The downstream processing of proteins consists of purification and formulation, which has a significant contribution to the overall production cost (*e.g.* nearly 70% in the case of monoclonal antibody (mAb)).[15] The purification stage starts with the removal of particulates such as cell debris by centrifugation or filtration, and proceeds to the further purification of the protein by chromatography and filtration.[16–18] Although chromatography can achieve high protein purity, scaling up chromatography is significantly more costly than other purification methods such as filtration and crystallisation due to the high cost of packing material and buffer. For instance, mAb is industrially purified by protein-A chromatography, whose resin costs €5000 to €14000 per litre.[19] As a result, protein crystallisation has become a topic of interest for the large-scale bioseparation of proteins, in order to selectively isolate certain proteins from mixtures.[20,21]

Building on the crystallisation theories discussed in the previous chapters of this book as well as existing reviews and books on protein crystallisation,[20,22–30] this chapter aims to offer a process development perspective on continuous protein crystallisation. The general protein crystallisation process is briefly discussed to establish the background knowledge for a more detailed review of the current developments in this field.

10.2 Protein Crystals

Since the reported crystallisation of haemoglobin in 1840,[31] the general principles for protein crystallisation are still not fully understood after nearly two centuries of intensive research.[20,26] The comprehensive characterisation of protein crystals can provide useful insight into the crystallisation mechanisms, but it is often difficult to grow crystals beyond micrometre size for accurate structure determination.[27]

Similar to small-molecule pharmaceutical compounds, proteins are more stable in the crystalline form.[32–36] However, the crystallisation process is much more complicated for proteins due to their complex three-dimensional configurations.[37–42] In order for a protein to crystallise, the protein needs to remain in the active form, whose three-dimensional structure allows the protein to perform the intended biological functions. The hydrogen bonds and hydrophobic interactions among protein crystals are much weaker in comparison to crystalline small molecules due to the large amount of incorporated water molecules, which can account for 51 ± 14% of the total crystal volume.[25,43–48] The resulting flexibility of protein molecules leads to the variance of the final crystalline state, making the nucleation during the protein crystallisation process much more difficult to predict than for small molecules.