[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.

[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.

[0014a] In one aspect, the disclosure relates to a method of reducing the viscosity of an antigen binding protein, which comprises making one or more modifications in the sequence of the antigen binding protein selected from:

a. if the antigen binding protein comprises the VH3|3-33 germline subfamily, modifying the VH3 sequence to comprise one or more substitutions selected from $1X^4$, $17X^5$, and $85X^6$, wherein X^4 is selected from D and E, X^5 is selected from G, A, V, I, L, and M, and W, and X^6 is selected from G.A, V, I, L, and M;
b. if the antigen binding protein comprises the VK3|L16 germline subfamily, modifying the VK3 sequence to comprise one or more substitutions selected from $4X^{10}$, $13X^{11}$, $76X^{12}$, 78F, $95X^{13}$, $97X^{14}$, and 98P, wherein X^{10} is selected from G, A, V, I, L, and M, X^{11} is selected from G, A, V, I, L, and M, X^{12} is selected from D and E, X^{13} is selected from R, K and H, and X^{14} is selected from D and E;
c. if the antigen binding protein comprises the VK3|L6 germline subfamily, modifying the VK3 sequence to comprise one or more substitutions selected from $76X^{12}$ and $95X^{13}$;
d. modifying the Fc domain sequence to comprise one or more substitutions selected from $253X^{15}$, $440X^{16}$, and $439X^{17}$, wherein X^{15} is selected from G, A, V, I, L, and M, X^{16} is selected from R, K, and H, and X^{17} is selected from D and E, wherein the Fc domain sequence comprises only one of $440X^{16}$ and $439X^{17}$; and
e. modifying the C-terminus of the Fc domain sequence to comprise $X^{18}X^{19}$ wherein X^{18} is one to four amino acids selected from D and E or from H, K, and R, and X^{19} is selected from P, M, G, A, V, I, L, S, T, N, Q, F, Y and W and is absent when X^{18} comprises D or E, is present when X^{18} comprises K or R at its C-terminal end, and is present or absent when X^{18} comprises H at its C-terminal end;

wherein the amino acids in subparagraphs a, b, c, and d are numbered according to the Aho numbering system and the amino acids in subparagraph e are numbered according to the EU numbering system.

[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 from $4X^{10}$, $13X^{11}$, $76X^{12}$, 78F, $95X^{13}$, $97X^{14}$, and 98P, wherein X^{10} is selected from G, A, V, I, L, and M, X^{11} is selected from G, A, V, I, L, and M, X^{12} is selected from D and E, X^{13} is selected from R, K and H, and X^{14} is selected from D and E. All residues are identified by the Aho numbering system. Preferred mutations for the VK3|L16 germline subfamily are 4L, 13L, 76D, 95R, 97E, and 98P.

[0016] If the antigen binding protein comprises the VK3|L6 germline subfamily, the method comprises modifying the VK3 sequence to comprise one or more substitutions selected from $76X^{12}$ and $95X^{13}$. Preferred mutations for the VK3|L6 germline subfamily are 76D and 95R.

[0017] The methods of this disclosure further comprise modifying the Fc domain to comprise one or more substitutions selected from $253X^{15}$, $440X^{16}$, and $439X^{17}$, wherein X^{15} is a small hydrophobic residue (G, A, V, I, L, or M), X^{16} is a basic residue (R, K, or H), and X^{17} is a charged negative residue (D or E), , wherein the Fc domain sequence comprises only one of $440X^{16}$ and $439X^{17}$. All residues are identified by the EU numbering system. Preferred mutations of the Fc domain are 253A, 440K, and 439E.

[0018] The methods of this disclosure further comprise modifying the C-terminus of the Fc domain sequence to comprise $X^{18}X^{19}$ wherein X^{18} is one to four amino acids selected from D and E or from H, K, and R, and X^{19} is selected from P, M, G, A, V, I, L, S, T, N, Q, F, Y and W and is absent when X^{18} comprises D or E, is present when X^{18} comprises K or R at its C-terminal end, and is present or absent when X^{18} comprises H at its C-terminal end. Preferred Fc C-terminal modifications comprise KP, KKP, KKKP (SEQ ID NO:380), E or EE at the C-terminus.

