activity. These rotations are often dependent on low energy interactions, such as hydrogen bonds and van der Waals forces, which may be very sensitive to environmental conditions. Current analytical technology is capable of evaluating the 3D structure of many proteins. Using multiple, relevant, state-of-the-art methods can help define protein tertiary structure and, to varying extents, quaternary structure and can add to the body of information supporting biosimilarity. At the same time, a protein's 3D conformation can often be difficult to precisely define using current physicochemical analytical technology. Any differences HOS between a proposed product and a reference product should be evaluated in terms of a potential effect on protein function and stability. Thus, functional assays are also critical tools for assessing the integrity of the HOSs.

A scientifically sound characterization that provides a comprehensive understanding of the chemical, physical, and biological characteristics of the proposed product is essential to the design of the manufacturing process and to the conduct of development studies. The body of knowledge that emerges will serve to support a demonstration of product quality and the effectiveness of a suitable control system during development and approval of the product.

Manufacturers should perform in-depth chemical, physical, and bioactivity comparisons with side-by-side analyses of an appropriate number of lots of the proposed product and the reference product and, where available and adequate, a comparison with a reference standard for suitable attributes (e.g., potency). The evaluation of multiple lots of a reference product and multiple lots of a proposed product enables estimation of product variability across lots. The number of lots needed to understand and estimate the lot-to-lot variability of both the reference and proposed products may differ on a case-by-case basis, and should be scientifically justified by the sponsor. The FDA encourages sponsors to consult to ensure that an appropriate number of lots are evaluated. Identification of specific lots of a reference product used in analytical similarity studies, together with expiration dates and time frames and when the lots were analyzed and used in other types of studies, should be provided. This information will be useful in justifying acceptance criteria to ensure product consistency, in addition to assessing similarity. However, the acceptance criteria should be based on the totality of the analytical data and not simply on the observed range of product attributes of the reference product. This is because some product attributes act in combination to affect a product's safety, purity, and potency profile; therefore, their potential interaction should be considered when evaluating similarity and setting specifications. For example, for some glycoproteins, the content and the distribution of tetra-antennary and *N*-acetyl-lactosamine repeats can affect in vivo potency and should not be evaluated independently of each other. Additionally, the data obtained from lots used in nonclinical and clinical studies and relevant information on the relationship between