asparagine residues to generate *N*-linked glycoproteins or to the hydroxyl groups of serine, threonine, or tyrosine to generate *O*-linked glycoforms. The *N*-linked repertoire contains >500 different oligosaccharide structures that may be differentially attached at multiple glycosylation sites to generate >1000 different types of glycan; a consequence of the activities of >250 glycosyltranferases (Marth and Grewal, 2008; NCI, 2016; Stanley, 2011; Zhong and Wright, 2013).

The potential for complexity/heterogeneity can be illustrated for mAbs. The full-length sequence of an IgG molecule includes ~40 asparagine/glutamine residues; therefore, random deamidation of one of these residues will generate 40 structural variants (isoforms); deamidation of two residues may generate $40 \times 40 = 1560$ variants; three: $40 \times 1560 = 59{,}280$ variants, and so on. When all possible overt PTMs (only) and combinations of PTMs are considered it has been calculated that a full-length IgG molecule may exhibit a heterogeneity embracing 10^8 isoforms (Kozlowski and Swann, 2006). Recombinant mAbs present a unique challenge as they must be evaluated on a case-by-case basis, each being constituted of heavy and light chains having unique variable region sequences. Fortunately, current technologies allow for early screening and selection of clones that do not have amino acid residues susceptible to PTMs within their complementarity determining regions (CDRs); other criteria may be selected to optimize solubility, stability, and so on. The constant region sequence can also be selected (i.e., Ig class and subclass) to define the final drug substance/product developed. A biosimilar candidate must be demonstrated to be structurally and functionally comparable (EMA, 2008; FDA, 2014).

4.3 PHYSICOCHEMICAL CHARACTERIZATION

It has been suggested that comparability between innovator and candidate biosimilar therapeutics may be better established through physicochemical characterization than limited clinical trials. This is posited on the premise that the extensive, and very expensive, clinical trials undertaken to achieve approval of the innovator product is not required for a candidate biosimilar; the added costs would negate the objective of making a less expensive drug available. A limited clinical trial within an outbred human population may yield unreliable results due to concomitant disease, associated and nonassociated differences between patients, and the like. It is essential to select a number of appropriate orthogonal techniques in establishing comparability; the range and sensitivity of methodologies currently available have been introduced in Chapter 2 of this book. The starting point for development of a biosimilar is characterization of the innovator product, employing multiple samples obtained from pharmacies, to inform the preliminary selection of clones and finally to confirm comparability. Unfortunately, the techniques employed and the results obtained by the innovator company are not in the public domain. The biopharmaceutical industry and patient interests could be better served if a consensus was established for the most appropriate techniques and protocols to be applied. The availability of a standard reference material, characterized within state-of-the-art methodologies, and available to any academic or commercial laboratory as an external control for their "in-house" protocols would be a step forward toward achieving these aims.