METHODS OF PRODUCING CONCENTRATED FORMULATED DRUG SUBSTANCES COMPRISING PROTEINS, AND CONCENTRATED FORMULATED DRUG SUBSTANCE MADE BY THE METHODS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Application Serial No. 63/554,394, filed February 16, 2024, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTIONS

[0002] The present inventions provide for improved methods of producing concentrated formulated drug substances comprising proteins of interest, as well as concentrated formulated drug substances made by the methods. For example, Fc-containing proteins, such as antibodies and Fc-fusion proteins, are amenable to the inventions. The inventions provide, among other things, improved approaches for use of arginine during the production process of concentrated formulated drug substances comprising proteins of interest.

BACKGROUND OF THE INVENTIONS

[0003] Concentrated antibody preparations for use as formulated drug substances (FDS) are desired in the medical field. Typically, FDS concentrations are 150 g/l or more.

[0004] Concentrated antibody preparations, however, are subject to increased viscosity, which is caused by weak intermolecular reactions due to hydrophobic interactions and attractions caused by charged regions. Although antibodies generally have a net positive charge, antibodies also have negatively charged regions, both charges participate in antibody self-association.

[0005] Arginine (usually Arg-HCl) has been added to antibody preparations to decrease viscosity, and is termed “arginine-spiking”. It has been reported that arginine disrupts antibody intermolecular reactions by interacting with aromatic groups and charged side chains. By decreasing viscosity, arginine allows for higher antibody concentrations during ultrafiltration/diafiltration (UF/DF) and other production processes.

[0006] Arginine tends to self-associate, which has a crowding effect on antibodies in solution. Arginine when present during UF/DF also is subject to volume exclusion and the Gibbs-Donnan effect. Volume exclusion refers to the expulsion of buffer through the semi-permeable membrane due to the larger volume occupied by the protein as volume increases. The Gibbs-Donnan effect refers to the movement of excipients through a semi permeable membrane and retention of monoclonal antibodies during UF/DF. See **FIG. 1**.

[0007] A final concentrated pool (FCP) is the protein pool that results from UF/DF, and can be referred to as a concentrated protein pool. Examples include concentrated Fc-containing protein pools and concentrated antibody pools.

[0008] Past approaches for the final concentrated pools would target arginine concentrations in the final concentrated pool to be within +/- 20% of the concentration in the formulated drug substance (FDS). These past approaches, however, are susceptible to volume exclusion and the Gibbs-Donnan effect. Accordingly, different arginine approaches are needed to facilitate the production of highly-concentrated protein preparations, such as concentrated antibodies and other Fc-containing proteins.

SUMMARY OF THE INVENTIONS

[0009] The present inventions facilitate the production of highly-concentrated preparations of proteins of interest, including but not limited to, antibodies and other Fc-containing proteins.

[0010] The inventions provide methods of producing a concentrated formulated drug substance including Fc-containing proteins, wherein the methods include the steps of: (a) providing a concentrated Fc-containing protein pool; (b) mixing the concentrated Fc-containing protein pool with an arginine salt solution to form a concentrated Fc-containing protein pool that has reduced viscosity; (c) purifying the concentrated Fc-containing protein pool from step (b) to form a concentrated formulated drug substance; and (d) mixing the concentrated formulated drug substance with an arginine salt solution to reduce viscosity, wherein the molarity of arginine after mixing in step (b) is less than about 80% of the molarity of arginine after the mixing in step (d). The arginine salt can be arginine-HCl. The purifying step can include ultrafiltration and diafiltration. The inventions provide methods of producing concentrated formulated drug substances that can have an Fc-containing protein concentration of at least about 150 g/l, 151 g/l, 152 g/l, 153 g/l, 154 g/l, 155 g/l, 156 g/l, 157 g/l, 158 g/l, 159 g/l, 160 g/l, 161 g/l, 162 g/l, 163 g/l, 164 g/l, 165 g/l, 166 g/l, 167 g/l, 168 g/l, 169 g/l, 170 g/l, 171 g/l, 172 g/l, 173 g/l, 174 g/l, 175 g/l, 176 g/l, 177 g/l, 178 g/l, 179 g/l, 180 g/l, 181 g/l, 182 g/l, 183 g/l, 184 g/l, 185 g/l, 186 g/l, 187 g/l, 188 g/l, 189 g/l, 190 g/l, 191 g/l, 192 g/l, 193 g/l, 194 g/l, 195 g/l, 196 g/l, 197 g/l, 198 g/l, 199 g/l, 200 g/l, 201 g/l, 202 g/l, 203 g/l, 204 g/l, 205 g/l, 206 g/l, 207 g/l, 208 g/l, 209 g/l, 210 g/l, 211 g/l, 212 g/l, 213 g/l, 214 g/l, 215 g/l, 216 g/l, 217 g/l, 218 g/l, 219 g/l, 220 g/l, 221 g/l, 222 g/l, 223 g/l, 224 g/l, 225 g/l, 226 g/l, 227 g/l, 228 g/l, 229 g/l, 230 g/l, 231 g/l, 232 g/l, 233 g/l, 234 g/l, 235 g/l, 236 g/l, 237 g/l,

238 g/l, 239 g/l, 240 g/l, 241 g/l, 242 g/l, 243 g/l, 244 g/l, 245 g/l, 246 g/l, 247 g/l, 248 g/l, 249 g/l, 250 g/l, 251 g/l, 252 g/l, 253 g/l, 254 g/l, 255 g/l, 256 g/l, 257 g/l, 258 g/l, 259 g/l or 260 g/l. The molarity of arginine after mixing in step (b) can be about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 79.1%, 79.2%, 79.3%, 79.4%, 79.5%, 79.6%, 79.7%, 79.8% or 79.9% of the molarity of arginine after the mixing in step (d).

[0011] The Fc-containing proteins used according to the inventions can be antibodies, and derivatives and fragments of antibodies. Antibodies include, but not limited to, monoclonal antibodies or fragments thereof, and bispecific antibodies or fragments thereof. The Fc-containing proteins also can be Fc-fusion proteins, including, but not limited to, receptor Fc-fusion proteins, such as trap proteins. The purifying can include ultrafiltration and diafiltration and/or other prufication methodologies available to the skilled person, including but not limited to chromatography, tangential flow filtration and centrigugation.

[0012] The molarity of arginine after mixing in step (b) can be about 40 mM to about 80 mM, about 40 mM to about 60 mM, about 40 mM to about 70 mM, about 20 mM to about 80 mM, about 20 mM to about 60 mM, about 20 mM to about 70 mM, about 30 mM to about 80 mM, about 30 mM to about 60 mM, or about 30 mM to about 70 mM. The molarity of arginine after mixing in step (b) can be about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, or

about 65 mM. The molarity of arginine after mixing in step (b) can be about 40 mM, about 50 mM, or about 60 mM.

[0013] The molarity of arginine after the mixing in step (d) can be about 100 mM to about 150 mM, about 100 mM to about 105 mM, about 100 mM to about 125 mM, about 55 mM to about 125 mM, about 55 mM to about 105 mM, about 55 mM to about 125 mM, about 75 mM to about 150 mM, about 75 mM to about 105 mM, or about 75 mM to about 125 mM. The molarity of arginine after the mixing in step (d) can be about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, or about 125 mM.

[0014] The Fc-containing protein is a human bispecific antibody that binds to different epitopes on MET. The molarity of arginine after mixing in step (b) is about 60 mM; and wherein the molarity of arginine after the mixing in step (d) is about 125 mM.

[0015] The Fc-containing protein is an anti-EGFR x anti-CD28 bispecific antibody. The molarity of arginine after mixing in step (b) is about 40 mM; and wherein the molarity of arginine after the mixing in step (d) is about 80 mM.

[0016] The Fc-containing protein is an anti-factor XI antibody. The molarity of arginine after mixing in step (b) is about 50 mM; and wherein the molarity of arginine after the mixing in step (d) is about 100 mM.

[0017] The inventions also provide concentrated formulated drug substances including Fc-containing proteins produced by methods that include the steps of: (a) providing a concentrated Fc-containing protein pool; (b) mixing the concentrated Fc-containing protein pool with an arginine salt solution to form a concentrated Fc-containing protein pool that has reduced viscosity; (c) purifying the

concentrated Fc-containing protein pool from step (b) to form a concentrated formulated drug substance; and (d) mixing the concentrated formulated drug substance with an arginine salt solution to reduce viscosity, wherein the molarity of arginine after mixing in step (b) is less than about 80% of the molarity of arginine after the mixing in step (d). The arginine salt can be arginine-HCl. The purifying step can include ultrafiltration and diafiltration.

[0018] The concentrated formulated drug substances provided according to the inventions can have an Fc-containing protein concentration of at least about 150 g/l, 151 g/l, 152 g/l, 153 g/l, 154 g/l, 155 g/l, 156 g/l, 157 g/l, 158 g/l, 159 g/l, 160 g/l, 161 g/l, 162 g/l, 163 g/l, 164 g/l, 165 g/l, 166 g/l, 167 g/l, 168 g/l, 169 g/l, 170 g/l, 171 g/l, 172 g/l, 173 g/l, 174 g/l, 175 g/l, 176 g/l, 177 g/l, 178 g/l, 179 g/l, 180 g/l, 181 g/l, 182 g/l, 183 g/l, 184 g/l, 185 g/l, 186 g/l, 187 g/l, 188 g/l, 189 g/l, 190 g/l, 191 g/l, 192 g/l, 193 g/l, 194 g/l, 195 g/l, 196 g/l, 197 g/l, 198 g/l, 199 g/l, 200 g/l, 201 g/l, 202 g/l, 203 g/l, 204 g/l, 205 g/l, 206 g/l, 207 g/l, 208 g/l, 209 g/l, 210 g/l, 211 g/l, 212 g/l, 213 g/l, 214 g/l, 215 g/l, 216 g/l, 217 g/l, 218 g/l, 219 g/l, 220 g/l, 221 g/l, 222 g/l, 223 g/l, 224 g/l, 225 g/l, 226 g/l, 227 g/l, 228 g/l, 229 g/l, 230 g/l, 231 g/l, 232 g/l, 233 g/l, 234 g/l, 235 g/l, 236 g/l, 237 g/l, 238 g/l, 239 g/l, 240 g/l, 241 g/l, 242 g/l, 243 g/l, 244 g/l, 245 g/l, 246 g/l, 247 g/l, 248 g/l, 249 g/l, 250 g/l, 251 g/l, 252 g/l, 253 g/l, 254 g/l, 255 g/l, 256 g/l, 257 g/l, 258 g/l, 259 g/l or 260 g/l. The molarity of arginine after mixing in step (b) can be about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 79.1%,

79.2%, 79.3%, 79.4%, 79.5%, 79.6%, 79.7%, 79.8% or 79.9% of the molarity of arginine after the mixing in step (d).

[0019] The Fc-containing proteins used according to the inventions can be antibodies, and derivatives and fragments of antibodies. Antibodies include, but not limited to, monoclonal antibodies or fragments thereof, and bispecific antibodies or fragments thereof. The Fc-containing proteins also can be Fc-fusion proteins, including, but not limited to, receptor Fc-fusion proteins, such as trap proteins.

[0020] The molarity of arginine after mixing in step (b) can be about 40 mM to about 80 mM, about 40 mM to about 60 mM, about 40 mM to about 70 mM, about 20 mM to about 80 mM, about 20 mM to about 60 mM, about 20 mM to about 70 mM, about 30 mM to about 80 mM, about 30 mM to about 60 mM, or about 30 mM to about 70 mM. The molarity of arginine after mixing in step (b) can be about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, or about 65 mM. The molarity of arginine after mixing in step (b) can be about 40 mM, about 50 mM, or about 60 mM.

[0021] The molarity of arginine after the mixing in step (d) can be about 100 mM to about 150 mM, about 100 mM to about 105 mM, about 100 mM to about 125 mM, about 55 mM to about 125 mM, about 55 mM to about 105 mM, about 55 mM to about 125 mM, about 75 mM to about 150 mM, about 75 mM to about 105 mM, or about 75 mM to about 125 mM. The molarity of arginine after the mixing in step (d) can be about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, or about 125 mM.

[0022] The Fc-containing protein is a human bispecific antibody that binds to different epitopes on MET. The molarity of arginine after mixing in step (b) is

about 60 mM; and wherein the molarity of arginine after the mixing in step (d) is about 125 mM.

[0023] The Fc-containing protein is an anti-EGFR x anti-CD28 bispecific antibody. The molarity of arginine after mixing in step (b) is about 40 mM; and wherein the molarity of arginine after the mixing in step (d) is about 80 mM.

[0024] The Fc-containing protein is an anti-factor XI antibody. The molarity of arginine after mixing in step (b) is about 50 mM; and wherein the molarity of arginine after the mixing in step (d) is about 100 mM.

[0025] The inventions also provide method of producing a concentrated formulated drug substance comprising Fc-containing proteins, wherein the method comprises the steps of: (a) providing a concentrated Fc-containing protein pool; (b) mixing the concentrated Fc-containing protein pool with an arginine salt solution to form a concentrated Fc-containing protein pool that has reduced viscosity; (c) purifying the concentrated Fc-containing protein pool from step (b) to form a concentrated formulated drug substance; and (d) mixing the concentrated formulated drug substance with an arginine salt solution to reduce viscosity, wherein the molarity of arginine after mixing in step (b) is less than about 95% of the molarity of arginine after the mixing in step (d). The arginine salt can be arginine-HCl.

[0026] The molarity of arginine after mixing in step (b) can be about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 94.1%, 94.2%, 94.3%, 94.5%, 94.6%, 94.7%, 94.8%, or 94.9% of the molarity of arginine after the mixing in step (d).

[0027] The Fc-containing proteins can be antibodies or fragments thereof. The antibodies can be monoclonal antibodies or fragments thereof, or bispecific antibodies or fragments thereof. The Fc-containing proteins can be Fc-fusion proteins. The Fc-fusion proteins can be receptor Fc-fusion proteins, optionally wherein the receptor Fc-fusion proteins are trap proteins. The purifying can include ultrafiltration and diafiltration and/or other prufication methodologies available to the skilled person, including but not limited to chromatography, tangential flow filtration and centrigugation.

