Sponsors should use appropriate analytical methodologies with adequate sensitivity and specificity for structural characterization of the proteins. Generally, such tests include the following comparisons of the proposed product and the reference product:

- Primary structures, such as amino acid sequence
- HOSs, including secondary, tertiary, and quaternary structures (including aggregation)
- Enzymatic PTMs, such as glycosylation and phosphorylation
- Other potential variations, such as protein deamidation and oxidation
- Intentional chemical modifications, such as PEGylation sites and characteristics

Sponsors should conduct extensive structural characterizations of both the proposed product and the reference product in multiple representative lots to understand the lot-to-lot variability of both products in the manufacturing processes. Lots used for the analyses should support the biosimilarity of both the clinical material used in the clinical study intended to support a demonstration of biosimilarity and the to-be-marketed proposed product to the reference product. Characterization of lots manufactured during process development of the proposed product may also be useful. Sponsors should justify the selection of the representative lots, including the number of lots.

In addition, the FDA recommends that sponsors analyze the finished dosage form of multiple lots of the proposed product and the reference product, assessing excipients and any formulation effect on purity, product- and process-related impurities, and stability. Differences in the formulation between the proposed product and the reference product are among the factors that may affect the extent and the nature of subsequent animal or clinical testing. A sponsor considering manufacturing changes after completing the initial analytical similarity assessment or after completing clinical testing intended to support a 351(k) application should perform an additional analytical similarity assessment with lots manufactured by the new process and the reference product and establish comparability of the proposed product manufactured by the old and new manufacturing processes. The nature and the extent of the changes may determine the scope of the analytical similarity and comparability studies and any necessary additional studies.

If the reference product or the proposed product cannot be adequately characterized by state-of-the-art technology, the application for the proposed product may not be appropriate for submission under Section 351(k) of the PHSA, and the sponsor should consult the FDA for guidance on the proper submission pathway.

3.4.5.2 Functional assays The pharmacologic activity of protein products should be evaluated by in vitro and/or in vivo functional assays.