**Review**

**Disulfide bond structures of IgG molecules**

**Structural variations, chemical modifications and possible impacts to stability and biological function**

Hongcheng Liu* and Kimberly May

Merck Research Laboratories; Union, NJ USA

**Key words:** recombinant monoclonal antibody, disulfide bond, trisulfide bond, free sulfhydryl, dehydroalanine, thioether, aggregation

The disulfide bond structures established decades ago for immunoglobulins have been challenged by findings from extensive characterization of recombinant and human monoclonal IgG antibodies. Non-classical disulfide bond structure was first identified in IgG4 and later in IgG2 antibodies. Although, cysteine residues should be in the disulfide bonded states, free sulfhydryls have been detected in all subclasses of IgG antibodies. In addition, disulfide bonds are susceptible to chemical modifications, which can further generate structural variants such as IgG antibodies with trisulfide bond or thioether linkages. Trisulfide bond formation has also been observed for IgG of all subclasses. Degradation of disulfide bond through β-elimination generates free sulfhydryls disulfide and dehydroalanine. Further reaction between free sulfhydryl and dehydroalanine leads to the formation of a non-reducible cross-linked species. Hydrolysis of the dehydroalanine residue contributes substantially to antibody hinge region fragmentation. The effect of these disulfide bond variations on antibody structure, stability and biological function are discussed in this review.

**Introduction**

The recombinant monoclonal IgG antibodies comprise a rapidly growing group of protein therapeutics. The disulfide bond structure of IgG is highly conserved through evolution and was once considered a uniform and homogeneous structural feature. However, detailed characterization of a large number of IgG molecules has revealed several new structural features in both recombinant and natural human IgG antibodies. These new findings and their effects on IgG structure, stability and biological function are reviewed here.

**Classical Disulfide Bond Structures**

Disulfide bond structures of the four subclasses of IgG were established in the 1960s.1,8 These disulfide bond structures are referred to as the classical disulfide bond structures because they are widely accepted. As shown in Figure 1, there are many similarities and some differences with regard to the disulfide bond structures in the four subclasses of IgG antibodies, IgG1, IgG2, IgG3, and IgG4. Each IgG contains a total of 12 intra-chain disulfide bonds; each disulfide bond is associated with an individual IgG domain. The two heavy chains are connected in the hinge region by a variable number of disulfide bonds: 2 for IgG1 and IgG4, 4 for IgG2 and 11 for IgG3. The light chain of the IgG1 is connected to the heavy chain by a disulfide bond between the last cysteine residue of the light chain and the fifth cysteine residue of the heavy chain. However, for IgG2, IgG3 and IgG4, the light chain is linked to the heavy chain by a disulfide bond between the last cysteine residue of the light chain and the third cysteine residue of the heavy chain.

The level of solvent exposure is different between intra-chain and inter-chain disulfide bonds. Cysteine residues that form inter-chain disulfide bonds are located in the hinge region with the exception of the third cysteine residue of the heavy chain in IgG4, IgG3 and IgG2, which is located between the interface of VH and CH1 domains.9 Therefore, inter-chain disulfide bonds are highly solvent exposed.9,12 On the other hand, intra-chain disulfide bonds are buried between the two layers of anti-parallel β-sheet structures within each domain and are not solvent exposed.8,12 The solvent exposure difference has important implications because exposed cysteine residues are considered more reactive than non-exposed cysteine residues.

**Non-Classical Linkage**

Disulfide bond structures other than the classical structures shown in Figure 1 have been observed mainly for IgG2 and IgG4, but not for IgG1 and IgG3. Only a trace amount of a disulfide bond variant with the two inter heavy chain disulfide bonds in the intra-chain form for IgG4 has been observed.13 IgG4 has repeated amino acid sequence in the hinge region and a total of 11 disulfide bonds in close proximity, which does not allow much flexibility for formation of disulfide bond variants.