[0028] The molarity of arginine after mixing in step (b) can be about 1.5 mM to about 285 mM, 1.5 mM to about 275 mM, 1.5 mM to about 250 mM, 1.5 mM to about 225 mM, 1.5 mM to about 200 mM, 1.5 mM to about 190 mM, 1.5 mM to about 180 mM, 1.5 mM to about 170 mM, 1.5 mM to about 160 mM, 1.5 mM to about 150 mM, 1.5 mM to about 140 mM, 1.5 mM to about 130 mM, 1.5 mM to about 120 mM, 1.5 mM to about 110 mM, 1.5 mM to about 100 mM, 1.5 mM to about 90 mM, 1.5 mM to about 80 mM, 1.5 mM to about 70 mM, 1.5 mM to about 60 mM, 1.5 mM to about 50 mM, 1.5 mM to about 40 mM, 1.5 mM to about 30 mM, 1.5 mM to about 20 mM, 1.5 mM to about 10 mM, 1.5 mM to about 5 mM, 5 mM to about 285 mM, 5 mM to about 275 mM, 5 mM to about 250 mM, 5 mM to about 225 mM, 5 mM to about 200 mM, 5 mM to about 190 mM, 5 mM to about 180 mM, 5 mM to about 170 mM, 5 mM to about 160 mM, 5 mM to about 150 mM, 5 mM to about 140 mM, 5 mM to about 130 mM, 5 mM to about 120 mM, 5 mM to about 110 mM, 5 mM to about 100 mM, 5 mM to about 90 mM, 5 mM to about 80 mM, 5 mM to about 70 mM, 5 mM to about 60 mM, 5 mM to about 50 mM, 5 mM to about 40 mM, 5 mM to about 30

mM, 5 mM to about 20 mM, 5 mM to about 10 mM, 10 mM to about 285 mM, 10 mM to about 275 mM, 10 mM to about 250 mM, 10 mM to about 225 mM, 10 mM to about 200 mM, 10 mM to about 190 mM, 10 mM to about 180 mM, 10 mM to about 170 mM, 10 mM to about 160 mM, 10 mM to about 150 mM, 10 mM to about 140 mM, 10 mM to about 130 mM, 10 mM to about 120 mM, 10 mM to about 110 mM, 10 mM to about 100 mM, 10 mM to about 90 mM, 10 mM to about 80 mM, 10 mM to about 70 mM, 10 mM to about 60 mM, 10 mM to about 50 mM, 10 mM to about 40 mM, 10 mM to about 30 mM, 10 mM to about 20 mM, 20 mM to about 285 mM, 20 mM to about 275 mM, 20 mM to about 250 mM, 20 mM to about 225 mM, 20 mM to about 200 mM, 20 mM to about 190 mM, 20 mM to about 180 mM, 20 mM to about 170 mM, 20 mM to about 160 mM, 20 mM to about 150 mM, 20 mM to about 140 mM, 20 mM to about 130 mM, 20 mM to about 120 mM, 20 mM to about 1 20 mM, 20 mM to about 100 mM, 20 mM to about 90 mM, 20 mM to about 80 mM, 20 mM to about 70 mM, 20 mM to about 60 mM, 20 mM to about 50 mM, 20 mM to about 40 mM, 20 mM to about 30 mM, 30 mM to about 285 mM, 30 mM to about 275 mM, 30 mM to about 250 mM, 30 mM to about 225 mM, 30 mM to about 200 mM, 30 mM to about 190 mM, 30 mM to about 180 mM, 30 mM to about 170 mM, 30 mM to about 160 mM, 30 mM to about 150 mM, 30 mM to about 140 mM, 30 mM to about 130 mM, 30 mM to about 120 mM, 30 mM to about 110 mM, 30 mM to about 100 mM, 30 mM to about 90 mM, 30 mM to about 80 mM, 30 mM to about 70 mM, 30 mM to about 60 mM, 30 mM to about 50 mM, 30 mM to about 40 mM, 40 mM to about 285 mM, 40 mM to about 275 mM, 40 mM to about 250 mM, 40 mM to about 225 mM, 40 mM to about 200 mM, 40 mM to about 190 mM, 40 mM to about 180 mM, 40 mM to about 170 mM, 40 mM to about 160 mM, 40 mM to about 150 mM, 40 mM to about 140 mM, 40 mM to about 130 mM, 40 mM to about 120 mM, 40 mM to about 110

mM, 40 mM to about 100 mM, 40 mM to about 90 mM, 40 mM to about 80 mM, 40 mM to about 70 mM, 40 mM to about 60 mM, 40 mM to about 50 mM, 50 mM to about 285 mM, 50 mM to about 275 mM, 50 mM to about 250 mM, 50 mM to about 225 mM, 50 mM to about 200 mM, 50 mM to about 190 mM, 50 mM to about 180 mM, 50 mM to about 170 mM, 50 mM to about 160 mM, 50 mM to about 150 mM, 50 mM to about 140 mM, 50 mM to about 130 mM, 50 mM to about 120 mM, 50 mM to about 110 mM, 50 mM to about 100 mM, 50 mM to about 90 mM, 50 mM to about 80 mM, 50 mM to about 70 mM, 50 mM to about 60 mM, 60 mM to about 285 mM, 60 mM to about 275 mM, 60 mM to about 250 mM, 60 mM to about 225 mM, 60 mM to about 200 mM, 60 mM to about 190 mM, 60 mM to about 180 mM, 60 mM to about 170 mM, 60 mM to about 160 mM, 60 mM to about 150 mM, 60 mM to about 140 mM, 60 mM to about 130 mM, 60 mM to about 120 mM, 60 mM to about 110 mM, 60 mM to about 100 mM, 60 mM to about 90 mM, 60 mM to about 80 mM, 60 mM to about 70 mM, 70 mM to about 285 mM, 70 mM to about 275 mM, 70 mM to about 250 mM, 70 mM to about 225 mM, 70 mM to about 200 mM, 70 mM to about 190 mM, 70 mM to about 180 mM, 70 mM to about 170 mM, 70 mM to about 160 mM, 70 mM to about 150 mM, 70 mM to about 140 mM, 70 mM to about 130 mM, 70 mM to about 120 mM, 70 mM to about 110 mM, 70 mM to about 100 mM, 70 mM to about 90 mM, 70 mM to about 80 mM, 80 mM to about 285 mM, 80 mM to about 275 mM, 80 mM to about 250 mM, 80 mM to about 225 mM, 80 mM to about 200 mM, 80 mM to about 190 mM, 80 mM to about 180 mM, 80 mM to about 170 mM, 80 mM to about 160 mM, 80 mM to about 150 mM, 80 mM to about 140 mM, 80 mM to about 130 mM, 80 mM to about 120 mM, 80 mM to about 110 mM, 80 mM to about 100 mM, 80 mM to about 90 mM, 90 mM to about 285 mM, 90 mM to about 275 mM, 90 mM to about 250 mM, 90 mM to about 225 mM, 90 mM to about 200 mM, 90 mM to

about 190 mM, 90 mM to about 180 mM, 90 mM to about 170 mM, 90 mM to about 160 mM, 90 mM to about 150 mM, 90 mM to about 140 mM, 90 mM to about 130 mM, 90 mM to about 120 mM, 90 mM to about 110 mM, 90 mM to about 100 mM, 100 mM to about 285 mM, 100 mM to about 275 mM, 100 mM to about 250 mM, 100 mM to about 225 mM, 100 mM to about 200 mM, 100 mM to about 190 mM, 100 mM to about 180 mM, 100 mM to about 170 mM, 100 mM to about 160 mM, 100 mM to about 150 mM, 100 mM to about 140 mM, 100 mM to about 130 mM, 100 mM to about 120 mM, 100 mM to about 110 mM, 110 mM to about 285 mM, 110 mM to about 275 mM, 110 mM to about 250 mM, 110 mM to about 225 mM, 110 mM to about 200 mM, 110 mM to about 190 mM, 110 mM to about 180 mM, 110 mM to about 170 mM, 110 mM to about 160 mM, 110 mM to about 150 mM, 110 mM to about 140 mM, 110 mM to about 130 mM, 110 mM to about 120 mM, 120 mM to about 285 mM, 120 mM to about 275 mM, 120 mM to about 250 mM, 120 mM to about 225 mM, 120 mM to about 200 mM, 120 mM to about 190 mM, 120 mM to about 180 mM, 120 mM to about 170 mM, 120 mM to about 160 mM, 120 mM to about 150 mM, 120 mM to about 140 mM, 110 mM to about 130 mM, 130 mM to about 285 mM, 130 mM to about 275 mM, 130 mM to about 250 mM, 130 mM to about 225 mM, 130 mM to about 200 mM, 130 mM to about 190 mM, 130 mM to about 180 mM, 130 mM to about 170 mM, 130 mM to about 160 mM, 130 mM to about 150 mM, 130 mM to about 140 mM, 140 mM to about 285 mM, 140 mM to about 275 mM, 140 mM to about 250 mM, 140 mM to about 225 mM, 140 mM to about 200 mM, 140 mM to about 190 mM, 140 mM to about 180 mM, 140 mM to about 170 mM, 140 mM to about 160 mM, 140 mM to about 150 mM, 150 mM to about 285 mM, 150 mM to about 275 mM, 150 mM to about 250 mM, 150 mM to about 225 mM, 150 mM to about 200 mM, 150 mM to about 190 mM, 150 mM to

about 180 mM, 150 mM to about 170 mM, 150 mM to about 160 mM, 160 mM to about 285 mM, 160 mM to about 275 mM, 160 mM to about 250 mM, 160 mM to about 225 mM, 160 mM to about 200 mM, 160 mM to about 190 mM, 160 mM to about 180 mM, 160 mM to about 170 mM, 170 mM to about 285 mM, 170 mM to about 275 mM, 170 mM to about 250 mM, 170 mM to about 225 mM, 170 mM to about 200 mM, 170 mM to about 190 mM, 170 mM to about 180 mM, 180 mM to about 285 mM, 180 mM to about 275 mM, 180 mM to about 250 mM, 180 mM to about 225 mM, 180 mM to about 200 mM, 180 mM to about 190 mM, 190 mM to about 285 mM, 190 mM to about 275 mM, 190 mM to about 250 mM, 190 mM to about 225 mM, 190 mM to about 200 mM, 200 mM to about 285 mM, 200 mM to about 275 mM, 200 mM to about 250 mM, 200 mM to about 225 mM, 225 mM to about 285 mM, 225 mM to about 275 mM, 225 mM to about 250 mM, 250 mM to about 285 mM, 250 mM to about 275 mM, or 275 mM to about 285 mM.

[0029] The molarity of arginine after the mixing in step (d) can be about 7.5 mM to about 297 mM, about 7.5 mM to about 290 mM, about 7.5 mM to about 270 mM, about 7.5 mM to about 250 mM, about 7.5 mM to about 230 mM, about 7.5 mM to about 210 mM, about 7.5 mM to about 190 mM, about 7.5 mM to about 170 mM, about 7.5 mM to about 150 mM, about 7.5 mM to about 130 mM, about 7.5 mM to about 110 mM, about 7.5 mM to about 90 mM, about 7.5 mM to about 70 mM, about 7.5 mM to about 50 mM, about 7.5 mM to about 30 mM, about 7.5 mM to about 10 mM, about 10 mM to about 290 mM, about 10 mM to about 270 mM, about 10 mM to about 250 mM, about 10 mM to about 230 mM, about 10 mM to about 210 mM, about 10 mM to about 190 mM, about 10 mM to about 170 mM, about 10 mM to about 150 mM, about 10 mM to about 130 mM, about 10 mM to about 110 mM, about 10 mM to about 90 mM, about 10 mM to about 70 mM, about 10 mM to about

50 mM, about 10 mM to about 30 mM, about 30 mM to about 290 mM, about 30 mM to about 270 mM, about 30 mM to about 250 mM, about 30 mM to about 230 mM, about 30 mM to about 210 mM, about 30 mM to about 190 mM, about 30 mM to about 170 mM, about 30 mM to about 150 mM, about 30 mM to about 130 mM, about 30 mM to about 110 mM, about 30 mM to about 90 mM, about 30 mM to about 70 mM, about 30 mM to about 50 mM, about 50 mM to about 290 mM, about 50 mM to about 270 mM, about 50 mM to about 250 mM, about 50 mM to about 230 mM, about 50 mM to about 210 mM, about 50 mM to about 190 mM, about 50 mM to about 170 mM, about 50 mM to about 150 mM, about 50 mM to about 130 mM, about 50 mM to about 110 mM, about 50 mM to about 90 mM, about 50 mM to about 70 mM, about 70 mM to about 290 mM, about 70 mM to about 270 mM, about 70 mM to about 250 mM, about 70 mM to about 230 mM, about 70 mM to about 210 mM, about 70 mM to about 190 mM, about 70 mM to about 170 mM, about 70 mM to about 150 mM, about 70 mM to about 130 mM, about 70 mM to about 110 mM, about 70 mM to about 90 mM, about 90 mM to about 290 mM, about 90 mM to about 270 mM, about 90 mM to about 250 mM, about 90 mM to about 230 mM, about 90 mM to about 210 mM, about 90 mM to about 190 mM, about 90 mM to about 170 mM, about 90 mM to about 150 mM, about 90 mM to about 130 mM, about 90 mM to about 110 mM, about 110 mM to about 290 mM, about 110 mM to about 270 mM, about 110 mM to about 250 mM, about 110 mM to about 230 mM, about 110 mM to about 210 mM, about 110 mM to about 190 mM, about 110 mM to about 170 mM, about 110 mM to about 150 mM, about 110 mM to about 130 mM, about 130 mM to about 290 mM, about 130 mM to about 270 mM, about 130 mM to about 250 mM, about 130 mM to about 230 mM, about 130 mM to about 210 mM, about 130 mM to about 190 mM, about 130 mM to about 170 mM, about 130 mM to about 150 mM,

about 150 mM to about 290 mM, about 150 mM to about 270 mM, about 150 mM to about 250 mM, about 150 mM to about 230 mM, about 150 mM to about 210 mM, about 150 mM to about 190 mM, about 150 mM to about 170 mM, about 170 mM to about 290 mM, about 170 mM to about 270 mM, about 170 mM to about 250 mM, about 170 mM to about 230 mM, about 170 mM to about 210 mM, about 170 mM to about 190 mM, about 190 mM to about 290 mM, about 190 mM to about 270 mM, about 190 mM to about 250 mM, about 190 mM to about 230 mM, about 190 mM to about 210 mM, about 210 mM to about 290 mM, about 210 mM to about 270 mM, about 210 mM to about 250 mM, about 210 mM to about 230 mM, about 230 mM to about 290 mM, about 230 mM to about 270 mM, about 230 mM to about 250 mM, about 250 mM to about 290 mM, about 250 mM to about 270 mM, or about 270 mM to about 290 mM.

