Yin et al., 2013). The formation of pGlu in antibodies (Liu et al., 2011) and therapeutic proteins is a concern for the biopharmaceutical industry because it introduces charge heterogeneity and variations may be considered to be evidence for lack of process control (Stanley, 2011). Importantly, N-terminal pGlu is also implicated in Alzheimer's disease and dementia because it increases the tendency for proteins to form insoluble fibrils; light chains are particularly prone to such processing with the formation of fibrils (Liu et al., 2011; Merlini et al., 2014). When producing recombinant mAbs in mammalian cells, the balance between production of heavy and light chains is critical and an excess of light chain production is optimal, the excess being secreted into the medium (Li et al., 2007). As there is no evidence of a specific benefit attached to the presence of N-terminal pGlu, to either the heavy or light chain, it may be best avoided during clone selection for a potential mAb therapeutic.

Sequencing studies reported the C-terminal residue of serum-derived IgG heavy chains to be glycine. However, when the genes for the IgG subclasses were sequenced, it was seen that they encoded for a C-terminal lysine residue. It was later shown that the lysine residue is cleaved, *in vivo*, by an endogenous carboxypeptidase B. Recombinant IgG molecules produced in mammalian cells exhibit mixed populations of molecules, with lysine present or absent on each heavy chain. The presence or absence of lysine results in charge heterogeneity, and the proportions of heavy chains bearing C-terminal lysine can vary between clones and the many parameters that define the production platform (Tang et al., 2013). Although the level of C-terminal lysine is not considered to be a CQA, it may be a valuable reporter for production consistency. A concept paper produced by the European Biopharmaceutical Enterprises (EBE), a specialized group of the European Federation of Pharmaceutical Industries and Associations (EFPIA) included the statements: "A number of scientific publications suggest that C-terminal lysine truncation has no impact on biological activity, PK/PD, immunogenicity and safety." And elsewhere in the document: "Lysine truncation does not appear to adversely affect product potency or safety. However, taking a conservative approach potential C-terminal lysine effects on all antibodies cannot be ruled out. Thus, lysine truncation should be characterized, and process consistency should be demonstrated during product development; regulatory agencies suggest that C-terminal lysine content should be reported for both the characterization and development phases" (EBE, 2013). Removal of C-terminal lysine results in the presence of a C-terminal glycine residue that, when produced in CHO cells, may be subject to amidation, introducing further structural and charge heterogeneity (Tsubaki et al., 2013). A recent report demonstrated that this problem has been circumnavigated by genetic engineering CHO cells to "knockdown" expression of the peptidylglycine α-amidating monooxygenase (PAM) enzyme (Skulj et al., 2014).

4.6 CYSTEINE AND DISULFIDE BOND FORMATION

The gene sequence for the human IgG1 subclass protein (Eu) encodes for five light chain and nine heavy chain cysteine residues; 28 for a H_2L_2 protein dimer. The standard structural cartoon for the human IgG1 protein (Eu) exhibits 12 intrachain and 4 interchain disulfide bridges. This general pattern of intrachain disulfide bridge formation is maintained for each of the other IgG subclasses; however, the number