Non-classical disulfide bond structures of IgG2 were first identified in recombinant monoclonal antibodies (mAbs) and then confirmed in human IgG2 molecules.14-16 In these publications, the classical disulfide bond structure was referred to as IgG2A.
while the two major non-classical structures were referred to as IgG_{2}B and IgG_{2}-A/B, the latter being considered a structural intermediate between IgG_{2}A and IgG_{2}B (Fig. 2). Distribution of different disulfide bond isoforms is dependent on the type of light chain, IgG_{2}A is the major form in molecules with \( \lambda \) light chain; IgG_{2}B is the major form in molecules with \( \kappa \) light chain.\(^{15} \) A conversion from the IgG\(_{2}\)A form to IgG\(_{2}\)B was observed during cell culture, in vitro incubation with serum and in patient serum.\(^{17} \) Molecular dynamic simulation study revealed that the sulfur atoms of inter-chain disulfide bonds are highly mobile and can be in close proximity.\(^{18} \) Therefore, it is not a surprise to observe the coexistence of multiple disulfide bond isoforms for IgG\(_{2}\) antibodies. In addition to isoforms from different intra-molecule disulfide bond linkages, disulfide bond linked IgG\(_{2}\) dimer was also found in recombinant IgG\(_{2}\) from cell culture and in human serum.\(^{19} \)

By far, IgG\(_{4}\) is the best known subclass of IgG molecule having non-classical disulfide bond structures (Fig. 3). Several interesting observations led to the ultimate finding of the non-classical disulfide bond structures. First, significant amounts of IgG\(_{4}\) were observed as half-molecules when analyzed by non-reducing sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE),\(^{20-24} \) but not by size-exclusion chromatography (SEC) run under native conditions,\(^{23} \) indicating that the two half-molecules are associated by non-covalent interactions rather than by covalent linkage. Second, polyclonal IgG\(_{4}\) is unable to cross-link antigen and behaves like a monovalent antibody,\(^{25} \) while monoclonal IgG\(_{4}\) can cross-link antigens.\(^{26} \) Third, IgG\(_{4}\) as a covalent-linked monomer demonstrates bispecificity in plasma.\(^{26} \) These observations were explained by the fact that the two inter heavy chain disulfide bonds of IgG\(_{4}\) are in equilibrium with intra-chain disulfide bond forms.\(^{13,23,24} \) IgG\(_{1}\) and IgG\(_{4}\) differ by one amino acid in the middle hinge region, \( i.e. \), there are two proline residues in IgG\(_{1}\) and a serine and a proline residue in IgG\(_{4}\). Stable inter heavy chain disulfide bonds of IgG\(_{4}\) were obtained by replacing the serine residue with a proline residue.\(^{13,23,24} \) Because of the instability of the inter heavy chain disulfide bonds, bispecific antibody can be formed in vitro in the presence of reducing reagents and in vivo by injection of equal amounts of two recombinant IgG\(_{4}\) antibodies specific for two different antigens into immunodeficient mice.\(^{27} \)

**Free Sulfhydryls**

Presumably, all cysteine residues in IgG are in the disulfide bonded state. However, free sulfhydryls has been routinely detected in IgG molecules, including IgG from serum and recombinant mAbs (Table 1).\(^{28-35} \) It is worthwhile to discuss two important observations in Table 1. First, higher level of free sulfhydryls was detected under denaturing conditions compared with native con-
The variable domain has a higher level of free sulfhydryls than that in the constant domain in the light chain. The CH3 domain has the highest level of free sulfhydryls followed by CH1, CH2 and the variable domain in the heavy chain. The lowest level of free sulfhydryls is associated with inter-chain disulfide bonds, suggesting that low level of free sulfhydryls is most likely due to incomplete formation of disulfide bonds. Because inter-chain disulfide bonds with higher solvent exposure level are more prone to degradation than intra-chain disulfide bonds, higher level of free sulfhydryls associated with inter-chain disulfide bonds is expected if free sulfhydryl is generated due to disulfide bond degradation. Distribution other than described above may indicate special cases where particular disulfide bonds are not efficiently formed.

Two special cases have been reported in the literature so far. In both cases, the intra-chain disulfide bond in the heavy chain variable domain is not completely formed at such a level that antibodies with this incomplete disulfide bond were detected by hydrophobic interaction or weak cation exchange chromatography. Complete formation of this particular disulfide bond can be achieved by the addition of copper sulfate to cell culture, suggesting that cell culture conditions can affect disulfide bond formation. Antibodies after in vitro incubation in serum or recovered from rat serum after administration showed significant reduction in incomplete disulfide bond formation.