[0030] The Fc-containing protein can be any antibody or trap protein, including but not limited to those disclosed herein. For instance, the antibody or trap protein can be one of dupilumab, cemiplimab, aflibercept and the antibodies of the Examples.

[0031] The inventions provide a concentrated formulated drug substance comprising Fc-containing proteins, which can be made by a method as disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

[0032] **FIG. 1** schematically depicts a retentate, semi-permeable membrane and permeate, and indicates movement of charged excipients through the semi-permeable membrane to the permeate side. The charged excipients are sufficiently small to pass through the semi-permeable membrane. In contrast, the

larger, monoclonal antibodies (mAb), although charged, are unable to pass though the semi-permeable membrane, which creates an unequal distribution of excipient ions. This results in a greater presence of excipient ions on the permeate side to balance the charges with the mAbs on the retentate side.

[0033] **FIG. 2** depicts protein concentration of a bispecific antibody (a human bispecific antibody that binds to different epitopes on MET (a transmembrane tyrosine kinase receptor that is activated by hepatocyte growth factor)) concentrated with Arg compared to control.

[0034] **FIG. 3** depicts data from an arginine adjustment study in ultrafiltration/diafiltration (UF/DF) using a PSMA x CD28 co-stimulatory bispecific antibody. The x-axis is the goal arginine spike concentration (mM). The y-axis is arginine loss in terms of protein concentration.

[0035] **FIG. 4** depicts data from an arginine adjustment study in ultrafiltration/diafiltration (UF/DF) using a human bispecific antibody that binds to different epitopes on MET (a transmembrane tyrosine kinase receptor that is activated by hepatocyte growth factor). The x-axis is the goal arginine spike concentration (mM). The y-axis is arginine loss in terms of protein concentration.

Detailed Description of the Inventions

[0036] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Definitions

[0037] The term “about” in the context of numerical values and ranges refers to values or ranges that approximate or are close to the recited values or

ranges such that the invention can perform as intended, such as having a desired rate, amount, density, degree, increase, decrease, percentage, value or presence of a form, variant, temperature or amount of time, as is apparent from the teachings contained herein. For example, “about” can signify values either above or below the stated value in a range of approx. +/- 10% or more or less depending on the ability to perform. Thus, this term encompasses values beyond those simply resulting from systematic error.

[0038] “Protein of interest” or “polypeptide of interest” (POI) can have any amino acid sequence, and includes any protein, polypeptide, or peptide that is desired to be expressed. Included are, but not limited to, viral proteins, bacterial proteins, fungal proteins, plant proteins and animal (including human) proteins. Protein types can include, but are not limited to, antibodies, antibody derivatives and fragments, receptors, Fc-containing proteins, Fc-fusion proteins, receptor Fc-fusion proteins, trap proteins (including mini-trap proteins), fusion proteins, antagonists, inhibitors, enzymes (such as those used in enzyme replacement therapy), factors, repressors, activators, ligands, reporter proteins, selection proteins, protein hormones, protein toxins, structural proteins, storage proteins, transport proteins, neurotransmitters and contractile proteins. Derivatives, components, domains, chains and fragments of the above also are included. The sequences can be natural, semi-synthetic or synthetic. A protein of interest is encoded by a “gene of interest” (GOI).

[0039] A “gene of interest” (GOI) encodes a “protein of interest” or “polypeptide of interest” and optionally can include other associated sequences. The sequences can be natural, semi-synthetic or synthetic. Native sequences,

mutant sequences and degenerate sequences can be GOIs. A gene of interest also can be referred to as a “transgene.”

[0040] “Polypeptide” or “peptide” refers to sequence(s) of amino acids covalently joined. Polypeptides include natural, semi-synthetic and synthetic proteins and protein fragments. “Polypeptide” and “protein” can be used interchangeably. Oligopeptides are considered shorter polypeptides.

[0041] “Antibodies” (also referred to as "immunoglobulins") are examples of proteins having multiple polypeptide chains and extensive post-translational modifications. The canonical immunoglobulin protein (for example, IgG) include four polypeptide chains - two light chains and two heavy chains. Each light chain is linked to one heavy chain via a cystine disulfide bond, and the two heavy chains are bound to each other via two cystine disulfide bonds. Immunoglobulins produced in mammalian systems are also glycosylated at various residues (for example, at asparagine residues) with various polysaccharides, and can differ from species to species, which may affect antigenicity for therapeutic antibodies. Butler and Spearman, "The choice of mammalian cell host and possibilities for glycosylation engineering", *Curr. Opin. Biotech.* 30:107-112 (2014).

[0042] Antibodies are often used as therapeutic biomolecules. An antibody includes immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain, CL. The VH and VL

regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (heavy chain CDRs may be abbreviated as HCDR1, HCDR2 and HCDR3; light chain CDRs may be abbreviated as LCDRI, LCDR2 and LCDR3. The term "high affinity" antibody refers to those antibodies having a binding affinity to their target of at least 10-9 M, at least 10-10 M; at least 10-11 M; or at least 10-12 M, as measured by surface plasmon resonance, for example, BIACORE™ or solution-affinity ELISA.

[0043] The phrase "bispecific antibody" includes an antibody capable of selectively binding two or more epitopes. Bispecific antibodies generally comprise two different heavy chains, with each heavy chain specifically binding a different epitope -- either on two different molecules (for example, antigens) or on the same molecule (for example, on the same antigen). If a bispecific antibody is capable of selectively binding two different epitopes (a first epitope and a second epitope), the affinity of the first heavy chain for the first epitope will generally be at least one to two, three or four orders of magnitude lower than the affinity of the first heavy chain for the second epitope, and vice versa. The epitopes recognized by the bispecific antibody can be on the same or a different target (for example, on the same or a different protein). Bispecific antibodies can be made, for example, by combining heavy chains that recognize different epitopes of the same antigen. For example, nucleic acid sequences encoding heavy chain variable sequences that recognize different epitopes of the same antigen can be fused to nucleic acid sequences encoding different heavy chain constant regions, and such sequences can be

expressed in a cell that expresses an immunoglobulin light chain. A typical bispecific antibody has two heavy chains each having three heavy chain CDRs, followed by (N-terminal to C-terminal) a CH1 domain, a hinge, a CH2 domain, and a CH3 domain, and an immunoglobulin light chain that either does not confer antigen-binding specificity but that can associate with each heavy chain, or that can associate with each heavy chain and that can bind one or more of the epitopes bound by the heavy chain antigen-binding regions, or that can associate with each heavy chain and enable binding or one or both of the heavy chains to one or both epitopes.

[0044] The phrase "heavy chain," or "immunoglobulin heavy chain" includes an immunoglobulin heavy chain constant region sequence from any organism, and unless otherwise specified includes a heavy chain variable domain. Heavy chain variable domains include three heavy chain CDRs and four FR regions, unless otherwise specified. Fragments of heavy chains include CDRs, CDRs and FRs, and combinations thereof. A typical heavy chain has, following the variable domain (from N-terminal to C-terminal), a CH1 domain, a hinge, a CH2 domain, and a CH3 domain. A functional fragment of a heavy chain includes a fragment that is capable of specifically recognizing an antigen (for example, recognizing the antigen with a KD in the micromolar, nanomolar, or picomolar range), that is capable of expressing and secreting from a cell, and that comprises at least one CDR.

[0045] The phrase "light chain" includes an immunoglobulin light chain constant region sequence from any organism, and unless otherwise specified includes human kappa and lambda light chains. Light chain variable (VL) domains typically include three light chain CDRs and four framework (FR) regions, unless otherwise specified. Generally, a full-length light chain includes, from amino terminus

to carboxyl terminus, a VL domain that includes FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, and a light chain constant domain. Light chains that can be used with these inventions include those, for example, that do not selectively bind either the first or second antigen selectively bound by the antigen-binding protein. Suitable light chains include those that can be identified by screening for the most commonly employed light chains in existing antibody libraries (wet libraries or in silico), where the light chains do not substantially interfere with the affinity and/or selectivity of the antigen-binding domains of the antigen-binding proteins. Suitable light chains include those that can bind one or both epitopes that are bound by the antigen-binding regions of the antigen-binding protein.

[0046] The phrase "variable domain" includes an amino acid sequence of an immunoglobulin light or heavy chain (modified as desired) that comprises the following amino acid regions, in sequence from N-terminal to C-terminal (unless otherwise indicated): FRI, CDRI, FR2, CDR2, FR3, CDR3, FR4. A "variable domain" includes an amino acid sequence capable of folding into a canonical domain (VH or VL) having a dual beta sheet structure wherein the beta sheets are connected by a disulfide bond between a residue of a first beta sheet and a second beta sheet.

[0047] The phrase "complementarity determining region," or the term "CDR," includes an amino acid sequence encoded by a nucleic acid sequence of an organism's immunoglobulin genes that normally (i.e., in a wild-type animal) appears between two framework regions in a variable region of a light or a heavy chain of an immunoglobulin molecule (for example, an antibody or a T cell receptor). A CDR can be encoded by, for example, a germline sequence or a rearranged or unrearranged sequence, and, for example, by a naive or a mature B cell or a T cell. In some circumstances (for example, for a CDR3), CDRs can be encoded by two or more

sequences (for example, germline sequences) that are not contiguous (for example, in a nucleic acid sequence that has not been rearranged) but are contiguous in a B cell nucleic acid sequence, for example, as the result of splicing or connecting the sequences (for example, V-D-J recombination to form a heavy chain CDR3).

[0048] “Antibody derivatives and fragments” include, but are not limited to: antibody fragments (for example, ScFv-Fc, dAB-Fc, half antibodies, Fab, F(ab’)2), multispecifics (for example, IgG-ScFv, IgG-dab, ScFV-Fc-ScFV, tri-specific) and Fc-Fusion Proteins (for example, Fc-Fusion (N-terminal), Fc-fusion (C-terminal), mono Fc-fusion, bi-specific Fc-fusion).

[0049] The phrase "Fc-containing protein" includes antibodies, bispecific antibodies, antibody derivatives containing an Fc, antibody fragments containing an Fc, Fc-fusion proteins, immunoadhesins, and other binding proteins that comprise at least a functional portion of an immunoglobulin CH2 and CH3 region. A "functional portion" refers to a CH2 and CH3 region that can bind a Fc receptor (for example, an FcyR; or an FcRn, (neonatal Fc receptor), and/or that can participate in the activation of complement. If the CH2 and CH3 region contains deletions, substitutions, and/or insertions or other modifications that render it unable to bind any Fc receptor and also unable to activate complement, the CH2 and CH3 region is not functional. Fc-fusion proteins include, for example, Fc-fusion (N-terminal), Fc-fusion (C-terminal), mono-Fc-fusion and bispecific Fc-fusion proteins.

[0050] “Fc" stands for fragment crystallizable, and is often referred to as a fragment constant. Antibodies contain an Fc region that is made up of two identical protein sequences. IgG has heavy chains known as γ-chains. IgA has heavy chains known as α-chains, IgM has heavy chains known as μ-chains. IgD has heavy chains known as σ-chains. IgE has heavy chains known as ε-chains. In

nature, Fc regions are the same in all antibodies of a given class and subclass in the same species. Human IgGs have four subclasses and share about 95% homology amongst the subclasses. In each subclass, the Fc sequences are the same. For example, human IgG1 antibodies will have the same Fc sequences. Likewise, IgG2 antibodies will have the same Fc sequences; IgG3 antibodies will have the same Fc sequences; and IgG4 antibodies will have the same Fc sequences. Alterations in the Fc region create charge variation.

[0051] Fc-containing proteins can comprise modifications in immunoglobulin domains, including where the modifications affect one or more effector function of the binding protein (for example, modifications that affect FcyR binding, FcRn binding and thus half-life, and/or CDC activity). Such modifications include, but are not limited to, the following modifications and combinations thereof, with reference to EU numbering of an immunoglobulin constant region: 238, 239, 248, 249, 250, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 297, 298, 301, 303, 305, 307, 308, 309, 311, 312, 315, 318, 320, 322, 324, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 337, 338, 339, 340, 342, 344, 356, 358, 359, 360, 361, 362, 373, 375, 376, 378, 380, 382, 383, 384, 386, 388, 389, 398, 414, 416, 419, 428, 430, 433, 434, 435, 437, 438, and 439.

[0052] For example, and not by way of limitation, the binding protein is an Fc-containing protein and exhibits enhanced serum half-life (as compared with the same Fc-containing protein without the recited modification(s)) and have a modification at position 250 (for example, E or Q); 250 and 428 (for example, L or F); 252 (for example, L/Y/F/W or T), 254 (for example, S or T), and 256 (for example, S/R/Q/E/D or T); or a modification at 428 and/or 433 (for example, L/R/SI/P/Q or K)

and/or 434 (for example, H/F or Y); or a modification at 250 and/or 428; or a modification at 307 or 308 (for example, 308P, V308F), and 434. In another example, the modification can comprise a 428L (for example, M428L) and 434S (for example, N434S) modification; a 428L, 2591 (for example, V259I), and a 308F (for example, V308F) modification; a 433K (for example, H433K) and a 434 (for example, 434Y) modification; a 252, 254, and 256 (for example, 252Y, 254T, and 256E) modification; a 250Q and 428L modification (for example, T250Q and M428L); a 307 and/or 308 modification (for example, 308F or 308P).

[0053] Some recombinant Fc-containing proteins contain receptors or receptor fragments, ligands or ligand fragments that have cognate binding partners in biological systems, and include "Receptor Fc-fusion proteins," which refer to recombinant molecules that contain a soluble receptor fused to an immunoglobulin Fc domain.

