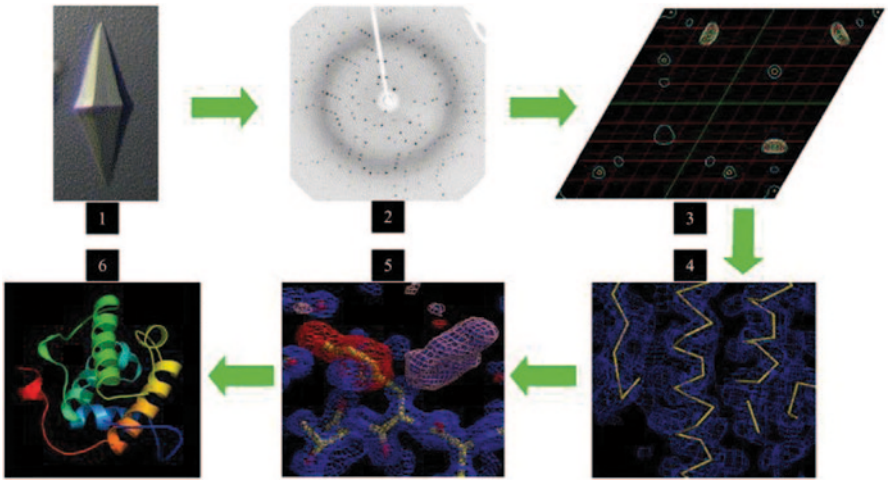

Fig. 3 Process of protein structure determination by single crystal diffraction and exploitation of the anomalous scattering phenomenon. A set of oscillation images (*2*) is obtained by exposing single crystals (*1*) of the protein under investigation to a beam of X-rays. The processed data are then used to "solve" the structure. Shown here is a Patterson map (*3*) (generated with the program XPREP) as used in the determination of a heavy atom substructure. Once the substructure is known, phases are calculated and refined. Phases and diffraction data permit the calculation of electron density maps and the generation of an initial trace of the model (*4*) (screenshot from XFIT). Multiple cycles of validation, model refitting and refinement against the diffraction data (*5*) (XFIT), and geometric restraints produce a model of the protein (*6*) (cartoon generated with PYMOL) for release to the structural biology community (Adapted with permission from [44])

Bulk Protein Crystallization (Process Development, Advantages, and Significance in Biopharmaceutical Development Process)

As discussed in the previous section, protein crystallography as a technique has made a significant contribution to drug discovery, design, and development. However, most of the applications have been in protein crystallography for structural biology and small molecule drug discovery and not in bulk crystallization for purification or polishing of proteins. While bulk crystallization is a popular approach applied in separation and purification of small molecule drugs and fine chemicals, its applications with proteins are limited to a handful of biopharmaceuticals such as insulin, lyzosyme, aprotinin, certain commercial enzymes, and some monoclonal antibodies. Peters et al. describe important criteria that distinguish bulk protein crystallization (a purification and polishing step for proteins) from traditional protein crystallization used for structure determination (Table 1) [42]. Among the several listed criteria, the precipitant or excipients used in crystallization are limited in bulk biopharmaceutical processes, since they have to be pharmaceutical grade, nontoxic, generally regarded as safe (GRAS), easily sourced, and inexpensive. Some aspects such as scalability, process compatibility, redissolution, and crystallization