yield are important in bulk biopharmaceutical crystallization processes which are not so significant for structural biology-related applications. Crystal size and packing quality (structural resolution) are important aspects in crystallization for structural elucidation.

The presence of impurities and related proteins and their influence on crystallization rate is an important consideration in bulk crystallization process development. Judge et al. describe the effect of protein impurities on lysozyme crystallization [25]. The effect of the protein impurities, avidin, ovalbumin, and conalbumin at concentrations up to 50%, on the solubility, crystal face growth rates, and crystal purity for lysozyme was investigated. The findings suggested that the presence of impurities had negligible effect on solubility and crystal purity (>99.99% pure crystals). However, the effect of the impurities on the face growth rates varied from none to a significant face-specific effect leading to growth cessation. These findings suggest the possibility of employing bulk crystallization as a unit operation (separation, polishing, and purification) in downstream processing of therapeutic proteins.

Process design, scale-up, and the design of industrial-scale protein crystallizer are important aspects for consideration in bulk protein crystallization. While industrial crystallizers for chemicals and small molecule drugs can employ methods including evaporation, cooling, precipitation, supercritical fluid-based crystallization, the choice becomes limited for proteins due to the macromolecular (and rather complex) nature, degradation, and potential stability issues with proteins. Hence, the choice of crystallization equipment and process design become extremely critical for bulk protein crystallization.

Gutka et al. describe the generation of larger hexagonal crystals of *Mtb* fructose-1,6-bisphosphatase enzyme by the traditional vapor diffusion method, by increasing the hanging drop volume from 2 µl to 4 and 6 µl (Fig. 4) [16]. It is important to note here that while larger crystals were easily obtained, the edges and surface properties of larger crystals were ill-defined (Fig. 4c).

While protein crystals for structural elucidation are traditionally obtained using vapor diffusion methodology, screening for large-scale protein crystallization development is often performed in batch crystallization mode, because of its inherent advantages. In fact, batch crystallization is now being widely accepted as a robust protein crystallization tool in the structural biology community as well [46]. While batch crystallization has its process advantages, successful transfer of an existing vapor diffusion-based crystallization method to microbatch and then its scale-up to process level remains a challenging task. Hekmat and coworkers describe the successful transfer of vapor diffusion-based crystallization to agitated batch crystallization at milliliter scale using lysozyme as a model protein [19]. The same research group also describes a stirred-tank crystallization-based approach [53] towards successful scale-up of batch crystallization conditions using lysozyme, lipase [18], and therapeutic mAbs [17, 52] as model proteins. Smejkal et al. describe a maximum local energy dissipation-based scale-up approach for stirred crystallization using lysozyme and Canakinumab Fab-fragment as model proteins. Faster onset of crystallization was observed for both lysozyme (ten times faster) and Cankinumab Fab-fragment (four times faster) under stirred crystallization conditions at 6 ml and 1 l