Hydrophobic Cohesion Overrides Charge Reversal in RNA–Cationic Surfactant Condensates

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The rational design of nucleic acid delivery systems requires a precise understanding of the driving forces behind polyelectrolyte–surfactant condensation. While these interactions are often attributed to simple electrostatic neutralization, this model fails to explain why condensates remain stable beyond the point of charge equivalence. In this study, we investigated the interactions between polyuridylic acid (PolyU) and a series of cationic alkyltrimethylammonium bromide surfactants using dynamic light scattering (DLS), zeta potential measurements, UV–Vis turbidimetry, and isothermal titration calorimetry (ITC). DLS and zeta potential data show a progressive increase in hydrodynamic diameter alongside the systematic neutralization of the PolyU backbone. Notably, charge reversal occurred beyond stoichiometric equivalence, yet UV–Vis turbidimetry confirmed that the condensates persisted deep into this overcharged regime. Resolubilization was only observed at significantly higher surfactant concentrations, suggesting that a non-Coulombic force maintains the integrity of the complex.
To quantify this behavior, ITC was conducted at 20, 30, and 37.5 °C. Fitting the calorimetric profiles to a multi-step binding model revealed two distinct regimes: an initial enthalpically-driven electrostatic phase and a subsequent cooperative, entropy-driven phase. This second phase is governed by hydrophobic tail aggregation along the RNA backbone. The extracted parameters (ΔG, ΔH, TΔS) indicate that entropic contributions, resulting from counterion release and alkyl chain dehydration, become increasingly dominant as chain length and temperature increase. These results show that hydrophobic cohesion, rather than electrostatic complementarity, is the primary force sustaining these complexes after charge reversal. This work provides a quantitative thermodynamic results for optimizing the stability of non-viral gene delivery vectors.

Keywords:
Isothermal titration calorimetry, titration, calorimetry, surfactant-polymer interactions, thermodynamics

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