**β-Elimination**

Under basic conditions, disulfide bonds can decompose through the β-elimination mechanism with the formation of dehydroalanine and persulfide, which can further revert back to a cysteine residue. Degradation of the inter light chain and heavy chain disulfide bond of IgG through the β-elimination mechanism followed by cross-linking of the resulting cysteine and dehydroalanine has led to the formation of a non-reducible thioether linkage, which was found at ~0.4% for a recombinant monoclonal IgG stored at 4°C and up to 13.6% for a heat-stressed sample. Subsequent hydrolysis of the dehydroalanine is another important mechanism in addition to peptide bond hydrolysis that leads to antibody fragmentation in the hinge region.

**Trisulfide Bond Formation**

Trisulfide bonding formation is a rare post-translational modification of proteins. The presence of trisulfide bonding was first reported for a recombinant monoclonal IgG, where one or two of the four inter heavy chain disulfide bonds may exist as a trisulfide bond. Trisulfide bonds were later detected in all subclasses of recombinant IgG antibodies, as well as in human IgG from patients with myeloma. In all cases, higher levels of trisulfide bonds were observed between the cysteine residues that normally form the inter light chain and heavy chain disulfide bonds. Trisulfide bonds in recombinant mAbs are believed to be formed during fermentation as a result of the reaction of an intact disulfide bond with dissolved hydrogen sulfide (H2S). This conclusion is supported by the observation that incubation of IgG with

![Figure 2. IgG, disulfide bond isoforms.](image)
from mutagenesis studies. Two mutants, one replacing the first middle hinge cysteine with a serine and the other replacing the serine in the middle hinge with a proline, resulted in more stable IgG4 molecules without affecting antigen binding activity. In a separate study, replacing the middle hinge serine with proline resulted in a more stable inter-chain disulfide bond and increased half-life, again without affecting antigen binding activity.

The effect of free sulfhydryl on the structure, stability and biological functions of IgG has been studied using individual domains, as well as intact IgG molecules. Individual domains of C ϵ domain, CH3 domain, and single-chain variable fragment without the complete intra-chain disulfide bond showed lower stability, but no substantial structural changes. It is expected that the lack of intra-chain disulfide bond in other domains will have similar destabilizing effect because all IgG domain share similar folding. Incomplete formation of the disulfide bond in the heavy chain variable domain of a recombinant monoclonal antibody resulted in a significant decrease in potency. A natural antibody derived from the ABPC48 mouse plasmacytoma, in which the second cysteine residue in the heavy chain variable domain was replaced by a tyrosine residue, is capable of binding antigen, suggesting further that a complete disulfide bond is not a prerequisite for antigen binding. Higher amounts of free sulfhydryl resulted in lower thermal stability of both recombinant and human IgG antibodies. The higher aggregation propensity of IgG2 compared with IgG1 is also attributed to higher level of free sulfhydryl of IgG2.

Partial reduction has been one of the commonly used methods to study the effect of inter-chain disulfide bond on the structure, stability and biological functions of IgG. Although a global conformational change was not observed, partial reduction increased the flexibility of the hinge region, probably as a result of reduction of inter-chain disulfide bonds, resulting in further separation of the two CH2 domains. An apparent increase in the hydrodynamic sizes of human IgG1, IgG2, and IgG4, but decreased size for IgG4, were also observed upon partial reduction and alkylation, which is again attributed to the structural change in the hinge region and CH2 domain. Highly dependent on the experimental conditions, partial reduction either has no

**Table 1. Level of free sulfhydryl in IgG**

| Type of IgG               | Mole of free SH/Mole of IgG | Reference |
|---------------------------|-----------------------------|-----------|
| Human IgG1                | 0.24                        | ND* 28, 29|
| Human IgG2                | 0.1–1.1                     | 30        |
| Human IgG4, IgG4, and recombinant IgG4 | ND* 0.9–2.2 | 31 |
| Recombinant IgG1, IgG2 and IgG4 | 0.02–0.08, 0.08–0.09 | 32 |
| Recombinant IgG1          | ND*                         | 33        |
| Recombinant IgG2          | 0.06                        | 34        |
| Recombinant IgG4          | 0.158                       | 35        |