[0054] "Fc-fusion proteins" comprise part or all of two or more proteins, one of which is an Fc portion of an immunoglobulin molecule, that are not fused in their natural state. Fc-fusion proteins include Fc-Fusion (N-terminal), Fc-Fusion (C-terminal), Mono Fc-Fusion and Bi-specific Fc-Fusion. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, for example, by Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88: 10535-39 (1991); Byrn et al., Nature 344:677-70, 1990; and Hollenbaugh et al., "Construction of Immunoglobulin Fusion Proteins", in Current Protocols in Immunology, Suppl. 4, pages 10.19.1 - 10.19.11 (1992). "Receptor Fc-fusion proteins" comprise one or more of one or more extracellular domain(s) of a receptor coupled to an Fc moiety, which can comprise a hinge region followed by a CH2 and CH3 domain of an immunoglobulin. The Fc-

fusion protein can contain two or more distinct receptor chains that bind to a single or more than one ligand(s). Some receptor Fc-fusion proteins may contain ligand binding domains of multiple different receptors. Receptor Fc-fusion proteins are also referred to as "traps," "trap molecules" or “trap proteins.” For example, such trap proteins include an IL-1 trap (for example, Rilonacept, which contains the IL-IRAcP ligand binding region fused to the IL-1R1 extracellular region fused to Fc of hIgGI ; see U.S. Pat. No. 6,927,044, or a VEGF Trap (for example, Aflibercept, which contains the Ig domain 2 of the VEGF receptor FltI fused to the Ig domain 3 of the VEGF receptor FlkI fused to Fc of hIgG1. See U. S. Pat. Nos. 7,087,411 and 7,279,159.

[0055] Rilonacept and aflibercept are examples of marketed trap proteins that antagonize IL1R (see US Pat. No. 7,927,583) and VEGF (see US Pat. No. 7,087,411), respectively. Other recombinant Fc-containing proteins include those recombinant proteins containing a peptide fused to an Fc domain. Recombinant Fc-containing proteins are described in C. Huang, "Receptor-Fc fusion therapeutics, traps, and MIMETIBODY™ technology," 20(6) Curr. Opin. Biotechnol. 692-9 (2009).

[0056] “Purification” in its various grammatical forms includes, but is not limited to, the use of one or more procedures such as depth filtration, tangential flow filtration, affinity capture chromatography, ionic exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, protein A chromatography, asymmetric field-flow fractionation, centrifugation and others available to the skilled person.

[0057] As used herein, “viscosity” refers to the rate of transfer of momentum of liquid. It is a quantity expressing the magnitude of internal friction, as measured by the force per unit area resisting a flow in which parallel layers unit

distance apart has unit speed relative to one another. In liquids, viscosity refers to the “thickness” of a liquid.

[0058] All numerical limits and ranges set forth herein include all numbers or values thereabout or there between of the numbers of the range or limit. The ranges and limits described herein expressly denominate and set forth all integers, decimals and fractional values defined and encompassed by the range or limit.

Detailed description

[0059] The present inventions provide a new adjustment strategy to permit high protein concentrations while minimizing arginine variability caused by the Gibbs-Donnan effect in final concentrated pools (FCP) of antibodies. Ultimately, the inventions facilitate FDS protein (for example, antibodies) of at least 150 g/l, 151 g/l, 152 g/l, 153 g/l, 154 g/l, 155 g/l, 156 g/l, 157 g/l, 158 g/l, 159 g/l, 160 g/l, 161 g/l, 162 g/l, 163 g/l, 164 g/l, 165 g/l, 166 g/l, 167 g/l, 168 g/l, 169 g/l, 170 g/l, 171 g/l, 172 g/l, 173 g/l, 174 g/l, 175 g/l, 176 g/l, 177 g/l, 178 g/l, 179 g/l, 180 g/l, 181 g/l, 182 g/l, 183 g/l, 184 g/l, 185 g/l, 186 g/l, 187 g/l, 188 g/l, 189 g/l, 190 g/l, 191 g/l, 192 g/l, 193 g/l, 194 g/l, 195 g/l, 196 g/l, 197 g/l, 198 g/l, 199 g/l, 200 g/l, 201 g/l, 202 g/l, 203 g/l, 204 g/l, 205 g/l, 206 g/l, 207 g/l, 208 g/l, 209 g/l, 210 g/l, 211 g/l, 212 g/l, 213 g/l, 214 g/l, 215 g/l, 216 g/l, 217 g/l, 218 g/l, 219 g/l, 220 g/l, 221 g/l, 222 g/l, 223 g/l, 224 g/l, 225 g/l, 226 g/l, 227 g/l, 228 g/l, 229 g/l, 230 g/l, 231 g/l, 232 g/l, 233 g/l, 234 g/l, 235 g/l, 236 g/l, 237 g/l, 238 g/l, 239 g/l, 240 g/l, 241 g/l, 242 g/l, 243 g/l, 244 g/l, 245 g/l, 246 g/l, 247 g/l, 248 g/l, 249 g/l, 250 g/l, 251 g/l, 252 g/l, 253 g/l, 254 g/l, 255 g/l, 256 g/l, 257 g/l, 258 g/l, 259 g/l, 260 g/l. Exemplary ranges of concentrations include 150 g/l to 260 g/l, 160 g/l to 250 g/l, 170 g/l to 240 g/l, 180 g/l to 240 g/l, 190 g/l top 240 g/l, 190 g/l to 235 g/l, 200 g/l to 235 g/l, 210 g/l to 235 g/l, 220 g/l to 235 g/l, 230 g/l to

240 g/l, 230 g/l to 235 g/l. The ranges above can be combined to form additional ranges.

[0060] The present inventions use the minimum amount of arginine needed to meet FCP quality attributes of minimizing turbidity, particle presence of opalescence. Rather, arginine can be added later in the process. Preferably, the arginine concentration in the FCP is less than 80% the arginine concentration in the FDS. For example, the arginine content in the FCP can be about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 79.1%, 79.2%, 79.3%, 79.4%, 79.5%, 79.6%, 79.7%, 79.8% or 79.9% of the amount of arginine in the FDS. Exemplary ranges of percentages for the arginine content in the FCP can include about 1% to 79%, 3% to 75%, 5% to 70%, 10% to 70%, 15% to 65%, 20% to 60%, 25% to 55%, 30% to 50%, 35% to 50%, 40% to 50%, 45% to 50%, 46% to 50%, 47% to 51%, 47% to 50%, 48% to 51%, 48% to 50%, 49% to 51%, 49% to 50%, 50% to 55%, 50% to 54%, 50% to 53%, 50% to 52% and 50% to 51% of the amount of arginine in the FDS.

[0061] Arginine can be added to the FCP at a concentration between about 20 mM to about 100 mM, about 20 mM to about 95 mM, about 20 mM to about 90 mM, about 20 mM to about 85 mM, about 20 mM to about 80 mM, about 20 mM to about 75 mM, about 20 mM to about 70 mM, about 20 mM to about 65 mM, about 20 mM to about 60 mM, about 20 mM to about 55 mM, about 20 mM to about 50 mM, about 20 mM to about 45 mM, about 20 mM to about 40 mM, about 20 mM to

about 35 mM, about 20 mM to about 30 mM, about 30 mM to about 100 mM, about 30 mM to about 95 mM, about 30 mM to about 90 mM, about 30 mM to about 85 mM, about 30 mM to about 80 mM, about 30 mM to about 75 mM, about 30 mM to about 70 mM, about 30 mM to about 65 mM, about 30 mM to about 60 mM, about 30 mM to about 55 mM, about 30 mM to about 50 mM, about 30 mM to about 45 mM, about 30 mM to about 40 mM, about 40 mM to about 100 mM, about 40 mM to about 95 mM, about 40 mM to about 90 mM, about 40 mM to about 85 mM, about 40 mM to about 80 mM, about 40 mM to about 75 mM, about 40 mM to about 70 mM, about 40 mM to about 65 mM, about 40 mM to about 60 mM, about 40 mM to about 55 mM, about 40 mM to about 50 mM, about 50 mM to about 100 mM, about 50 mM to about 95 mM, about 50 mM to about 90 mM, about 50 mM to about 85 mM, about 50 mM to about 80 mM, about 50 mM to about 75 mM, about 50 mM to about 70 mM, about 50 mM to about 65 mM, about 50 mM to about 60 mM, about 60 mM to about 100 mM, about 60 mM to about 95 mM, about 60 mM to about 90 mM, about 60 mM to about 85 mM, about 60 mM to about 80 mM, about 60 mM to about 75 mM, about 60 mM to about 70 mM, about 70 mM to about 100 mM, about 70 mM to about 95 mM, about 70 mM to about 90 mM, about 70 mM to about 85 mM, about 70 mM to about 80 mM, about 80 mM to about 100 mM, about 80 mM to about 95 mM, about 80 mM to about 90 mM, or about 90 mM to about 100 mM. Preferably, the arginine concentration in the FCP is between about 56 mM to about 84 mM. Preferably, the arginine concentration in the FCP is between about 31 mM to about 87 mM.

[0062] Exemplary arginine concentrations in the FCP can include about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, or about 100 mM.

Preferably, the arginine concentration in the FCP is about 60 mM. Preferably, the arginine concentration in the FCP is about 70 mM.

[0063] Arginine can be added to the FDS at a concentration between about 40 mM to about 200 mM, about 40 mM to about 175 mM, about 40 mM to about 150 mM, about 40 mM to about 125 mM, about 40 mM to about 100 mM, about 40 mM to about 90 mM, about 40 mM to about 80 mM, about 40 mM to about 70 mM, about 40 mM to about 60 mM, about 40 mM to about 50 mM, about 50 mM to about 200 mM, about 50 mM to about 175 mM, about 50 mM to about 150 mM, about 50 mM to about 125 mM, about 50 mM to about 100 mM, about 50 mM to about 90 mM, about 50 mM to about 80 mM, about 50 mM to about 70 mM, about 50 mM to about 60 mM, about 60 mM to about 200 mM, about 60 mM to about 175 mM, about 60 mM to about 150 mM, about 60 mM to about 125 mM, about 60 mM to about 100 mM, about 60 mM to about 90 mM, about 60 mM to about 80 mM, about 60 mM to about 70 mM, about 70 mM to about 200 mM, about 70 mM to about 175 mM, about 70 mM to about 150 mM, about 70 mM to about 125 mM, about 70 mM to about 100 mM, about 70 mM to about 90 mM, about 70 mM to about 80 mM, about 80 mM to about 200 mM, about 80 mM to about 175 mM, about 80 mM to about 150 mM, about 80 mM to about 125 mM, about 80 mM to about 100 mM, about 80 mM to about 90 mM, about 90 mM to about 200 mM, about 90 mM to about 175 mM, about 90 mM to about 150 mM, about 90 mM to about 125 mM, about 90 mM to about 100 mM, about 100 mM to about 200 mM, about 100 mM to about 175 mM, about 100 mM to about 150 mM, about 100 mM to about 125 mM, about 125 mM to about 200 mM, about 125 mM to about 175 mM, about 125 mM to about 150 mM, about 150 mM to about 200 mM, about 150 mM to about 175 mM, or about 175 mM to about 200 mM. Preferably, the arginine concentration in the FDS is between about 56 mM

to about 84 mM. Preferably, the arginine concentration in the FDS is between about 100 mM to about 150 mM.

[0064] Exemplary arginine concentrations in the FDS can include about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 110 mM, about 115 mM, about 120 mM, about 125 mM, about 130 mM, about 140 mM, about 145 mM, about 150 mM, about 155 mM, about 160 mM, about 165 mM, about 170 mM, about 175 mM, about 180 mM, about 185 mM, about 190 mM, about 195 mM, or about 200 mM. Preferably, the arginine concentration in the FDS is about 70 mM. Preferably, the arginine concentration in the FCP is about 125 mM.

[0065] For example, arginine adjustment concentration can be used for a human bispecific antibody that binds to different epitopes on MET, and where the FCP target arginine spike can be about 40 mM to about 80 mM, about 40 mM to about 75 mM, about 40 mM to about 70 mM, about 40 mM to about 65 mM, about 40 mM to about 45 mM, about 45 mM to about 80 mM, about 45 mM to about 75 mM, about 45 mM to about 70 mM, about 45 mM to about 65 mM, about 45 mM to about 60 mM, about 45 mM to about 55 mM, about 45 mM to about 50 mM, about 50 mM to about 80 mM, about 50 mM to about 75 mM, about 50 mM to about 70 mM, about 50 mM to about 65 mM, about 50 mM to about 60 mM, about 50 mM to about 55 mM, about 55 mM to about 80 mM, about 55 mM to about 75 mM, about 55 mM to about 70 mM, about 55 mM to about 65 mM, about 55 mM to about 60 mM, about 60 mM to about 80 mM, about 60 mM to about 75 mM, about 60 mM to about 70 mM, about 60 mM to about 65 mM, about 65 mM to about 80 mM, about 65 mM to about 75 mM, about 65 mM to about 70 mM, about 70 mM to about 80 mM, about 70 mM

to about 75 mM, or about 75 mM to about 80 mM. For example, the FCP target arginine spike can be about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, or about 80 mM. For example, the FCP can be about 55 mM, about 56 mM, about 57 mM, about 58 mM, about 59 mM, about 60 mM, about 61 mM, about 62 mM, about 63 mM, about 64 mM, or about 65 mM. For example, the FCP target arginine spike can be about 60 mM.

[0066] For example, final arginine concentration for the human bispecific antibody that binds to different epitopes on MET (e.g., the FDS), and where the arginine concentration in the FDS can be about 100 mM to about 150 mM, about 105 mM to about 150 mM, about 110 mM to about 150 mM, about 115 mM to about 150 mM, about 120 mM to about 150 mM, about 125 mM to about 150 mM, about 130 mM to about 150 mM, about 135 mM to about 150 mM, about 140 mM to about 150 mM, about 145 mM to about 150 mM, about 100 mM to about 145 mM, about 105 mM to about 145 mM, about 110 mM to about 145 mM, about 115 mM to about 145 mM, about 120 mM to about 145 mM, about 125 mM to about 145 mM, about 130 mM to about 145 mM, about 135 mM to about 145 mM, about 140 mM to about 145 mM, about 100 mM to about 140 mM, about 105 mM to about 140 mM, about 110 mM to about 140 mM, about 115 mM to about 140 mM, about 120 mM to about 140 mM, about 125 mM to about 140 mM, about 130 mM to about 140 mM, about 135 mM to about 140 mM, about 100 mM to about 135 mM, about 105 mM to about 135 mM, about 110 mM to about 135 mM, about 115 mM to about 135 mM, about 120 mM to about 135 mM, about 125 mM to about 135 mM, about 130 mM to about 135 mM, about 100 mM to about 130 mM, about 105 mM to about 130 mM, about 110 mM to about 130 mM, about 115 mM to about 130 mM, about 120 mM to

about 130 mM, about 125 mM to about 130 mM, about 100 mM to about 125 mM, about 105 mM to about 125 mM, about 110 mM to about 125 mM, about 115 mM to about 125 mM, about 120 mM to about 125 mM, about 100 mM to about 120 mM, about 105 mM to about 120 mM, about 110 mM to about 120 mM, about 115 mM to about 120 mM, about 100 mM to about 115 mM, about 105 mM to about 115 mM, about 110 mM to about 115 mM, about 100 mM to about 110 mM, about 105 mM to about 110 mM, or about 100 mM to about 105 mM.

