3.4.2.1 Nature of protein products and related scientific considerations Unlike small-molecule drugs, whose structure can usually be completely defined and entirely reproduced, proteins are typically more complex and are unlikely to be shown to be structurally identical to a reference product. Many potential differences in the protein structure can arise. Because even minor structural differences (including certain changes in glycosylation patterns) can significantly affect a protein's safety and/or effectiveness, it is important to evaluate these differences.

In general, proteins can differ in at least three ways: (a) primary amino acid sequence; (b) modification of amino acids, such as sugar moieties (glycosylation) or other side chains; and (c) HOS (protein folding and protein–protein interactions). Modifications to amino acids may lead to heterogeneity and can be difficult to control. Protein modifications and HOS can be affected by formulation and environmental conditions, including light, temperature, moisture, packaging materials, container closure systems, and delivery device materials. Additionally, process-related impurities as well as product-related ones may increase the likelihood and/or the severity of an immune response to a protein product, and certain excipients may limit the ability to characterize the protein product.

Advances in analytical sciences enable some protein products to be extensively characterized with respect to their physicochemical and biological properties, such as HOSs and functional characteristics. These analytical methodologies have increasingly improved the ability to identify and characterize not only the drug substance of a protein product but also the excipients and product- and process-related impurities.

Despite such significant improvements in analytical techniques, however, the current analytical methodology may not be able to detect all relevant structural and functional differences between two protein products. In addition, there may be an incomplete understanding of the relationship between a product's structural attributes and its clinical performance. Thus, as set forth in the PHSA, data derived from analytical studies, animal studies, and a clinical study or studies are required to demonstrate biosimilarity unless the FDA determines an element unnecessary.

3.4.2.2 Manufacturing process considerations Different manufacturing processes may alter a protein product in a way that could affect the safety or the effectiveness of the product. For example, differences in biological systems used to manufacture a protein product may cause different PTMs, which in turn may affect the safety and/or the effectiveness of the product. Thus, when the manufacturing process for a marketed protein product is changed, the application holder must assess the effects of the change and demonstrate—through appropriate analytical testing, functional assays, and/or in some cases animal and/or clinical studies—that the change does not have an adverse effect on the identity, the