Liquid-Liquid Phase Separation and Crystallization in Protein Solutions Induced by Multivalent Counterions

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We have studied the phase behavior of model globular protein in solution in the presence of multivalent counterions. It was shown that negatively charged globular proteins at neutral pH in the presence of multivalent counterions undergo a “reentrant condensation (RC)” phase behavior [1,2], i.e. a phase-separated regime occurs in between two critical salt concentrations, \( c^* < c^{**} \) (FIG.1), which can be a meta-stable liquid-liquid phase separation (LLPS). This reentrant phase behavior corresponds to an effective charge inversion of proteins as confirmed by zeta-potential measurements and supported by Monte Carlo simulations [2]. Crystallization from the condensed regime follows different mechanisms. Close to \( c^* \), crystals grow following a one-step procedure, i.e. nucleation and growth mechanism; close to \( c^{**} \), crystallization follows a two-step procedure, i.e., crystals growth follows a meta-stable LLPS. X-ray diffraction analyses on the high quality single crystals provide direct evidence of crystal structure and cation binding sites. Our finding of RC and LLPS induced by multivalent cations provides a new way to tune protein interactions with predictable phase behavior as well as controlled protein crystallization.

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FIG. 1. Phase diagram of BSA solution as a function of protein and salt concentration. The symbols indicate individual samples in the respective regimes. Both amorphous aggregates and dense liquid phases were observed in Regime II.