Non-Classical Pathway for Protein Crystallization Revealed by Time-Resolved SAXS

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Recent progress in protein and colloid crystallization as well as biomineralization has shown non-classical features in the early stage of nucleation [1-3]. While the classical nucleation theory predicts that the solute molecules reversibly aggregate in the supersaturated solution and form nuclei with the exact density and structure of the crystals in the final stage, the non-classical pathway suggests an intermediate phase (clusters or dense liquid phase) exists in between the initial solution and the final crystalline state [1-3]. The free energy landscape of the non-classical pathway show an additional free energy minimum corresponding to the intermediate phase. If the free energy of the intermediate phase is higher than that of the initial solution, it is unstable and the intermediate phase exists as mesoscopic clusters. If the free energy of the intermediate phase is lower than that of the initial solution, then the metastable phase can be a dense liquid phase [3].

Here we show that pre-assembled protein clusters formed via cation bridging can serve as a building block of crystallization. Globular proteins, human serum albumin and beta-lactoglobulin have been crystallized from solution in the presence of multivalent metal ions. These negatively charged globular proteins undergo a reentrant condensation phase behavior [4]. Crystallization near phase boundaries follows different mechanisms [5-7]. Time-resolved SAXS measurements demonstrate that protein clusters act as precursors of nucleation with a reduced energy barrier [7]. Crystallographic analysis provides direct evidence of the crystal structure and cation binding sites [5-7], which enhance interactions between protein contacts. The limited binding number (2 ~ 4) ensures the flexibility of proteins within clusters, which is crucial for the conformation relaxation during nucleation.
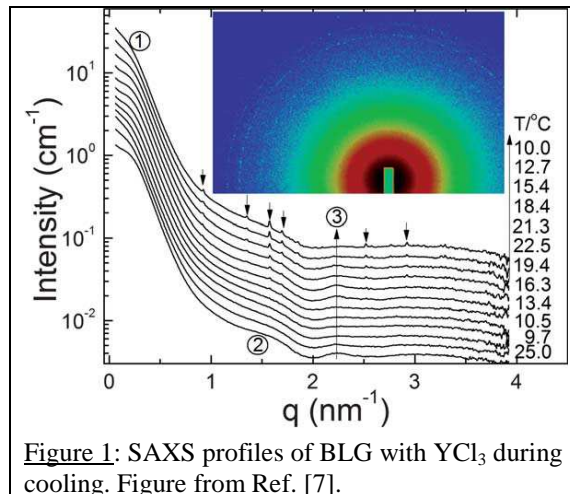

Figure 1: SAXS profiles of BLG with YCl$_3$ during cooling. Figure from Ref. [7].

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