[0067] For example, the FDS target arginine spike can be about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, about 125 mM, about 130 mM, about 135 mM, about 140 mM, about 145 mM, about or 150 mM. For example, the FDS target arginine concentration can be about 120 mM, about 121 mM, about 122 mM, about 123 mM, about 124 mM, about 125 mM, about 126 mM, about 127 mM, about 128 mM, about 129 mM, or about 130 mM. For example, the FDS target arginine spike can be about 125 mM.

[0068] Arginine adjustment concentration can be used for an anti-EGFR x anti-CD28 bispecific antibody.

[0069] For example, arginine adjustment concentration can be used for an anti-EGFR x anti-CD28 bispecific antibody, and where the FCP target arginine spike can be about 20 mM to about 60 mM, about 20 mM to about 55 mM, about 20 mM to about 50 mM, about 20 mM to about 45 mM, about 20 mM to about 40 mM, about 20 mM to about 35 mM, about 20 mM to about 30 mM, about 20 mM to about 25 mM, about 25 mM to about 60 mM, about 25 mM to about 55 mM, about 25 mM to about 50 mM, about 25 mM to about 45 mM, about 25 mM to about 40 mM, about 25 mM to about 35 mM, about 25 mM to about 30 mM, about 30 mM to about 60 mM, about 30 mM to about 55 mM, about 30 mM to about 50 mM, about 30 mM to

about 45 mM, about 30 mM to about 40 mM, about 30 mM to about 35 mM, about 35 mM to about 60 mM, about 35 mM to about 55 mM, about 35 mM to about 50 mM, about 35 mM to about 45 mM, about 35 mM to about 40 mM, about 40 mM to about 60 mM, about 40 mM to about 55 mM, about 40 mM to about 50 mM, about 40 mM to about 45 mM, about 45 mM to about 60 mM, about 45 mM to about 55 mM, about 45 mM to about 50 mM, about 50 mM to about 60 mM, about 50 mM to about 55 mM, or about 55 mM to about 60 mM.

[0070] For example, the FCP target arginine spike can be about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, or about 60 mM. For example, the FCP target arginine spike can be about 35 mM, about 36 mM, about 37 mM, about 38 mM, about 39 mM, about 40 mM, about 41 mM, about 42 mM, about 43 mM, about 44 mM, or about 45 mM. For example, the arginine concentration in the FCP can be about 60 mM.

[0071] For example, final arginine concentration for the an anti-EGFR x anti-CD28 bispecific antibody (e.g., the FDS), and where the arginine concentration in the FDS can be about 55 mM to about 105 mM, about 55 mM to about 100 mM, about 55 mM to about 95 mM, about 55 mM to about 90 mM, about 55 mM to about 85 mM, about 55 mM to about 80 mM, about 55 mM to about 75 mM, about 55 mM to about 70 mM, about 55 mM to about 65 mM, about 55 mM to about 60 mM, about 60 mM to about 105 mM, about 60 mM to about 100 mM, about 60 mM to about 95 mM, about 60 mM to about 90 mM, about 60 mM to about 85 mM, about 60 mM to about 80 mM, about 60 mM to about 75 mM, about 60 mM to about 70 mM, about 60 mM to about 65 mM, about 65 mM to about 105 mM, about 65 mM to about 100 mM, about 65 mM to about 95 mM, about 65 mM to about 90 mM, about 65 mM to about 85 mM, about 65 mM to about 80 mM, about 65 mM to about 75 mM, about 65 mM

to about 70 mM, about 70 mM to about 105 mM, about 70 mM to about 100 mM, about 70 mM to about 95 mM, about 70 mM to about 90 mM, about 70 mM to about 85 mM, about 70 mM to about 80 mM, about 70 mM to about 75 mM, about 75 mM to about 105 mM, about 75 mM to about 100 mM, about 75 mM to about 95 mM, about 75 mM to about 90 mM, about 75 mM to about 85 mM, about 75 mM to about 80 mM, about 80 mM to about 105 mM, about 80 mM to about 100 mM, about 80 mM to about 95 mM, about 80 mM to about 90 mM, about 80 mM to about 85 mM, about 85 mM to about 105 mM, about 85 mM to about 100 mM, about 85 mM to about 95 mM, about 85 mM to about 90 mM, about 90 mM to about 105 mM, about 90 mM to about 100 mM, about 90 mM to about 95 mM, about 95 mM to about 105 mM, about 95 mM to about 100 mM, or about 100 mM to about 105 mM.

[0072] For example, final arginine concentration for the an anti-EGFR x anti-CD28 bispecific antibody (e.g., the FDS), and where the arginine concentration in the FDS can be about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, or about 105 mM. For example, the final arginine concentration in the FDS can be 75 mM, about 76 mM, about 77 mM, about 78 mM, about 79 mM, about 80 mM, about 81 mM, about 82 mM, about 83 mM, about 84 mM, or about 85 mM. For example, the final arginine concentration in the FDS can be about 80 mM.

[0073] For example, arginine adjustment concentration can be used for an anti-factor XI antibody, and where the FCP target arginine spike can be about 30 mM to about 70 mM, about 30 mM to about 65 mM, about 30 mM to about 60 mM, about 30 mM to about 55 mM, about 30 mM to about 50 mM, about 30 mM to about 45 mM, about 30 mM to about 40 mM, about 30 mM to about 35 mM, about 35 mM to about 70 mM, about 35 mM to about 65 mM, about 35 mM to about 60 mM, about

35 mM to about 55 mM, about 35 mM to about 50 mM, about 35 mM to about 45 mM, about 35 mM to about 40 mM, about 40 mM to about 70 mM, about 40 mM to about 65 mM, about 40 mM to about 60 mM, about 40 mM to about 55 mM, about 40 mM to about 50 mM, about 40 mM to about 45 mM, about 45 mM to about 70 mM, about 45 mM to about 65 mM, about 45 mM to about 60 mM, about 45 mM to about 55 mM, about 45 mM to about 50 mM, about 50 mM to about 70 mM, about 50 mM to about 65 mM, about 50 mM to about 60 mM, about 50 mM to about 55 mM, about 55 mM to about 70 mM, about 55 mM to about 65 mM, about 55 mM to about 60 mM, about 60 mM to about 70 mM, about 60 mM to about 65 mM, or about 65 mM to about 70 mM. For example, arginine adjustment concentration can be used for the anti-factor XI antibody, and where the FCP target arginine spike can be about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, or about 70 mM. For example, arginine adjustment concentration can be used for the anti-factor XI antibody, and where the FCP target arginine spike can be about 45 mM, about 46 mM, about 47 mM, about 48 mM, about 49 mM, about 50 mM, about 51 mM, about 52 mM, about 52 mM, about 53 mM, about 54 mM, or about 55 mM. For example, arginine adjustment concentration can be used for the anti-factor XI antibody, and where the FCP target arginine spike can be about 50 mM.

[0074] For example, final arginine concentration for the anti-factor XI antibody (e.g., the FDS), and where the arginine concentration in the FDS can be about 75 mM to about 125 mM, about 75 mM to about 120 mM, about 75 mM to about 115 mM, about 75 mM to about 110 mM, about 75 mM to about 105 mM, about 75 mM to about 100 mM, about 75 mM to about 95 mM, about 75 mM to about 90 mM, about 75 mM to about 85 mM, about 75 mM to about 80 mM, about 80 mM

to about 125 mM, about 80 mM to about 120 mM, about 80 mM to about 115 mM, about 80 mM to about 110 mM, about 80 mM to about 105 mM, about 80 mM to about 100 mM, about 80 mM to about 95 mM, about 80 mM to about 90 mM, about 80 mM to about 85 mM, about 85 mM to about 125 mM, about 85 mM to about 120 mM, about 85 mM to about 115 mM, about 85 mM to about 110 mM, about 85 mM to about 105 mM, about 85 mM to about 100 mM, about 85 mM to about 95 mM, about 85 mM to about 90 mM, about 90 mM to about 125 mM, about 90 mM to about 120 mM, about 90 mM to about 115 mM, about 90 mM to about 110 mM, about 90 mM to about 105 mM, about 90 mM to about 100 mM, about 90 mM to about 95 mM, about 95 mM to about 125 mM, about 95 mM to about 120 mM, about 95 mM to about 115 mM, about 95 mM to about 110 mM, about 95 mM to about 105 mM, about 95 mM to about 100 mM, about 100 mM to about 125 mM, about 100 mM to about 120 mM, about 100 mM to about 115 mM, about 100 mM to about 110 mM, about 100 mM to about 105 mM, about 105 mM to about 125 mM, about 105 mM to about 120 mM, about 105 mM to about 115 mM, about 105 mM to about 110 mM, about 110 mM to about 125 mM, about 110 mM to about 120 mM, about 110 mM to about 115 mM, about 115 mM to about 125 mM, about 115 mM to about 120 mM, or about 120 mM to about 125 mM.

[0075] For example, final arginine concentration for the anti-factor XI antibody (e.g., the FDS), and where the arginine concentration in the FDS can be about 75 mM, about 80 mM, about 85 mM, about 85 mM, about 90 mM, about 95 mM, about 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, or about 125 mM. For example, final arginine concentration for the anti-factor XI antibody (e.g., the FDS), and where the arginine concentration in the FDS can be about 95 mM, about 96 mM, about 97 mM, about

98 mM, about 99 mM, about 100 mM, about 101 mM, about 102 mM, about 103 mM, about 104 mM, or about 105 mM. For example, final arginine concentration for the anti-factor XI antibody (e.g., the FDS), and where the arginine concentration in the FDS can be about 100 mM.

[0076] For example, the Fc-containing protein can be a human bispecific antibody that binds to different epitopes on MET. The molarity of arginine after mixing in step (b) can be about 60 mM; and wherein the molarity of arginine after the mixing in step (d) can be about 125 mM.

[0077] For example, the Fc-containing protein can be an anti-EGFR x anti-CD28 bispecific antibody. The molarity of arginine after mixing in step (b) can be about 40 mM; and wherein the molarity of arginine after the mixing in step (d) can be about 80 mM.

[0078] For example, the Fc-containing protein can be an anti-factor XI antibody. The molarity of arginine after mixing in step (b) can be about 50 mM; and wherein the molarity of arginine after the mixing in step (d) can be about 100 mM.

[0079] The inventions allow for high protein concentration in the FDS while decreasing variability of the FCP arginine concentration. According to the inventions, an increase in protein concentration can be achieved that allows for smaller volumetric dosing. Antibody FDS concentrations up to approximately 240 g/l have been achieved.

[0080] Pharmaceutically acceptable excipients, such as, but not limited to, surfactants, amino acids, and stabilizers can be included in the inventions described herein. Choice of pharmaceutically acceptable excipient can be determined by the skilled person such that certain excipients can be included and other excipients can be excluded. Optionally, the pH of the FCP and/or FDS can be

about pH 5 to about 6.5. For example, the pH of the FCP and/or FDS can be about pH 5, about pH 5.2, about pH 5.4, about pH 5.6, about pH 5.8, about pH 6, about pH 6.2, about pH 6.4, or about pH 6.5.

[0081] For example, a surfactant (e.g., polysorbate) can be added to the FDS and/or FCP at a concentration between about 0.1 mg/ml to about 4 mg/ml, about 0.1 mM to about 3.6 mM, about 0.1 mM to about 3.2 mM, about 0.1 mM to about 2.8 mM, about 0.1 mM to about 2.4 mM, about 0.1 mM to about 2 mM, about 0.1 mM to about 1.6 mM, about 0.1 mM to about 1.2 mM, about 0.1 mM to about 0.8 mM, about 0.1 mM to about 0.4 mM, about 0.1 mM to about 0.2 mM, about 0.2 mM to about 4 mM, about 0.2 mM to about 3.6 mM, about 0.2 mM to about 3.2 mM, about 0.2 mM to about 2.8 mM, about 0.2 mM to about 2.4 mM, about 0.2 mM to about 2 mM, about 0.2 mM to about 1.6 mM, about 0.2 mM to about 1.2 mM, about 0.2 mM to about 0.8 mM, about 0.2 mM to about 0.4 mM, about 0.4 mM to about 4 mM, about 0.4 mM to about 3.6 mM, about 0.4 mM to about 3.2 mM, about 0.4 mM to about 2.8 mM, about 0.4 mM to about 2.4 mM, about 0.4 mM to about 2 mM, about 0.4 mM to about 1.6 mM, about 0.4 mM to about 1.2 mM, about 0.4 mM to about 0.8 mM, about 0.8 mM to about 4 mM, about 0.8 mM to about 3.6 mM, about 0.8 mM to about 3.2 mM, about 0.8 mM to about 2.8 mM, about 0.8 mM to about 2.4 mM, about 0.8 mM to about 2 mM, about 0.8 mM to about 1.6 mM, about 0.8 mM to about 1.2 mM, about 1.2 mM to about 4 mM, about 1.2 mM to about 3.6 mM, about 1.2 mM to about 3.2 mM, about 1.2 mM to about 2.8 mM, about 1.2 mM to about 2.4 mM, about 1.2 mM to about 2 mM, about 1.2 mM to about 1.6 mM, about 1.6 mM to about 4 mM, about 1.6 mM to about 3.6 mM, about 1.6 mM to about 3.2 mM, about 1.6 mM to about 2.8 mM, about 1.6 mM to about 2.4 mM, about 1.6 mM to about 2 mM, about 2 mM to about 4 mM, about 2 mM to about 3.6 mM, about 2 mM to about

3.2 mM, about 2 mM to about 2.8 mM, about 2 mM to about 2.4 mM, about 2.4 mM to about 4 mM, about 2.4 mM to about 3.6 mM, about 2.4 mM to about 3.2 mM, about 2.4 mM to about 2.8 mM, about 2.8 mM to about 4 mM, about 2.8 mM to about 3.6 mM, about 2.8 mM to about 3.2 mM, about 3.2 mM to about 4 mM, about 3.2 mM to about 3.6 mM, or about 3.6 mM to about 4 mM.

