of interchain bridges and their architecture varies between and within the IgG subclasses (Liu and May, 2012; Macdougall et al., 2012). Heterogeneity of disulfide bridge formation has been reported for normal serum-derived IgG, myeloma proteins, and recombinant mAbs. Formation of the H_2L_2 dimer occurs following release of heavy and light chains into the endoplasmic reticulum (ER), with evidence that binding of the constant region of the light chain (C_L) to the heavy chain C_H1 domain "catalyses" the generation of a correctly folded H_2L_2 structure (Li et al., 2007). This nascent form explores multiple dynamic structures, with the formation of native and nonnative disulfide bonds that are transiently formed and reduced until a low-energy conformation is achieved (Aricescu and Owens, 2013; Stanley, 2011). It should be noted that little or no processing of the high mannose oligosaccharide will have occurred at this point; therefore, the conformation of the secreted IgG-Fc will not be achieved until oligosaccharide processing is completed.

The IgG1 molecule establishes the "standard" pattern with two interheavy chain disulfide bridges and a single light-heavy chain bridge; IgG2, IgG3, and IgG4 express 3, 11, and 2 interheavy chain bridges, respectively. The cysteine residues that form interchain disulfide bridges are clustered within the hinge region and may be subject to reduction and reformation when present in a reducing environment. Heterogeneity in disulfide bond formation in IgG2 was first reported for recombinant IgG2 proteins but, later, was also observed for normal serum derived IgG2 (Correia, 2010; Dillon et al., 2008; Liu et al., 2011; Wypych et al., 2008). The interconversion of these isoforms is dynamic and promoted by a reducing environment provided by the presence of thioredoxin reductase, released into culture media by effete cells; it can be ameliorated by control of dissolved oxygen levels (Davies et al., 2013; Hutterer et al., 2013; Kao et al., 2013; Koterba et al., 2012; Rispens et al., 2013, 2014). An *in vitro* model revealed that susceptibility to reduction/oxidation differed between IgG subclasses and light chain types, with sensitivity being in the order IgG1λ > IgG1κ > IgG2λ > IgG2κ (Koterba et al., 2012).

A core hinge region sequence of –Cys–Pro–Pro–Cys–, present in IgG1, IgG2, and IgG3, forms a partial helical structure that does not allow for intraheavy chain disulfide bridge formation. However, the homologous sequence in the IgG4 subclass is –Cys–Pro–Ser–Cys–, and this does allow for intraheavy chain disulfide bridge formation. Consequently, natural and recombinant IgG4 antibody populations are a mixture of molecules exhibiting inter- and intrahinge heavy chain disulfide bridge isoforms, generated *in vivo* and *in vitro* (Correia, 2010; Liu et al., 2011; Rispens et al., 2013). The IgG4 form having intrahinge heavy chain bridges is susceptible to dissociation into half-molecules (HLs) that may reassociate randomly to generate bispecific molecules—that is, a molecule that is monovalent for two nonidentical antigens (epitopes). This phenomenon is referred to as Fab arm exchange. The exchange is facilitated by the presence of an arginine residue at position 409 (R409) in the IgG4 heavy chain, in place of the lysine 409 (K409) present in IgG1, IgG2, IgG3 molecules. There exists a polymorphic variant of IgG4 that also has K409 and is not subject to Fab arm exchange (Davies et al., 2013; Rispens et al., 2013, 2014). Lateral noncovalent interactions between the two C_H3 domains of R409 IgG4 are reduced so that, under physiological conditions and in the absence of hinge region intraheavy chain disulfide bridges, they dissociate to form HL heteromonomers.