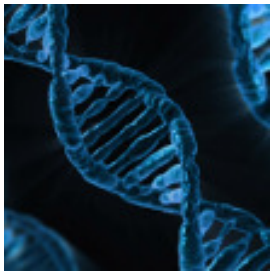

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Inducing protein reentrant condensation with polyvalent anions

Reentrant condensation (RC) describes the ability some polyvalent ions have to precipitate proteins at low concentrations and then resolubilise them at higher concentrations. Whether RC will be induced is dependent on protein net charge, ion net charge, ion concentration and ion species. Trivalent cations such as yttrium and lanthanum induce RC of acidic proteins and their mechanisms have been studied extensively. We use protein solubility, static light scattering, dynamic light scattering, and zeta potential measurements to understand the molecular mechanism for RC by polyvalent anions such as pyrophosphate (SPP) and tripolyphosphate (STPP), which have been studied to a smaller degree than the cations.

Basic proteins have a net positive charge and repel each other through long-range electrostatics when no counter anions are present. The initial lysozyme concentration (precipitation) occurs when the polyvalent anions STPP/SPP interact with solvent-exposed basic residues via electrostatics, which neutralize the long-range repulsive interactions between lysozyme molecules and allows protein cross-linking (precipitation) to take place. Further increasing SPP/STPP concentration after this causes resolubilization because lysozyme net charge has been reversed from positive to negative. While this mechanism follows what has been proposed for the behavior of acidic proteins with trivalent cations, there are also fundamental differences. Lysozyme precipitation occurs almost immediately when in the two-phase region possibly indicating a fundamental difference in the nature of the protein-protein interactions. Interestingly, citrate does not induce reentrant condensation of lysozyme at similar strengths of protein-protein attractions despite having a similar charge, size and shape to SPP; highlighting the importance of ion-specific effects.

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