[0082] For example, the amino acid (e.g., histidine or proline) can be added to the FDS and/or FCP at a concentration between about 2 mM to about 16 mM, about 2 mM to about 14 mM, about 2 mM to about 12 mM, about 2 mM to about 10 mM, about 2 mM to about 8 mM, about 2 mM to about 6 mM, about 2 mM to about 4 mM, about 4 mM to about 20 mM, about 4 mM to about 18 mM, about 4 mM to about 16 mM, about 4 mM to about 14 mM, about 4 mM to about 12 mM, about 4 mM to about 10 mM, about 4 mM to about 8 mM, about 4 mM to about 6 mM, about 6 mM to about 20 mM, about 6 mM to about 18 mM, about 6 mM to about 16 mM, about 6 mM to about 14 mM, about 6 mM to about 12 mM, about 6 mM to about 10 mM, about 6 mM to about 8 mM, about 8 mM to about 20 mM, about 8 mM to about 18 mM, about 8 mM to about 16 mM, about 8 mM to about 14 mM, about 8 mM to about 12 mM, about 8 mM to about 10 mM, about 10 mM to about 20 mM, about 10 mM to about 18 mM, about 10 mM to about 16 mM, about 10 mM to about 14 mM, about 10 mM to about 12 mM, about 12 mM to about 20 mM, about 12 mM to about 18 mM, about 12 mM to about 16 mM, about 12 mM to about 14 mM, about 14 mM to about 20 mM, about 14 mM to about 18 mM, about 14 mM to about 16 mM, about 16 mM to about 20 mM, about 16 mM to about 18 mM, or about 18 mM to about 20 mM.

[0083] For example, the stabilizer (e.g., sucrose) can be added to the FDS and/or FCP at a concentration between about 1 % (w/v) to about 10 % (w/v),

about 1 % (w/v) to about 8 % (w/v), about 1 % (w/v) to about 6 % (w/v), about 1 % (w/v) to about 4 % (w/v), about 1 % (w/v) to about 2 % (w/v), about 2 % (w/v) to about 10 % (w/v), about 2 % (w/v) to about 8 % (w/v), about 2 % (w/v) to about 6 % (w/v), about 2 % (w/v) to about 4 % (w/v), about 4 % (w/v) to about 10 % (w/v), about 4 % (w/v) to about 8 % (w/v), about 4 % (w/v) to about 6 % (w/v), about 6 % (w/v) to about 10 % (w/v), about 6 % (w/v) to about 8 % (w/v), or about 8 % (w/v) to about 10 % (w/v).

[0084] The inventions can be employed in the production of biological and pharmaceutical products. For example, for antibodies, the inventions are amendable for research and production use for diagnostics and therapeutics based upon all major antibody classes, namely IgG, IgA, IgM, IgD and IgE. IgG is a preferred class, such as IgG1 (including IgG1λ and IgG1κ), IgG2 and IgG4. Exemplary antibodies to be produced according to the inventions include, but are not limited to, Fianlimab, Linvoseltamab, Nezastomig, Odronextamab, Vonsetamig, Davutamig, Itepekimab, Mibavademab, Ubamatamab, Garetosmab, Pozalimab, Alirocumab, Atoltivimab, Maftivimab, Odesivimab, Odesivivmab-ebgn, Casirivimab, Imdevimab, Cemiplimab, Cemplimab-rwlc, Dupilumab, Evinacumab, Evinacumab-dgnb, Fasimumab, Nesvacumab, Trevogrumab, Rinucumab and Sarilumab. Exemplary antibodies to be produced according to the inventions include, but are not limited to, Abciximab, Adalimumab, Adalimumab-atto, Ado-trastuzumab, Alemtuzumab, Alirocumab, Atezolizumab, Avelumab, Basiliximab, Belimumab, Benralizumab, Bevacizumab, Bezlotoxumab, Blinatumomab, Brentuximab vedotin, Brodalumab, Canakinumab, Capromab pendetide, Certolizumab pegol, Cemiplimab, Cetuximab, Denosumab, Dinutuximab, Dupilumab, Durvalumab, Eculizumab, Elotuzumab, Emicizumab-kxwh, Emtansinealirocumab, Evinacumab, Evolocumab,

Fasinumab, Golimumab, Guselkumab, Ibritumomab tiuxetan, Idarucizumab, Infliximab, Infliximab-abda, Infliximab-dyyb, Ipilimumab, Ixekizumab, Mepolizumab, Necitumumab, Nesvacumab, Nivolumab, Obiltoxaximab, Obinutuzumab, Ocrelizumab, Oatumumab, Olaratumab, Omalizumab, Panitumumab, Pembrolizumab, Pertuzumab, Ramucirumab, Ranibizumab, Raxibacumab, Reslizumab, Rinucumab, Rituximab, Sarilumab, Secukinumab, Siltuximab, Tocilizumab, Tocilizumab, Trastuzumab, Trevogrumab, Ustekinumab, and Vedolizumab. Antibodies can include a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a multispecific antibody, a bispecific antibody, an antigen binding antibody fragment, a single chain antibody, a diabody, triabody or tetrabody, a Fab fragment or a F(ab')2 fragment, an IgD antibody, an IgE antibody, an IgM antibody, an IgG antibody, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody. The antibody can be an IgG1 antibody. The antibody can be an IgG2 antibody. The antibody can be an IgG3 antibody. The antibody can be an IgG4 antibody. The antibody can be a chimeric IgG2/IgG4 antibody. The antibody can be a chimeric IgG2/IgG1 antibody. The antibody can be a chimeric IgG2/IgG1/IgG4 antibody.

[0085] In addition to the antibodies described in the Examples and Figures, the antibody can be selected from the group consisting of an anti-Programmed Cell Death 1 antibody (e.g. an anti-PD1 antibody as described in U.S. Pat. Appln. Pub. No. US2015/0203579A1), an anti-Programmed Cell Death Ligand-1 (e.g. an anti-PD-L1 antibody as described in in U.S. Pat. Appln. Pub. No. US2015/0203580A1), an anti-Dll4 antibody, an anti-Angiopoetin-2 antibody (e.g. an anti-ANG2 antibody as described in U.S. Pat. No. 9,402,898), an anti-Angiopoetin-Like 3 antibody (e.g. an anti-AngPtl3 antibody as described in U.S. Pat. No.

9,018,356), an anti-platelet derived growth factor receptor antibody (e.g. an anti-PDGFR antibody as described in U.S. Pat. No. 9,265,827), an anti-Erb3 antibody, an anti-Prolactin Receptor antibody (e.g. anti-PRLR antibody as described in U.S. Pat. No. 9,302,015), an anti-Complement 5 antibody (e.g. an anti-C5 antibody as described in U.S. Pat. Appln. Pub. No US2015/0313194A1), an anti-TNF antibody, an anti-epidermal growth factor receptor antibody (e.g. an anti-EGFR antibody as described in U.S. Pat. No. 9,132,192 or an anti-EGFRvIII antibody as described in U.S. Pat. Appln. Pub. No. US2015/0259423A1), an anti-Proprotein Convertase Subtilisin Kexin-9 antibody (e.g. an anti-PCSK9 antibody as described in U.S. Pat. No. 8,062,640 or U.S. Pat. Appln. Pub. No. US2014/0044730A1), an anti-Growth And Differentiation Factor-8 antibody (e.g. an anti-GDF8 antibody, also known as anti-myostatin antibody, as described in U.S. Pat Nos. 8,871,209 or 9,260,515), an anti-Glucagon Receptor (e.g. anti-GCGR antibody as described in U.S. Pat. Appln. Pub. Nos. US2015/0337045A1 or US2016/0075778A1), an anti-VEGF antibody, an anti-IL1R antibody, an interleukin 4 receptor antibody (e.g. an anti-IL4R antibody as described in U.S. Pat. Appln. Pub. No. US2014/0271681A1 or U.S. Pat Nos. 8,735,095 or 8,945,559), an anti-interleukin 6 receptor antibody (e.g. an anti-IL6R antibody as described in U.S. Pat. Nos. 7,582,298, 8,043,617 or 9,173,880), an anti-IL1 antibody, an anti-IL2 antibody, an anti-IL3 antibody, an anti-IL4 antibody, an anti-IL5 antibody, an anti-IL6 antibody, an anti-IL7 antibody, an anti-interleukin 33 (e.g. anti-IL33 antibody as described in U.S. Pat. Appln. Pub. Nos. US2014/0271658A1 or US2014/0271642A1), an anti-Respiratory syncytial virus antibody (e.g. anti-RSV antibody as described in U.S. Pat. Appln. Pub. No. US2014/0271653A1), an anti-Cluster of differentiation 3 (e.g. an anti-CD3 antibody, as described in U.S. Pat. Appln. Pub. Nos. US2014/0088295A1 and US20150266966A1, and in U.S.

Application No. 62/222,605), an anti-Cluster of differentiation 20 (e.g. an anti-CD20 antibody as described in U.S. Pat. Appln. Pub. Nos. US2014/0088295A1 and US20150266966A1, and in U.S. Pat. No. 7,879,984), an anti-CD19 antibody, an anti-CD28 antibody, an anti-Cluster of Differentiation 48 (e.g. anti-CD48 antibody as described in U.S. Pat. No. 9,228,014), an anti-Fel d1 antibody (e.g. as described in U.S. Pat. No. 9,079,948), an anti-Middle East Respiratory Syndrome virus (e.g. an anti-MERS antibody as described in U.S. Pat. Appln. Pub. No. US2015/0337029A1), an anti-Ebola virus antibody (e.g. as described in U.S. Pat. Appln. Pub. No. US2016/0215040), an anti-Zika virus antibody, an anti-Lymphocyte Activation Gene 3 antibody (e.g. an anti-LAG3 antibody, or an anti-CD223 antibody), an anti-Nerve Growth Factor antibody (e.g. an anti-NGF antibody as described in U.S. Pat. Appln. Pub. No. US2016/0017029 and U.S. Pat. Nos. 8,309,088 and 9,353,176), an anti-Activin A antibody, an anti-SARS-CoV-2 antibody (variants), an anti-platelet derived growth factor-b antibody (e.g., anti-PDGF-b antagonist), an anti-Natriuretic Peptide Receptor 1 antibody (e.g., anti-NPR1 antibody/antagonist), an anti-leptin receptor (LEPR) antibody, an anti-interleukin 2 receptor subunit gamma antibody (e.g., anti-IL2Rg antibody), an anti-factor XI antibody, an anti-transmembrane serine protease 6 antibody (e.g., anti-TMPRSS6 antibody), and an anti-bet v 1 antibody. The bispecific antibody can be selected from the group consisting of an anti-CD3 x anti-CD20 bispecific antibody (as described in U.S. Pat. Appln. Pub. Nos. US2014/0088295A1 and US20150266966A1), an anti-CD3 x anti-Mucin 16 bispecific antibody (e.g., an anti-CD3 x anti-Muc16 bispecific antibody), and an anti-CD3 x anti-Prostate-specific membrane antigen bispecific antibody (e.g., an anti-CD3 x anti-PSMA bispecific antibody), an anti-BCMA (B cell maturation antigen) x anti-CD3 bispecific antibody, an anti-PSMA x anti-CD28 bispecific antibody, an anti-

PD-L1 antibody x anti-IL2Ra, an anti-MET x anti-MET (two distinct epitopes of Mesenchymal Epithelial Transition (MET)) bispecific antibody, an anti-CD28 x anti-Mucin 16 bispecific antibody (e.g., an anti-CD28 x anti-Muc16 bispecific antibody), an anti-CD22 x anti-CD28 bispecific antibody, an anti-epidermal growth factor receptor antibody x anti-CD28 bispecific antibody (e.g. an anti-EGFR x anti-CD28 bispecific antibody). See also U.S. Patent Publication No. US 2019/0285580 A1.

[0086] The inventions also are amenable to the production of other molecules, including fusion proteins. Preferred fusion proteins include Receptor-Fc-fusion proteins, such as Trap proteins. The protein of interest is a recombinant protein that contains an Fc moiety and another domain, (e.g., an Fc-fusion protein). The Fc-fusion protein can be a receptor Fc-fusion protein, which contains one or more extracellular domain(s) of a receptor coupled to an Fc moiety. The Fc moiety can comprise a hinge region followed by a CH2 and CH3 domain of an IgG. The receptor Fc-fusion protein can contain two or more distinct receptor chains that bind to either a single ligand or multiple ligands. For example, an Fc-fusion protein is a TRAP protein, such as for example an IL-1 trap (e.g., rilonacept, which contains the IL-1RAcP ligand binding region fused to the IL-1R1 extracellular region fused to Fc of hIgG1; see U.S. Pat. No. 6,927,044, or a VEGF trap (e.g., aflibercept or ziv-aflibercept, which contains the Ig domain 2 of the VEGF receptor Flt1 fused to the Ig domain 3 of the VEGF receptor Flk1 fused to Fc of hIgG1; see U.S. Pat. Nos. 7,087,411 and 7,279,159). The Fc-fusion protein can be a ScFv-Fc-fusion protein, which contains one or more of one or more antigen binding domain(s), such as a variable heavy chain fragment and a variable light chain fragment, of an antibody coupled to an Fc moiety.

[0087] Other proteins lacking Fc portions, such as recombinantly produced enzymes and mini-traps, also can be made according to the inventions . Mini-traps are trap proteins that use a multimerizing component (MC) instead of an Fc portion, and are disclosed in U.S. Patent Nos. 7,279,159 and 7,087,411. Derivatives, components, domains, chains and fragments of the above also are included.

[0088] The inventions are further described by the following Examples, which do not limit the inventions in any manner and are applicable to all sections of the descriptions of the inventions and the aspects of the inventions. The order of performance of the below examples can be altered or combined as determined by the person of skill in the art in view of the teachings and data contained herein.

Example 1 – Arginine Spike Normalized to Concentration of an PSMA x CD28 co-stimulatory bispecific antibody

[0089] Turning to **FIG. 3**, the x-axis is the goal arginine spike concentration (mM), and the y-axis is arginine loss in terms of protein concentration. The x-axis is the target concentration of arginine in the product prior to final concentration by UF/DF. It will be lower at the end of the process as a result of the loss from the Donnan effect.

[0090] The data show two responses as a function of increasing arginine spike concentration. First, the loss of arginine increases as function of increasing the arginine spike concentration demonstrating the Donnan effect as the increased arginine concentration provides more force for transfer across the UF/DF membrane. Second, there is increased variability in the arginine loss as the spike

concentration is increased. Therefore, operating with lower arginine spikes will decrease arginine loss and variability in the arginine concentration in the FCP.