ND*, not determined.
impact\textsuperscript{9,65} or reduces complement activation efficiency.\textsuperscript{64,66} The effect of partial reduction on binding to Fc receptors and, consequently, antibody-dependent cell-mediated cytotoxicity (ADCC) is also not consistent, e.g., no effect\textsuperscript{67,68} and significantly reduced activity\textsuperscript{59,60} were observed for different antibodies. One of the critical issues is the degree of reduction of the intra-chain disulfide bonds in different studies. It has been reported that only inter-chain disulfide bonds of human IgG are susceptible to reduction under native conditions.\textsuperscript{71} However, reduction of intra-chain disulfide bonds of rabbit IgG under native conditions may be possible.\textsuperscript{62,64}

Although levels of free sulfhydryls appear to be low, their presence poses some challenges for recombinant monoclonal antibody formulation. It was found that the majority of the IgG\textsubscript{1} dimer is formed due to formation of intermolecular disulfide bonds,\textsuperscript{72} which could result from free sulfhydryls. Dimerization may be possible.\textsuperscript{62,64} It is possible that antibodies with incomplete disulfide bonds are more susceptible to unfolding under various stress conditions and, therefore, have a higher propensity for covalent aggregation through disulfide bond formation. IgG antibodies with higher levels of free sulfhydryls also have a greater tendency to expose hydrophobic regions, which can drive the formation of non-covalent aggregates through inter-molecule hydrophobic interactions.

**Conclusion**

Heterogeneity is a common feature of recombinant mAbs as a result of post-translational modifications and variation related specifically to disulfide bond structures. A potential major contributor to heterogeneity is the degree of reduction of intra-chain disulfide bonds. It has been clearly demonstrated that intra-chain disulfide bonds are in general more stable after antibody assembly. Therefore, the low levels of free sulfhydryl associated with these intra-chain disulfide bonds are probably due to incomplete formation of disulfide bonds. On the other hand, inter-chain disulfide bonds are exposed and less stable, which explains why increased heterogeneity is associated with these bonds. It is thus reasonable to hypothesize that non-classical disulfide bond structures, trisulfide bonding and thioether linkages formation may occur after antibody assembly.

Close attention should be paid to these new disulfide bond-related structures during the development of recombinant mAbs because changes in structures and stability have been observed. Theoretically, administration of non-native disulfide bonded structures to humans has the potential to trigger immune response. Lowering stability can also ultimately lead to non-native structures because of the higher propensity to unfold and form aggregates. More experiments are thus warranted to improve understanding of the effects of disulfide bond related structural variants on the stability, structure and biological functions of IgG molecules.

**Acknowledgments**

We thank Larry Dick, Huijuan Li and Yi Du for their critical review of this manuscript.

**References**

1. Milstein C. The disulfide bridges of immunoglobulin kappa-chains. Biochem J 1966; 101:358-51; PMID:1651119.

2. Pink JR, Milstein C. Inter-heavy chain disulfide bridge in immune globulins. Nature 1967; 214:92-4; PMID:4166384; http://dx.doi.org/10.1038/21492a0.

3. Frangione B, Milstein C. Disulphide bridges of immunoglobulin G 1-heavy chains. Nature 1967; 216:393-41; PMID:4169396; http://dx.doi.org/10.1038/216393a0.

4. Pink JR, Milstein C. Disulfide bridges of a human immunoglobulin G protein. Nature 1967; 216:941-2; PMID:4169397; http://dx.doi.org/10.1038/216941a0.

5. Frangione B, Milstein C, Franklin EC. Intrachain disulfide bridges in immunoglobulin G heavy chains. The Fc fragment. Biochem J 1968; 106:15-21; PMID:4889360.

6. Frangione B, Milstein C. Variations in the S-S bridges of immunoglobulins G: interchain disulfide bridges of gammaG\textsubscript{2}, myeloma proteins. J Mol Biol 1968; 113:893-906; PMID:4178816; http://dx.doi.org/10.1016/0022-2836(68)90364-2.

7. Edelman GM, Cunningham BA, Gall WE, Gottlieb RD, Rutishauser U, Wadzal MJ. The covalent structure of an entire gammaG\textsubscript{2} immunoglobulin molecule. Proc Natl Acad Sci USA 1969; 63:78-85; PMID:5237909; http://dx.doi.org/10.1073/pnas.63.1.78.

8. Frangione B, Milstein C, Pink JR. Structural studies of immunoglobulin G. Nature 1969; 221:145-8; PMID:5782707; http://dx.doi.org/10.1038/221145a0.

