kinetics) should be performed to evaluate whether the proposed product and the reference product are highly similar. A meaningful assessment as to whether the proposed product is highly similar to the reference product depends on, among other things, the capabilities of available state-of-the-art analytical assays to assess, for example, the molecular weight of the protein, the complexity of the protein (HOS and PTMs), the degree of heterogeneity, the functional properties, the impurity profiles, and the degradation profiles denoting stability. The capability of the methods used in these analytical assessments, as well as their limitations, should be described by the sponsor. Physicochemical and functional characterization studies should be sufficient to establish relevant quality attributes including those that define a product's identity, quantity, safety, purity, and potency. The product-related impurities, product-related substances, and process-related impurities should be identified, characterized as appropriate, quantified, and compared with multiple lots of the proposed product to multiple lots of the reference product, to the extent feasible and relevant, as part of an assessment of the potential impact on the safety, the purity, and the potency of the product.

The primary structure of some protein products can be highly heterogeneous, which could affect the expected clinical performance of a protein product. Protein heterogeneity may arise in a number of ways. Replication errors in the DNA encoding the protein sequence and amino acid misincorporation may occur during translation, although the level of these errors is typically small. In addition, most protein products undergo some PTM that can alter the functions of the protein by attaching other biochemical groups such as phosphate and various lipids and carbohydrates; by proteolytic cleavage following translation; by changing the chemical nature of an amino acid (e.g., formylation); or by many other mechanisms. Such modifications can result from intracellular activities during cell culture or by deliberate modification of the protein, for example, PEGylation. Other PTMs can be a consequence of manufacturing process operations; for instance, glycation may occur with exposure to the product to reducing sugars. Also, storage conditions may be permissive for certain degradation pathways such as oxidation, deamidation, or aggregation. All of these product-related variants may alter the biological properties of the expressed recombinant protein. Therefore, identification and determination of the relative levels of these protein variants should be included in the comparative analytical characterization studies.

The 3D conformation of a protein is a major factor in its biological function. Proteins generally exhibit complex 3D conformations (tertiary structure and, in some cases, quaternary structure) because of their large size and the rotational characteristics of protein alpha carbons. The resulting flexibility enables dynamic, but subtle, changes in protein conformation over time, some of which may be required for functional