Example 2 – Arginine Spike Normalized to Concentration of an human bispecific antibody that binds to different epitopes on MET

[0091] Turning to **FIG. 4**, the x-axis is the goal arginine spike concentration (mM), and the y-axis is arginine loss in terms of protein concentration. The x-axis is the starting or target concentration the load of the arginine concentration. It will be lower at the end of the process as a result of the loss from the Donnan effect.

[0092] Similar to Example 1, the data here show two responses as a function of increasing arginine spike concentration. First, the loss of arginine increases as function of increasing the arginine spike concentration demonstrating the Donnan effect as the increased arginine concentration provides more force for transfer across the UF/DF membrane. Second, there is increased variability in the arginine loss as the spike concentration is increased. Therefore, operating with lower arginine spikes will decrease arginine loss and variability in the arginine concentration in the FCP. **FIG. 2** depicts protein concentration after concentrating with and without Arg-HCl.

Example 3 – Analysis of the Data of Examples 1 and 2

[0093] Examples 1 and 2 demonstrate that the inventions provide greater acceptable FCP arginine range. See Table 1. Antibody concentrations of about 240 g/l were achieved while targeting less than 50% of the FDS concentration

target of 125 mM in the FCP. Further data is provided below in Table 1:

Table 1

	Acceptable Arginine Spike Concentration Range[(1)]	FCP Arginine Tolerance Range	Operating range based on % Margin Arginine Adjustment Effect on Arginine Response
Previous Approach	71-88 mM	48-72 mM	78.8%
New Approach	50-109 mM	31.3-86-7 mM [(2)]	34.7%
Results	42 mM wider range before failure	31.4 mM wider range	44.1% less effect on FCP arginine

[0094] (1) is determined via a UF/DF model simulations with a uniform distribution of arginine spike concentration. Values represent edge of failure (greater than 100 ppm).

(2) is:

FCP Arginine Conc. Limit (mM) =

$$\frac{(\text{DS Arg. Conc. (mM)} * \text{DS V. (L)}) - (\text{DS Suc. Exc. Arg. Conc. (mM)} * \text{DS Suc. Exc. V. (L)})}{\text{FCP Volume (L)}}$$

Legend: DS = Drug Substance; Suc. = Sucrose; Exc. = Excipient; Conc. = Concentration; Volume: V.

[0095] The inventions allow for high protein concentration in the FDS, decrease variability of FCP concentration, widen the acceptable FCP arginine range and decrease the statistically significant of arginine adjustment concentration on FCP arginine concentration.

[0096] It is to be understood that the description, specific examples and data are given by way of illustration and are not intended to limit the present inventions. Various changes and modifications within the present inventions, including combining teaching in whole and in part, will become apparent to the

skilled artisan from the discussion, disclosure and data contained herein, and thus are considered part of the inventions.

Example 4 – Parameters for Arginine Spike for a human bispecific antibody that binds to different epitopes on MET

[0097] Another strategy can decrease the cost, widen the acceptable window for FCP arginine concentration and the arginine spike concentration, minimize the Donnan effect during UF/DF, and decrease batch failure as a result of arginine adjustment concentration. Table 2 shows additional data from UF/DF for a human bispecific antibody that binds to different epitopes on MET (a transmembrane tyrosine kinase receptor that is activated by hepatocyte growth factor) (see Example 2).

[0098] Table 2

	Target Arg Spike	FCP Arg Target	FCP Arg % Tolerance of Target	Acceptable Arg Spike Conc. Range[1]
Previous strategy	Match FDS (125 mM)	125 mM (100-150 mM)	±20%	68-98 mM for 90 mM spike
New strategy	Minimize to target protein conc. (60 mM)	60 mM (31.3-86.7 mM)[2]	±44%	50-109 mM for 80 mM spike (bispecific Ab)
Impact	Saves ~47% arginine stock	Greater tolerance at FCP to enable successful FDS target		29 mM wider range for FCP arginine success

(1) Determined through UF/DF DoE model simulations with a uniform distribution of arginine spike concentration; values represent edge of failure (>100 ppm)(2) FCP Arginine Conc. Limit (mM)

[0099] For example, arginine adjustment concentration will be used for a human bispecific antibody that binds to different epitopes on MET, and where the FCP target arginine spike will be about 40 mM to about 80 mM, about 40 mM to about 75 mM, about 40 mM to about 70 mM, about 40 mM to about 65 mM, about

40 mM to about 45 mM, about 45 mM to about 80 mM, about 45 mM to about 75 mM, about 45 mM to about 70 mM, about 45 mM to about 65 mM, about 45 mM to about 60 mM, about 45 mM to about 55 mM, about 45 mM to about 50 mM, about 50 mM to about 80 mM, about 50 mM to about 75 mM, about 50 mM to about 70 mM, about 50 mM to about 65 mM, about 50 mM to about 60 mM, about 50 mM to about 55 mM, about 55 mM to about 80 mM, about 55 mM to about 75 mM, about 55 mM to about 70 mM, about 55 mM to about 65 mM, about 55 mM to about 60 mM, about 60 mM to about 80 mM, about 60 mM to about 75 mM, about 60 mM to about 70 mM, about 60 mM to about 65 mM, about 65 mM to about 80 mM, about 65 mM to about 75 mM, about 65 mM to about 70 mM, about 70 mM to about 80 mM, about 70 mM to about 75 mM, or about 75 mM to about 80 mM. For example, the FCP target arginine spike will be about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, or about 80 mM. For example, the FCP target arginine spike will be about 60 mM.

[00100] For example, final arginine concentration for the human bispecific antibody that binds to different epitopes on MET (e.g., the FDS), and where the arginine concentration in the FDS will be about 100 mM to about 150 mM, about 105 mM to about 150 mM, about 110 mM to about 150 mM, about 115 mM to about 150 mM, about 120 mM to about 150 mM, about 125 mM to about 150 mM, about 130 mM to about 150 mM, about 135 mM to about 150 mM, about 140 mM to about 150 mM, about 145 mM to about 150 mM, about 100 mM to about 145 mM, about 105 mM to about 145 mM, about 110 mM to about 145 mM, about 115 mM to about 145 mM, about 120 mM to about 145 mM, about 125 mM to about 145 mM, about 130 mM to about 145 mM, about 135 mM to about 145 mM, about 140 mM to about 145 mM, about 100 mM to about 140 mM, about 105 mM to about 140 mM,

about 110 mM to about 140 mM, about 115 mM to about 140 mM, about 120 mM to about 140 mM, about 125 mM to about 140 mM, about 130 mM to about 140 mM, about 135 mM to about 140 mM, about 100 mM to about 135 mM, about 105 mM to about 135 mM, about 110 mM to about 135 mM, about 115 mM to about 135 mM, about 120 mM to about 135 mM, about 125 mM to about 135 mM, about 130 mM to about 135 mM, about 100 mM to about 130 mM, about 105 mM to about 130 mM, about 110 mM to about 130 mM, about 115 mM to about 130 mM, about 120 mM to about 130 mM, about 125 mM to about 130 mM, about 100 mM to about 125 mM, about 105 mM to about 125 mM, about 110 mM to about 125 mM, about 115 mM to about 125 mM, about 120 mM to about 125 mM, about 100 mM to about 120 mM, about 105 mM to about 120 mM, about 110 mM to about 120 mM, about 115 mM to about 120 mM, about 100 mM to about 115 mM, about 105 mM to about 115 mM, about 110 mM to about 115 mM, about 100 mM to about 110 mM, about 105 mM to about 110 mM, or about 100 mM to about 105 mM.

[00101] For example, the FDS target arginine spike will be about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, about 125 mM, about 130 mM, about 135 mM, about 140 mM, about 145 mM, about or 150 mM. For example, the FDS target arginine spike will be about 125 mM.

Example 5 Parameters for Arginine Spike and an anti-EGFR x anti-CD28 bispecific antibody

[00102] Arginine adjustment concentration will be used for an anti-EGFR x anti-CD28 bispecific antibody.

[00103] For example, arginine adjustment concentration will be used for an anti-EGFR x anti-CD28 bispecific antibody, and where the FCP target arginine

spike will be about 20 mM to about 60 mM, about 20 mM to about 55 mM, about 20 mM to about 50 mM, about 20 mM to about 45 mM, about 20 mM to about 40 mM, about 20 mM to about 35 mM, about 20 mM to about 30 mM, about 20 mM to about 25 mM, about 25 mM to about 60 mM, about 25 mM to about 55 mM, about 25 mM to about 50 mM, about 25 mM to about 45 mM, about 25 mM to about 40 mM, about 25 mM to about 35 mM, about 25 mM to about 30 mM, about 30 mM to about 60 mM, about 30 mM to about 55 mM, about 30 mM to about 50 mM, about 30 mM to about 45 mM, about 30 mM to about 40 mM, about 30 mM to about 35 mM, about 35 mM to about 60 mM, about 35 mM to about 55 mM, about 35 mM to about 50 mM, about 35 mM to about 45 mM, about 35 mM to about 40 mM, about 40 mM to about 60 mM, about 40 mM to about 55 mM, about 40 mM to about 50 mM, about 40 mM to about 45 mM, about 45 mM to about 60 mM, about 45 mM to about 55 mM, about 45 mM to about 50 mM, about 50 mM to about 60 mM, about 50 mM to about 55 mM, or about 55 mM to about 60 mM.

[00104] For example, the FCP target arginine spike will be about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, or about 60 mM. For example, the arginine concentration in the FCP will be about 60 mM.

[00105] For example, final arginine concentration for the an anti-EGFR x anti-CD28 bispecific antibody (e.g., the FDS), and where the arginine concentration in the FDS will be about 55 mM to about 105 mM, about 55 mM to about 100 mM, about 55 mM to about 95 mM, about 55 mM to about 90 mM, about 55 mM to about 85 mM, about 55 mM to about 80 mM, about 55 mM to about 75 mM, about 55 mM to about 70 mM, about 55 mM to about 65 mM, about 55 mM to about 60 mM, about 60 mM to about 105 mM, about 60 mM to about 100 mM, about

60 mM to about 95 mM, about 60 mM to about 90 mM, about 60 mM to about 85 mM, about 60 mM to about 80 mM, about 60 mM to about 75 mM, about 60 mM to about 70 mM, about 60 mM to about 65 mM, about 65 mM to about 105 mM, about 65 mM to about 100 mM, about 65 mM to about 95 mM, about 65 mM to about 90 mM, about 65 mM to about 85 mM, about 65 mM to about 80 mM, about 65 mM to about 75 mM, about 65 mM to about 70 mM, about 70 mM to about 105 mM, about 70 mM to about 100 mM, about 70 mM to about 95 mM, about 70 mM to about 90 mM, about 70 mM to about 85 mM, about 70 mM to about 80 mM, about 70 mM to about 75 mM, about 75 mM to about 105 mM, about 75 mM to about 100 mM, about 75 mM to about 95 mM, about 75 mM to about 90 mM, about 75 mM to about 85 mM, about 75 mM to about 80 mM, about 80 mM to about 105 mM, about 80 mM to about 100 mM, about 80 mM to about 95 mM, about 80 mM to about 90 mM, about 80 mM to about 85 mM, about 85 mM to about 105 mM, about 85 mM to about 100 mM, about 85 mM to about 95 mM, about 85 mM to about 90 mM, about 90 mM to about 105 mM, about 90 mM to about 100 mM, about 90 mM to about 95 mM, about 95 mM to about 105 mM, about 95 mM to about 100 mM, or about 100 mM to about 105 mM.

[00106] For example, the final arginine concentration for the an anti-EGFR x anti-CD28 bispecific antibody (e.g., the FDS), will be about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, or about 105 mM. For example, the final arginine concentration in the FDS will be about 80 mM.

Example 6: Parameters for Arginine Spike and an anti-factor XI antibody

[00107] For example, arginine adjustment concentration will be used for an anti-factor XI antibody, and where the FCP target arginine spike will be about 30 mM to about 70 mM, about 30 mM to about 65 mM, about 30 mM to about 60 mM, about 30 mM to about 55 mM, about 30 mM to about 50 mM, about 30 mM to about 45 mM, about 30 mM to about 40 mM, about 30 mM to about 35 mM, about 35 mM to about 70 mM, about 35 mM to about 65 mM, about 35 mM to about 60 mM, about 35 mM to about 55 mM, about 35 mM to about 50 mM, about 35 mM to about 45 mM, about 35 mM to about 40 mM, about 40 mM to about 70 mM, about 40 mM to about 65 mM, about 40 mM to about 60 mM, about 40 mM to about 55 mM, about 40 mM to about 50 mM, about 40 mM to about 45 mM, about 45 mM to about 70 mM, about 45 mM to about 65 mM, about 45 mM to about 60 mM, about 45 mM to about 55 mM, about 45 mM to about 50 mM, about 50 mM to about 70 mM, about 50 mM to about 65 mM, about 50 mM to about 60 mM, about 50 mM to about 55 mM, about 55 mM to about 70 mM, about 55 mM to about 65 mM, about 55 mM to about 60 mM, about 60 mM to about 70 mM, about 60 mM to about 65 mM, or about 65 mM to about 70 mM.

[00108] For example, arginine adjustment concentration will be used for the anti-factor XI antibody, and where the FCP target arginine spike will be about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, or about 70 mM. For example, arginine adjustment concentration will be used for the anti-factor XI antibody, and where the FCP target arginine spike will be about 50 mM.

[00109] For example, final arginine concentration for the anti-factor XI antibody (e.g., the FDS), and where the arginine concentration in the FDS will be about 75 mM to about 125 mM, about 75 mM to about 120 mM, about 75 mM to

about 115 mM, about 75 mM to about 110 mM, about 75 mM to about 105 mM, about 75 mM to about 100 mM, about 75 mM to about 95 mM, about 75 mM to about 90 mM, about 75 mM to about 85 mM, about 75 mM to about 80 mM, about 80 mM to about 125 mM, about 80 mM to about 120 mM, about 80 mM to about 115 mM, about 80 mM to about 110 mM, about 80 mM to about 105 mM, about 80 mM to about 100 mM, about 80 mM to about 95 mM, about 80 mM to about 90 mM, about 80 mM to about 85 mM, about 85 mM to about 125 mM, about 85 mM to about 120 mM, about 85 mM to about 115 mM, about 85 mM to about 110 mM, about 85 mM to about 105 mM, about 85 mM to about 100 mM, about 85 mM to about 95 mM, about 85 mM to about 90 mM, about 90 mM to about 125 mM, about 90 mM to about 120 mM, about 90 mM to about 115 mM, about 90 mM to about 110 mM, about 90 mM to about 105 mM, about 90 mM to about 100 mM, about 90 mM to about 95 mM, about 95 mM to about 125 mM, about 95 mM to about 120 mM, about 95 mM to about 115 mM, about 95 mM to about 110 mM, about 95 mM to about 105 mM, about 95 mM to about 100 mM, about 100 mM to about 125 mM, about 100 mM to about 120 mM, about 100 mM to about 115 mM, about 100 mM to about 110 mM, about 100 mM to about 105 mM, about 105 mM to about 125 mM, about 105 mM to about 120 mM, about 105 mM to about 115 mM, about 105 mM to about 110 mM, about 110 mM to about 125 mM, about 110 mM to about 120 mM, about 110 mM to about 115 mM, about 115 mM to about 125 mM, about 115 mM to about 120 mM, or about 120 mM to about 125 mM.