9. Lefranc MP, Pommie C, Kaar Q, Dupraz E, Bosc N, Guiraudou D, et al. IMGT unique numbering for immunoglobulin and T cell receptor constant domains and Ig superfamily C-like domains. Dev Comp Immunol 2005; 29:195-203; PMID:15572068; http://dx.doi.org/10.1016/j.devimm.2004.07.003.

10. Lefranc MP, Pommie C, Ruiz M, Giudicelli V, Foulquier E, Truong L, et al. IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev Comp Immunol 2003; 27:55-57; PMID:12477501; http://dx.doi.org/10.1016/S0145-305X(02)00019-3.

11. Amzel LM, Poljak RJ. Three-dimensional structure of immunoglobulins. Annu Rev Biochem 1979; 48:961-97; PMID:4988932; http://dx.doi.org/10.1146/annurev.bi.48.070179.004525.

12. Ikach H, Gego Y, Hamaguchi K. Reduction of the buried intrachain disulfide bond of the constant fragment of the immunoglobulin light chain: global unfolding under physiological conditions. Biochemistry 1986; 25:2009-13; PMID:3085710; http://dx.doi.org/10.1021/bi00356a026.

13. Bloom JW, Madanat MS, Marriott D, Wong T, Chan SY. Intrachain disulfide bond in the core hinge region of human IgG\textsubscript{1}. Protein Sci 1997; 6:407-15; PMID:9041643; http://dx.doi.org/10.1002/pro.5560060217.

14. Wypych J, Li M, Guo A, Zhang Z, Martinez T, Allen MJ, et al. Human IgG\textsubscript{1} antibodies display disulfide-mediated structural isomers. J Biol Chem 2008; 283:16194-205; PMID:18339624; http://dx.doi.org/10.1074/jbc.M709987200.

15. Dillon TM, Ricci MS, Veicina C, Flynn GC, Liu YD, Rehder DS, et al. Structural and functional characterization of disulfide isoforms of the human IgG\textsubscript{1} subclass. J Biol Chem 2008; 283:16206-15; PMID:18339626; http://dx.doi.org/10.1074/jbc.M709988200.

16. Martinez T, Guo A, Allen MJ, Han M, Pace D, Jones J, et al. Disulfide connectivity of human immunoglobulin G\textsubscript{1} structural isoforms. Biochemistry 2008; 47:7496-508; PMID:18549248; http://dx.doi.org/10.1021/bi078576c.

17. Liu YD, Chen X, Eck JZ, Plant M, Dillon TM, Flynn GC. Human IgG\textsubscript{1} antibody disulfide rearrangement in vivo. J Biol Chem 2008; 283:29266-72; PMID:18713741; http://dx.doi.org/10.1074/jbc.M804787200.

18. Wang X, Kumar S, Singh S. Disulfide bond scrambling in IgG\textsubscript{1}, monoclonal antibodies: insights from molecular dynamics simulations. Pharm Res 2011; http://dx.doi.org/10.1007/s11095-011-0503-9;PMID:21671135.

19. Yoo EM, Wims LA, Chan LA, Morrison SL. Human IgG\textsubscript{1} can form covalent dimers. J Immunol 2003; 170:3134-8; PMID:12626570.

20. Petersen JG, Dorrington KJ. An in vitro system for studying the kinetics of interchain disulfide bond formation in immunoglobulin G. J Biol Chem 1974; 249:5633-41; PMID:4212934.

21. Colcher D, Mileenic D, Roselli M, Raubitschek A, Xaranont G, King D, et al. Characterization and biodistribution of recombinant and recombinant/chimeric constructs of monoclonal antibody B72.3. Cancer Res 1989; 40:1738-45; PMID:2924317.
Rajan R, Matsumura M. Characterization of anti-
body aggregation: role of buried, unpaired cysteines
Rych S, Gokarn YR, Hultgen H, Stevenson RJ,
Brych SR, et al. Contributions of a disulfide bond
to the structure, stability and dimerization of human IgG antibody CH3 domain. Protein Sci 2008; 17:95-
106; PMID:18536460; http://dx.doi.org/10.1100/j.
pbro.2010.01.0019.

