay to control biomolecular condensation.

P forms star-shaped dimers comprising long negatively charged, flexible N-terminal arms and C-terminal globular domains characterised by a large dipole moment (~700 D). Using light scattering, small-angle X-ray scattering, analytical ultracentrifugation and calorimetry, we demonstrate that P exhibits thermoresponsive LLPS with a lower critical solution temperature (LCST), indicative of entropically driven condensation. Phase separation exhibits reentrant behaviour as a function of salt concentration or pH, revealing a delicate balance between electrostatic screening and dipolar attractions.

Our results show that self-association occurs below the macroscopic saturation concentration through conformation-specific interactions, highlighting the importance of pre-condensation molecular organisation. We propose a minimal three-state model, whereby the disordered N-terminal regions modulate the effective interactions between the anisotropic macrodipolar domains. This results in a narrow window of phase stability and a calorimetric apparent second-order transition.

Light and X-ray scattering at an absolute scale reveals that condensed droplets are 30 times more concentrated than the surrounding supernatant. We measured the surface tension of the condensates using atomic force microscopy and used a combination of high-sensitivity light scattering, the Gouy method and small-angle X-ray scattering (SAXS). We propose that dipole–dipole interactions, together with allosteric regulation of surface dipole accessibility, constitute subtle mechanisms underlying the in vitro formation of condensates, a process crucial for viral replication.

Keywords:
LLPS, LCST, rabies virus, viral factories

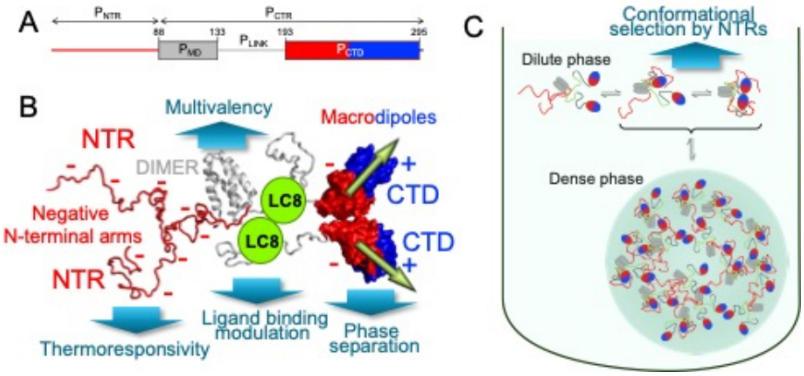

Figure | RABV P thermoresponsive phase separation. (A) Schematic representation of RABV P protein. Boxes = folded domains, Lines = IDRs. (B) Structural model of RABV P showing the roles of the different moieties in controling PS. (C) Illustration of the demixing into dilute and dense phases.

References:

Bouchama et al., Rabies Virus Phosphoprotein Exhibits Thermoresponsive Phase Separation with a Lower Critical Solution Temperature. J Mol Biol 437, 168889 (2025). DOI:10.1016/j.jmb.2024.168889