**Tata Institute of Fundamental Research**
Survey No. 36/P, Gopanpally Village, Serilingampally, Ranga Reddy Dist., Hyderabad – 500 046

# Internal Webinar

## Trivalent Cation-Induced Protein Condensation Beyond Electrostatic Interactions: Insights from THz Spectroscopy and Other Biophysical Probes

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Trivalent metal ions are known to trigger liquid–liquid phase separation (LLPS) in negatively charged globular proteins such as bovine serum albumin (BSA), primarily through charge screening and subsequent aggregation. This phenomenon is particularly intriguing for exploring the potential universality of multivalent ion-induced phase behaviour. In this study, we used dynamic light scattering (DLS), optical microscopy, circular dichroism (CD), and terahertz Fourier-transform infrared (THz-FTIR) spectroscopy to investigate how mono-, di-, and trivalent ions influence the hydration and phase behaviour of BSA at room temperature and upon heating above its denaturation temperature. Our findings reveal that trivalent ions consistently induce LLPS without altering the protein's secondary structure, while monovalent and divalent ions promote gelation at elevated temperatures. Fluorescence anisotropy measurements using ANS as a probe show that LLPS phases retain internal mobility even at high temperatures, in contrast to the restricted dynamics seen in gelled systems. THz-FTIR spectroscopy (0.2–22.5 THz) provides unique insight into ion-specific hydration dynamics, which correlate strongly with the onset of LLPS. Additionally, we map the concentration-dependent phase diagrams for various lanthanide ions, emphasizing that hydration effects play a crucial role in the emergence of distinct phase regimes in protein solutions. Together, these findings highlight that trivalent cation-induced protein condensation involves mechanisms beyond simple electrostatic interactions, with hydration playing a pivotal and ion-specific role.

***Friday, May 30th 2025***

***14:30 Hrs***