[0019] The foregoing methods are preferably applied to the high viscosity antibodies shown in Figures 1A and 1B hereinafter. The part of the method involving the VH1|1-18 sequence is preferably applied to antibodies AF, AK, AL, AN, and AO from Figure 1B. The part of the method involving the VH3|3-33 sequence is preferably applied to antibodies AQ, AM, AI, and AG from Figure 1B. The part of the method involving the VK3|L16 sequence is preferably applied to antibodies AF and AQ from Figure 1B. The part of the method involving the VK3|L6 sequence is preferably applied to antibody AJ.

[0020] The methods of this disclosure further comprise a method of preparing an antigen binding protein that reaches maximum serum concentration faster than does a parental

antibody when the antigen binding protein and the parental antibody are administered at the same concentration, which comprises introducing sequence modification $440X^{16}$ in the parental antibody wherein X^{16} is selected from R, K, and H. In a preferred method, the sequence-modified antigen binding protein reaches maximum serum concentration after subcutaneous injection at least twice as fast as the parental antibody. Also within this disclosure is a method of preparing an antigen binding protein that reaches a maximum serum concentration after subcutaneous injection that is higher than that of a parental antibody when the antigen binding protein and the parental antibody are administered at the same concentration, which comprises introducing sequence modification $440X^{16}$ in the parental antibody wherein X^{16} is selected from R, K, and H. In a preferred method, the sequence-modified antigen binding protein reaches a maximum serum concentration that is at least about 25% higher than that of the parental antibody. In each of these methods, the preferred X^{16} is K and the preferred parental antibody is a PCSK9 polypeptide (antibody AK most preferred).

[0021] The disclosure further relates to a mutant antigen binding protein, which comprises one or more sequences selected from:

a. a VH1|1-18 germline subfamily sequence comprising one or more substitutions selected from $82X^1$, $94X^2$, 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;

b. a VH3|3-33 germline subfamily sequence comprising one or more substitutions selected from $1X^4$, $17X^5$, and $85X^6$, wherein X^4 is selected from D and E, X^5 is selected from G, A, V, I, L, and M, and X^6 is selected from G.A, V, I, L, and M;

c. a VK3|L16 germline subfamily, comprising one or more substitutions selected from $4X^{10}$, $13X^{11}$, $76X^{12}$, 78F, $95X^{13}$, $97X^{14}$, and 98P, wherein X^{10} is selected from G, A, V, I, L, and M, X^{11} is selected from G, A, V, I, L, and M, X^{12} is selected from D and E, X^{13} is selected from R, K and H, and X^{14} is selected from D and E, wherein the mutant antigen binding protein does not comprise only substitution 78F;

d. a VK3|IL6 germline subfamily, comprising one or more substitutions selected from $76X^{12}$ and $95X^{13}$;

e. an Fc domain sequence comprising one or more substitutions selected from $253X^{10}$, $440X^{11}$, and $439X^{12}$, wherein X^{10} is selected from G, A, V, I, L, and M, X^{11} is selected from R, K, and H, and X^{12} is selected from D and E, wherein the antigen binding protein comprises at least one of $253X^{15}$ or modifications selected from subparagraphs a, b, c, d and f when X^{16} is K and X^{17} is E and the antigen binding protein specifically binds CD20; and

f. an Fc domain sequence comprising at the C-terminus $X^{18}X^{19}$ wherein X^{18} is one to four amino acids selected from D and E or from H, K, and R, and X^{19} is selected from P, M, G, A, V, I, L, S, T, N, Q, F, Y and W and is absent when X^{18} comprises D or E, is present when X^{18} comprises K or R at its C-terminal end, and is present or absent when X^{18} comprises H at its C-terminal end, and wherein the antigen binding protein comprises at least one of $253X^{15}$ or substitutions selected from subparagraphs a through e when PGKP (SEQ ID NO:381), PGKKP (SEQ ID NO:382), PGKKKP (SEQ ID NO:383), or PGE appears at the C-terminus and the antigen binding protein specifically binds CD20 or CD38. Preferred Fc C-terminal modifications comprise KP, KKP, KKKP (SEQ ID NO:380), E or EE at the C-terminus;

wherein the variable region amino acids are numbered according to the Aho numbering system and all amino acids from conserved regions including Fc are according to EU numbering.

