The challenge in generating a biosimilar antibody therapeutic is well illustrated in a report of the analysis of a candidate Herceptin biosimilar (Xie et al., 2010). Values for methionine oxidation, deamidation of asparagine and glutamine were reported; however, a note added in press stated that the values were higher than those obtained by the innovator company. It was therefore posited that additional chemical modifications had been introduced into the protein when reconstituting the innovator Herceptin prior to analysis. This illustrates both the susceptibility of such large molecules to chemical modification *in vitro* and the sensitivity of techniques available for determining comparability. A gross error resulted from generating an IgG1 constant region sequence different from that of the innovator product (Xie et al., 2010). This suggests that rather than sequencing the innovator product prior to embarking on the project, the sequence of Herceptin had been sourced from a sequence database that has been shown to be in error (Jefferis and Lefranc, 2009).

Given the potential structural heterogeneity of endogenous and recombinant biologics, it is outside the scope of this chapter to attempt to summarize all published studies for all currently approved biologics. Therefore, I shall limit this chapter mostly to the consequences of structural heterogeneities for human IgG antibodies, with some appropriate diversions. As previously noted, the heterogeneity of normal polyclonal IgG presents a complexity that has not been amenable to detailed analysis in past decades; however, mass spectrometry is beginning to provide viable approaches (Leblanc et al., 2014; Yang and Zubarev, 2010). The recombinant mAb therapeutics has been a focus of interest for PTM/CM studies due to their impact on structure and function. The usual approach is to determine the PTM/CM profile for an mAb and then to subject it to prolonged exposure to conditions known to induce specific CMs. Subsequent qualitative and quantitative analyses identify the degree of susceptibility of individual amino acid residues to modification (Correia, 2010). An alternative, and possibly more relevant, approach is to recover an mAb therapeutic from patient blood and to determine the PTM/CMs effected *in vivo* after a given time interval (Haberger et al., 2014). Earlier priorities in the development of mAbs centered on antigen specificity and affinity of binding as they transitioned from murine to chimeric, humanized, and fully human antibodies. Residues within light and heavy chain CDRs susceptible to PTM and CM modifications have been shown to compromise binding activity. However, currently available techniques allow for sequence analysis at the clone selection stage, such that molecules with CDR residues susceptible to modification can be discarded (e.g., asparagine, methionine, lysine).

4.5 N- AND C-TERMINAL RESIDUES

Unique N-terminal sequence may be obtained for the heavy and light chains of most monoclonal IgG paraproteins; for others, however, the N-terminal amino acid yield may not be quantitative or appear to be entirely "blocked" (Nakajima et al., 2011). This results when a gene encodes for the incorporation of N-terminal glutamic acid or glutamine residues with subsequent cyclization and the generation of pyroglutamic acid (pGlu), which may occur both *in vivo* and *in vitro* (Kumar and Bachhawat, 2012; Liu et al., 2011; Nakajima et al., 2011; Perez-Garmendia and Gevorkian, 2013;