Nielsen RW, Tachibana C, Hansen N, Winther J.
Thijs S, Maeyer J, Augustine JG, Frederick CA, Lilie
H. Charactertization of lower molecular weight anti-
body bands of recombinant monoclonal IgG antibodies on non-reducing SDS-PAGE. Biotechnol Lett 2007;
29:1611-22; PMID:1766583; http://dx.doi.org/10.1016/j.
biolett.2007.09.027.

Aubert HB, Bernheim JL, Oborski F, Chemseddine A.
Effect of copper sulfate on performance of a serum-free
CHO cell culture process and the level of free thiol
linking structure to function. Dev Biol (Basel) 2005; 22:
127-137; PMID:15637256.

Schuurman J, Van Dooren HR, Tan KY, Aalberse RC.
Normal human immunoglobulin G4 is bispecific: it has two different antigen-combining sites. Immunology 1999; 97:693-8; PMID:10457225; http://dx.doi.org/10.1111/j.1600-0749.1999.t01-10-
0005.x.

van der Neut Kolfschoten M, Schuurman J, Losen M,
Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 2007; 317:154-7; PMID:17872445; http://dx.doi.org/10.1126/science.1146603.

Gevondyan NM, Volynskaia AM, Gevondyan VS.
Four free cysteine residues in human IgG4 of healthy donors. Biochemistry (Moscow) 2006; 71:279-
84; PMID:16545064; http://dx.doi.org/10.1134/S0006237706070721.

Lacy ER, Baker M, Brigham-Burke M. Free sulphydryl measurement as an indicator of antibody stability. Anal Biochem 2008; 382:66-8; PMID:18675772; http://dx.doi.org/10.1016/j.ab.2007.06.016.

Zhang W, Csapmany M. Free sulphydryl in recombinant monoclonal antibodies. Biotechnol Prog 2002; 18:590-
13; PMID:12502667; http://dx.doi.org/10.1021/bp0102511z.

Liu H, Gaya-Bulseco G, Chiumae C, Newby-Kew A. Characterization of lower molecular weight antibody bands of recombinant monoclonal IgG antibodies on non-reducing SDS-PAGE. Biotechnol Lett 2007; 29:1611-22; PMID:1766583; http://dx.doi.org/10.1016/j.biolett.2007.09.027.

Chiumae C, Gaya-Bulseco G, Liu H. Identification and localization of cysteine residues in monoclonal antibodies by fluorescence labeling and mass spectrometry. Anal Chem 2009; 81:6449-57; PMID:19572546; http://dx.doi.org/10.1021/ac900815z.
68. McNabb T, Koh TY, Dorrington KJ, Painter RH. Structure and function of immunoglobulin domains. V. Binding, University of immunoglobulin G and fragments to placental membrane preparations. J Immunol 1976; 117:882-8; PMID:956658.

69. Michaelson TE, Wisloff F, Natvig JB. Structural requirements in the Fc region of rabbit IgG antibodies necessary to induce cytotoxicity by human lymphocytes. Scand J Immunol 1975; 4:71-8; PMID:1079629; http://dx.doi.org/10.1111/j.1365-3083.1975.tb02601.x.

70. Barnett Foster DE, Dorrington KJ, Painter RH. Structure and function of immunoglobulin domains. VII. Studies on the structural requirements of human immunoglobulin G for granulocyte binding. J Immunol 1978; 120:1952-6; PMID:659887.

71. Liu H, Chumsae C, Guza-Bulseco G, Hurkmans K, Radziejewksi CH. Ranking the susceptibility of disulfide bonds in human IgG1 antibodies by reduction, differential alkylation and LC-MS analysis. Anal Chem 2010; 82:5219–26; PMID:20491447; http://dx.doi.org/10.1021/ac100575n.

72. Remmel RL Jr, Callahan WJ, Krishnan S, Zhou L, Bondarenko PV, Nichols AC, et al. Active dimer of Epratuzumab provides insight into the complex nature of an antibody aggregate. J Pharm Sci 2006; 95:126-45; PMID:16315222; http://dx.doi.org/10.1002/jps.20515.

73. Van Buren N, Rehder D, Gadgil H, Matsumura M, Jacob J. Elucidation of two major aggregation pathways in an IgG antibody. J Pharm Sci 2009; 98:3013-30; PMID:18680168; http://dx.doi.org/10.1002/jps.21514.