[0010] The Aho numbering approach was utilized in the past to improve stability and other biophysical properties. Ewert et al. (2003), "Structure-based improvement of the biophysical properties of immunoglobulin VH domains with a generalizable approach," Biochemistry 42: 1517-1528; Ewert et al. (2003), "Biophysical properties of human antibody variable domains," J. Mol. Biol. 325: 531-553; Ewert et al. (2004), "Stability improvement of antibodies for extracellular and intracellular applications: CDR grafting to stable frameworks and structure-based framework engineering," Methods 34: 184-199; and Rothlisberger et al. (2005), "Domain interactions in the Fab fragment: a comparative evaluation of the single-chain Fv and Fab format engineered with variable domains of different stability," J Mol. Biol. 347: 773-789. The Aho numbering system was also utilized in the past to reduce propensity for aggregaton. Borras et al. (2013), US Pat. No. 8,545,849.

SUMMARY

[0011] This disclosure relates to methods for reducing viscosity of antigen binding proteins by modifying sequences in framework regions and/or the Fc domain that are shown to be associated with high viscosity.

[0011a] In one aspect, the present invention refers to a method of reducing the viscosity of an antigen binding protein that comprises the VH1|1-18 germline subfamily, which comprises modifying the VH1 sequence to comprise substitutions $82X^1$, $94X^2$, and $95X^3$, wherein X^1 is selected from R, K and H, X^2 is selected from S, T, N and Q and X^3 is selected from R, K, and H; wherein the amino acids are numbered according to the Aho numbering system.

[0012] In the process details which follow, all variable region amino acids are identified by Aho numbering, all amino acids from conserved regions are identified by EU numbering. Aho numbering is aligned and correlated with the other main numbering schemes including EU (Edelman et al. (1969), "The covalent structure of an entire gamma immunoglobulin molecule," Proc. Natl. Acad. Sci. U. S. A. 63, 78-85), Kabat (Kabat et al. (1991), Sequences of proteins of immunological interest, Fifth Edition. NIH Publication No. 91-3242), Chothia (Chothia et al., (1992), "Structural repertoire of the human VH segments," J.Mol.Biol. **227:** 799-817); (Tomlinson et al., (1995), " The structural repertoire of the human V kappa domain," EMBO J 14: 4628-4638). Any of the four numbering systems can be interchangeably used to identify the preferred amino acid substitutions described in this specification.

[0013] If the antigen binding protein comprises the VH1|1-18 germline subfamily, the method comprises modifying the VH1 sequence to comprise one or more substitutions selected

from $82X^1$, $94X^2$, and $95X^3$, wherein X^1 is a basic residues (R, K or H), X^2 is a polar, uncharged residue (S, T, N or Q) and X^3 is a basic residue (R, K, or H). All residues are identified by the Aho numbering system. Preferred mutations for the VH1|1-18 germline subfamily are 82R, 94S, and 95R. The method as applied to the VH1|1-18 germline subfamily may further comprise substitution $59X^{20}$ wherein X^{20} is a basic residue (R, K or H), with the mutation 59K preferred.

[0014] If the antigen binding protein comprises the VH3|3-33 germline subfamily, the method comprises modifying the VH3 sequence to comprise one or more substitutions selected from $1X^4$, $17X^5$, and $85X^6$, wherein X^4 is a charged negative residue (D or E), X^5 is a small hydrophobic residue (G, A, V, I, L, or M), and X^6 is a small hydrophobic residue (G.A, V, I, L, or M). All residues are identified by the Aho numbering system. Preferred mutations for the VH3|3-33 germline subfamily are 1E, 17G, and 85A.

[0015] If the antigen binding protein comprises the VK3|L16 germline subfamily, the method comprises modifying the VK3 sequence to comprise one or more substitutions selected

What is claimed is:

1. A method of reducing the viscosity of an antigen binding protein that comprises the VH1|1-18 germline subfamily, which comprises modifying the VH1 sequence to comprise substitutions $82X^1$, $94X^2$, and $95X^3$, wherein X^1 is selected from R, K and H, X^2 is selected from S, T, N and Q and X^3 is selected from R, K, and H; wherein the amino acids are numbered according to the Aho numbering system.

2. The method of claim 1, wherein the antigen binding protein comprises except as substituted the sequences of an antibody selected from antibodies AF, AK, AL, AN and AO in Figure 1B (SEQ ID NOS: 114 and 116; 134 and 136; 138 and 140; 146 and 148; and 150 and 152).

3. The method of any one of claims 1 to 2, which comprises modifying the VH1 sequence of an antigen binding protein comprising the VH1|1-18 germline subfamily to comprise substitutions 82R, 94S, and 95R.

4. The method of any one of claims 1 to 2, further comprising modifying the VH1 sequence of an antigen binding protein comprising the VH1|1-18 germline subfamily to comprise substitution $59X^{20}$ wherein X^{20} is selected from R, K and H.

5. The method of claim 3, wherein the antigen binding protein except as substituted comprises the sequences of an antibody selected from antibodies AF, AK, AL, AN and AO from Figure 1B (SEQ ID NOS. 114 and 116; 134 and 136; 138 and 140; 146 and 148; and 150 and 152).