[0022] Preferred antigen binding proteins in accordance with this disclosure are those wherein the foregoing modifications are applied to antibodies of Figures 1A and 1B hereinafter. Also preferred are antigen binding proteins wherein:

[0023] the VH1|1-18 germline subfamily sequence comprises one or more substitutions selected from 82R, 94S, and 95R, with antigen binding proteins having all such substitutions most preferred;

[0024] the VH3|3-33 germline subfamily sequence comprises one or more of substitutions 1E, 17G, and 85A, with antigen binding proteins having all such substitutions most preferred;

[0025] the VK3|L16 germline subfamily sequence comprises one or more substitutions selected from 4L, 13L, 76D, 95R, 97E, and 98P, with antigen binding proteins having all such substitutions most preferred;

[0026] the VK3|L6 germline subfamily sequence comprises one or more substitutions selected from 76D and 95R, with antigen binding proteins having both such substitutions most preferred;

[0027] the Fc domain sequence comprises one or more substitutions selected from 253A, 440K, and 439E, with antigen binding proteins having all such substitutions most preferred; and

[0028] the Fc domain C-terminus comprises a sequence selected from KP, KKP, KKKP (SEQ ID NO:380), and E. .

[0029] All of the foregoing preferred amino acid substitutions in variable regions are identified by the Aho numbering system. All residues in conserved regions including Fc are identified by the EU numbering system.

[0030] Preferred antigen binding proteins in accordance with this disclosure include: antibodies AF, AK, AL, AN and AO from Figure 1B having one or more, most preferably all, of the foregoing VH1|1-18 germline subfamily substitutions; antibodies AQ, AM, AI, and AG from Figure 1B having one or more, most preferably all, of the VH3|3-33 germline subfamily substitutions; antibodies AF and AQ having one or more, most preferably all, of the VK3|L16 germline subfamily substitutions; antibody AJ from Figure 1B having one or more, most preferably all, of the VK3|L6 germline subfamily substitutions; and antibodies BA, AH, and AN from Figure 1B having one or more, preferably all, of the Fc substitutions noted above.

[0031] Due to the foregoing sequence modifications, the disclosure further relates to antigen binding proteins that specifically bind to PCSK9 comprising a heavy chain sequence selected from SEQ ID NOS: 352, 353, 354, 366, and 368, preferably also comprising a light chain sequence of SEQ ID NO: 351.

[0032] Due to the foregoing sequence modifications, the disclosure also relates to antigen binding proteins that specifically bind c-fms comprising a heavy chain sequence selected from SEQ ID NOS: 356, 357, and 358, preferably further comprising a light chain sequence of SEQ ID NO: 355.

[0033] Due to the foregoing sequence modifications, the disclosure also relates to antigen binding proteins that specifically bind GIPR comprising a heavy chain sequence selected from SEQ ID NOS: 359, 361, 362, 364, and 368, preferably further comprising a light chain sequence selected from SEQ ID NOS: 360, 363, 365, and 367.

[0034] All modified antigen binding proteins are useful for the same indications as described previously for the unmodified antibodies.

[0035] Each of the antigen binding proteins from Figures 1A and 1B having mutated heavy chains is preferred to further comprise a light chain sequence as noted in the unmodified parent antibody of Figures 1A and 1B. Each of the foregoing antigen binding proteins having a mutated light chain is preferred to further comprise a heavy chain sequence as noted above or as appearing in the unmodified parent antibody of Figures 1A and 1B.

