is an attractive option in biopharmaceutical processing, it requires elaborate studies and data-driven development approaches including but not limited to scale-down models, transportation validation, experimental verification of controlled and uncontrolled freeze-thawing processes [27–29]. Moreover, since the process involves volumes of frozen liquid, it presents a potential logistics and business limitation. Recently, there has been a focus on several drying technologies (including lyophilization) for protein therapeutics; each of these drying methods can have a significant impact on product quality and should be rationally selected [1]. Spray-drying and supercritical fluid-drying are two such emerging techniques besides freeze-drying with specific applications in protein biopharmaceutical development [21–23, 33, 48]. Bowen et al. describe a bulk drug substance storage approach based on spray-drying of mAbs [5]. The findings suggested that the physical stability of the spray-dried mAbs was comparable or greater than that of corresponding freeze-dried samples. Finally, extensive characterization of the spray-dried mAbs with regards to reconstitution time and reconstituted solution properties suggested that the said process was feasible for powder-based biologic bulk storage applications of mAbs.

Bulk freeze-drying has been successfully adapted for pharmaceutical processing of small molecules particularly cytotoxic anticancer drugs and antibiotics [4]. Insulin is a classic biopharmaceutical wherein the drug substance is processed as a freeze-dried solid. This chapter discusses the potential of bulk crystallization and bulk freeze-drying applied in succession as final polishing steps in typical biopharmaceutical manufacturing processes.

Protein Crystallography: Principle, History and Applications in Drug Development

Principle underlying protein crystallography can be understood by carefully evaluating the protein phase diagram. Figure 2 describes the schematic illustration of a typical protein crystallization phase diagram formed as a result of varying protein concentration and varying adjustable parameters typically precipitant or additive concentration, pH, and temperature [9]. Protein crystals are formed in the metastable zone which can be achieved by varying both the protein concentration and one or more of the adjustable parameters. The underlying principle of macromolecular crystallization and approaches to protein crystallization have been reviewed by McPherson [35, 36, 37].

The successful application of crystallography in structural elucidation of proteins dates back in to the late 1950s with the first-reported structure of sperm whale myoglobin by Sir John Cowdery Kendrew and Max Perutz, for which they shared the Nobel Prize in Chemistry in 1962 [26]. In the early 1990s, the National Institute of Health, through the Protein Structure Initiative (PSI), funded several centers for high-throughput structure determination of proteins [40]. Large-scale protein purification, crystallization and X-ray crystallography formed the core of such a collaborative effort which provided novel structural information on proteins of