[0036] The disclosure further comprises antigen binding proteins as described above that have improved pharmacokinetic properties. The disclosure comprises an

antigen binding protein optionally having any of the aforementioned sequence modifications wherein:

a. the antigen binding protein comprises the sequence modification $440X^{16}$ relative to a parental antibody lacking the $440X^{16}$ sequence modification,
b. the antigen binding protein reaches maximum serum concentration after subcutaneous injection faster than does the parental antibody when the antigen binding protein and the parental antibody are administered at the same concentration, and
c. the antigen binding protein reaches a maximum serum concentration after subcutaneous injection that is higher than that of the parental antibody when the antigen binding protein and the parental antibody are administered at the same concentration.

The preferred parental antibody for such an antigen binding protein is a PCSK9 binding polypeptide, with antibody AK most preferred. The preferred substituent X^{16} in such an antigen binding protein is K. Further within this disclosure is a method of treating hypercholesterolemia with such an antigen binding protein.

[0037] The disclosure also relates to isolated nucleic acids encoding the antigen binding proteins of the disclosure, as well as vectors comprising the nucleic acids, host cells comprising the vectors, and methods of making and using the antigen binding proteins.

[0038] In other examples, the present disclosure provides compositions comprising the antigen binding proteins and kits comprising the antigen binding proteins, as well as articles of manufacture comprising the antigen binding proteins.

BRIEF DESCRIPTION OF THE FIGURES

[0039] Figures 1A and 1B show a table of viscosity values measured for IgG1 and IgG2 monoclonal antibodies formulated at 150 mg/mL in a formulation buffer including 20 mM acetate and 9% sucrose at pH 5.2 (without polysorbate). Figures 1A and 1B show the targets of the antibodies studied as well as their light and heavy chain types, germline subfamilies, concentration, pI, and viscosity. Each antibody in Figures 1A and 1B has the amino acid sequences as noted in the figures and the Sequence Listing. The heavy and light chain amino

CLAIMS

What is claimed is:

1. A method of reducing the viscosity of an antigen binding protein, which comprises making one or more modifications in the sequence of the antigen binding protein selected from:
   a. if the antigen binding protein comprises the VH3|3-33 germline subfamily, modifying the VH3 sequence to comprise one or more substitutions selected from $1X^4$, $17X^5$, and $85X^6$, wherein X^4 is selected from D and E, X^5 is selected from G, A, V, I, L, and M, and W, and X^6 is selected from G.A, V, I, L, and M;
   b. if the antigen binding protein comprises the VK3|L16 germline subfamily, modifying the VK3 sequence to comprise one or more substitutions selected from $4X^{10}$, $13X^{11}$, $76X^{12}$, 78F, $95X^{13}$, $97X^{14}$, and 98P, wherein X^{10} is selected from G, A, V, I, L, and M, X^{11} is selected from G, A, V, I, L, and M, X^{12} is selected from D and E, X^{13} is selected from R, K and H, and X^{14} is selected from D and E;
   c. if the antigen binding protein comprises the VK3|L6 germline subfamily, modifying the VK3 sequence to comprise one or more substitutions selected from $76X^{12}$ and $95X^{13}$;
   d. modifying the Fc domain sequence to comprise one or more substitutions selected from $253X^{15}$, $440X^{16}$, and $439X^{17}$, wherein X^{15} is selected from G, A, V, I, L, and M, X^{16} is selected from R, K, and H, and X^{17} is selected from D and E, wherein the Fc domain sequence comprises only one of $440X^{16}$ and $439X^{17}$; and
   e. modifying the C-terminus of the Fc domain sequence to comprise $X^{18}X^{19}$ wherein X^{18} is one to four amino acids selected from D and E or from H, K, and R, and X^{19} is selected from P, M, G, A, V, I, L, S, T, N, Q, F, Y and W and is absent when X^{18} comprises D or E, is present when X^{18} comprises K or R at its C-terminal end, and is present or absent when X^{18} comprises H at its C-terminal end;

   wherein the amino acids in subparagraphs a, b, c, and d are numbered according to the Aho numbering system and the amino acids in subparagraph e are numbered according to the EU numbering system.