[00110] For example, final arginine concentration for the anti-factor XI antibody (e.g., the FDS), and where the arginine concentration in the FDS will be about 75 mM, about 80 mM, about 85 mM, about 85 mM, about 90 mM, about 95 mM, about 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM,

about 115 mM, about 120 mM, or about 125 mM. For example, the final arginine concentration for the anti-factor XI antibody (e.g., the FDS) will be about 100 mM.

Example 7: Parameters for Arginine Spike and dupilumab

[00111] For example, arginine adjustment concentration will be used for dupilumab, and where the molarity of arginine after mixing in step (b) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 94.1%, 94.2%, 94.3%, 94.5%, 94.6%, 94.7%, 94.8%, or 94.9% of the molarity of arginine after the mixing in step (d).

[00112] For example, arginine adjustment concentration will be used for dupilumab, and where the molarity of arginine after mixing in step (b) is about 1.5 mM to about 285 mM, 1.5 mM to about 275 mM, 1.5 mM to about 250 mM, 1.5 mM to about 225 mM, 1.5 mM to about 200 mM, 1.5 mM to about 190 mM, 1.5 mM to about 180 mM, 1.5 mM to about 170 mM, 1.5 mM to about 160 mM, 1.5 mM to about 150 mM, 1.5 mM to about 140 mM, 1.5 mM to about 130 mM, 1.5 mM to about 120 mM, 1.5 mM to about 110 mM, 1.5 mM to about 100 mM, 1.5 mM to about 90 mM, 1.5 mM to about 80 mM, 1.5 mM to about 70 mM, 1.5 mM to about 60 mM, 1.5 mM to about 50 mM, 1.5 mM to about 40 mM, 1.5 mM to about 30 mM, 1.5 mM to about 20 mM, 1.5 mM to about 10 mM, 1.5 mM to about 5 mM, 5 mM to about 285 mM, 5 mM to about 275 mM, 5 mM to about 250 mM, 5 mM to about 225 mM, 5 mM to about 200 mM, 5 mM to about 190 mM, 5 mM to about 180 mM, 5 mM to about 170

mM, 5 mM to about 160 mM, 5 mM to about 150 mM, 5 mM to about 140 mM, 5 mM to about 130 mM, 5 mM to about 120 mM, 5 mM to about 110 mM, 5 mM to about 100 mM, 5 mM to about 90 mM, 5 mM to about 80 mM, 5 mM to about 70 mM, 5 mM to about 60 mM, 5 mM to about 50 mM, 5 mM to about 40 mM, 5 mM to about 30 mM, 5 mM to about 20 mM, 5 mM to about 10 mM, 10 mM to about 285 mM, 10 mM to about 275 mM, 10 mM to about 250 mM, 10 mM to about 225 mM, 10 mM to about 200 mM, 10 mM to about 190 mM, 10 mM to about 180 mM, 10 mM to about 170 mM, 10 mM to about 160 mM, 10 mM to about 150 mM, 10 mM to about 140 mM, 10 mM to about 130 mM, 10 mM to about 120 mM, 10 mM to about 110 mM, 10 mM to about 100 mM, 10 mM to about 90 mM, 10 mM to about 80 mM, 10 mM to about 70 mM, 10 mM to about 60 mM, 10 mM to about 50 mM, 10 mM to about 40 mM, 10 mM to about 30 mM, 10 mM to about 20 mM, 20 mM to about 285 mM, 20 mM to about 275 mM, 20 mM to about 250 mM, 20 mM to about 225 mM, 20 mM to about 200 mM, 20 mM to about 190 mM, 20 mM to about 180 mM, 20 mM to about 170 mM, 20 mM to about 160 mM, 20 mM to about 150 mM, 20 mM to about 140 mM, 20 mM to about 130 mM, 20 mM to about 120 mM, 20 mM to about 1 20 mM, 20 mM to about 100 mM, 20 mM to about 90 mM, 20 mM to about 80 mM, 20 mM to about 70 mM, 20 mM to about 60 mM, 20 mM to about 50 mM, 20 mM to about 40 mM, 20 mM to about 30 mM, 30 mM to about 285 mM, 30 mM to about 275 mM, 30 mM to about 250 mM, 30 mM to about 225 mM, 30 mM to about 200 mM, 30 mM to about 190 mM, 30 mM to about 180 mM, 30 mM to about 170 mM, 30 mM to about 160 mM, 30 mM to about 150 mM, 30 mM to about 140 mM, 30 mM to about 130 mM, 30 mM to about 120 mM, 30 mM to about 110 mM, 30 mM to about 100 mM, 30 mM to about 90 mM, 30 mM to about 80 mM, 30 mM to about 70 mM, 30 mM to about 60 mM, 30 mM to about 50 mM, 30 mM to about 40 mM, 40 mM to about 285

mM, 40 mM to about 275 mM, 40 mM to about 250 mM, 40 mM to about 225 mM, 40 mM to about 200 mM, 40 mM to about 190 mM, 40 mM to about 180 mM, 40 mM to about 170 mM, 40 mM to about 160 mM, 40 mM to about 150 mM, 40 mM to about 140 mM, 40 mM to about 130 mM, 40 mM to about 120 mM, 40 mM to about 110 mM, 40 mM to about 100 mM, 40 mM to about 90 mM, 40 mM to about 80 mM, 40 mM to about 70 mM, 40 mM to about 60 mM, 40 mM to about 50 mM, 50 mM to about 285 mM, 50 mM to about 275 mM, 50 mM to about 250 mM, 50 mM to about 225 mM, 50 mM to about 200 mM, 50 mM to about 190 mM, 50 mM to about 180 mM, 50 mM to about 170 mM, 50 mM to about 160 mM, 50 mM to about 150 mM, 50 mM to about 140 mM, 50 mM to about 130 mM, 50 mM to about 120 mM, 50 mM to about 110 mM, 50 mM to about 100 mM, 50 mM to about 90 mM, 50 mM to about 80 mM, 50 mM to about 70 mM, 50 mM to about 60 mM, 60 mM to about 285 mM, 60 mM to about 275 mM, 60 mM to about 250 mM, 60 mM to about 225 mM, 60 mM to about 200 mM, 60 mM to about 190 mM, 60 mM to about 180 mM, 60 mM to about 170 mM, 60 mM to about 160 mM, 60 mM to about 150 mM, 60 mM to about 140 mM, 60 mM to about 130 mM, 60 mM to about 120 mM, 60 mM to about 110 mM, 60 mM to about 100 mM, 60 mM to about 90 mM, 60 mM to about 80 mM, 60 mM to about 70 mM, 70 mM to about 285 mM, 70 mM to about 275 mM, 70 mM to about 250 mM, 70 mM to about 225 mM, 70 mM to about 200 mM, 70 mM to about 190 mM, 70 mM to about 180 mM, 70 mM to about 170 mM, 70 mM to about 160 mM, 70 mM to about 150 mM, 70 mM to about 140 mM, 70 mM to about 130 mM, 70 mM to about 120 mM, 70 mM to about 110 mM, 70 mM to about 100 mM, 70 mM to about 90 mM, 70 mM to about 80 mM, 80 mM to about 285 mM, 80 mM to about 275 mM, 80 mM to about 250 mM, 80 mM to about 225 mM, 80 mM to about 200 mM, 80 mM to about 190 mM, 80 mM to about 180 mM, 80 mM to about 170 mM, 80 mM to

about 160 mM, 80 mM to about 150 mM, 80 mM to about 140 mM, 80 mM to about 130 mM, 80 mM to about 120 mM, 80 mM to about 110 mM, 80 mM to about 100 mM, 80 mM to about 90 mM, 90 mM to about 285 mM, 90 mM to about 275 mM, 90 mM to about 250 mM, 90 mM to about 225 mM, 90 mM to about 200 mM, 90 mM to about 190 mM, 90 mM to about 180 mM, 90 mM to about 170 mM, 90 mM to about 160 mM, 90 mM to about 150 mM, 90 mM to about 140 mM, 90 mM to about 130 mM, 90 mM to about 120 mM, 90 mM to about 110 mM, 90 mM to about 100 mM, 100 mM to about 285 mM, 100 mM to about 275 mM, 100 mM to about 250 mM, 100 mM to about 225 mM, 100 mM to about 200 mM, 100 mM to about 190 mM, 100 mM to about 180 mM, 100 mM to about 170 mM, 100 mM to about 160 mM, 100 mM to about 150 mM, 100 mM to about 140 mM, 100 mM to about 130 mM, 100 mM to about 120 mM, 100 mM to about 110 mM, 110 mM to about 285 mM, 110 mM to about 275 mM, 110 mM to about 250 mM, 110 mM to about 225 mM, 110 mM to about 200 mM, 110 mM to about 190 mM, 110 mM to about 180 mM, 110 mM to about 170 mM, 110 mM to about 160 mM, 110 mM to about 150 mM, 110 mM to about 140 mM, 110 mM to about 130 mM, 110 mM to about 120 mM, 120 mM to about 285 mM, 120 mM to about 275 mM, 120 mM to about 250 mM, 120 mM to about 225 mM, 120 mM to about 200 mM, 120 mM to about 190 mM, 120 mM to about 180 mM, 120 mM to about 170 mM, 120 mM to about 160 mM, 120 mM to about 150 mM, 120 mM to about 140 mM, 110 mM to about 130 mM, 130 mM to about 285 mM, 130 mM to about 275 mM, 130 mM to about 250 mM, 130 mM to about 225 mM, 130 mM to about 200 mM, 130 mM to about 190 mM, 130 mM to about 180 mM, 130 mM to about 170 mM, 130 mM to about 160 mM, 130 mM to about 150 mM, 130 mM to about 140 mM, 140 mM to about 285 mM, 140 mM to about 275 mM, 140 mM to about 250 mM, 140 mM to about 225 mM, 140 mM to

about 200 mM, 140 mM to about 190 mM, 140 mM to about 180 mM, 140 mM to about 170 mM, 140 mM to about 160 mM, 140 mM to about 150 mM, 150 mM to about 285 mM, 150 mM to about 275 mM, 150 mM to about 250 mM, 150 mM to about 225 mM, 150 mM to about 200 mM, 150 mM to about 190 mM, 150 mM to about 180 mM, 150 mM to about 170 mM, 150 mM to about 160 mM, 160 mM to about 285 mM, 160 mM to about 275 mM, 160 mM to about 250 mM, 160 mM to about 225 mM, 160 mM to about 200 mM, 160 mM to about 190 mM, 160 mM to about 180 mM, 160 mM to about 170 mM, 170 mM to about 285 mM, 170 mM to about 275 mM, 170 mM to about 250 mM, 170 mM to about 225 mM, 170 mM to about 200 mM, 170 mM to about 190 mM, 170 mM to about 180 mM, 180 mM to about 285 mM, 180 mM to about 275 mM, 180 mM to about 250 mM, 180 mM to about 225 mM, 180 mM to about 200 mM, 180 mM to about 190 mM, 190 mM to about 285 mM, 190 mM to about 275 mM, 190 mM to about 250 mM, 190 mM to about 225 mM, 190 mM to about 200 mM, 200 mM to about 285 mM, 200 mM to about 275 mM, 200 mM to about 250 mM, 200 mM to about 225 mM, 225 mM to about 285 mM, 225 mM to about 275 mM, 225 mM to about 250 mM, 250 mM to about 285 mM, 250 mM to about 275 mM, or 275 mM to about 285 mM.

[00113] For example, arginine adjustment concentration will be used for dupilumab, and where the molarity of arginine after the mixing in step (d) is about 7.5 mM to about 297 mM, about 7.5 mM to about 290 mM, about 7.5 mM to about 270 mM, about 7.5 mM to about 250 mM, about 7.5 mM to about 230 mM, about 7.5 mM to about 210 mM, about 7.5 mM to about 190 mM, about 7.5 mM to about 170 mM, about 7.5 mM to about 150 mM, about 7.5 mM to about 130 mM, about 7.5 mM to about 110 mM, about 7.5 mM to about 90 mM, about 7.5 mM to about 70 mM, about 7.5 mM to about 50 mM, about 7.5 mM to about 30 mM, about 7.5 mM to about 10

mM, about 10 mM to about 290 mM, about 10 mM to about 270 mM, about 10 mM to about 250 mM, about 10 mM to about 230 mM, about 10 mM to about 210 mM, about 10 mM to about 190 mM, about 10 mM to about 170 mM, about 10 mM to about 150 mM, about 10 mM to about 130 mM, about 10 mM to about 110 mM, about 10 mM to about 90 mM, about 10 mM to about 70 mM, about 10 mM to about 50 mM, about 10 mM to about 30 mM, about 30 mM to about 290 mM, about 30 mM to about 270 mM, about 30 mM to about 250 mM, about 30 mM to about 230 mM, about 30 mM to about 210 mM, about 30 mM to about 190 mM, about 30 mM to about 170 mM, about 30 mM to about 150 mM, about 30 mM to about 130 mM, about 30 mM to about 110 mM, about 30 mM to about 90 mM, about 30 mM to about 70 mM, about 30 mM to about 50 mM, about 50 mM to about 290 mM, about 50 mM to about 270 mM, about 50 mM to about 250 mM, about 50 mM to about 230 mM, about 50 mM to about 210 mM, about 50 mM to about 190 mM, about 50 mM to about 170 mM, about 50 mM to about 150 mM, about 50 mM to about 130 mM, about 50 mM to about 110 mM, about 50 mM to about 90 mM, about 50 mM to about 70 mM, about 70 mM to about 290 mM, about 70 mM to about 270 mM, about 70 mM to about 250 mM, about 70 mM to about 230 mM, about 70 mM to about 210 mM, about 70 mM to about 190 mM, about 70 mM to about 170 mM, about 70 mM to about 150 mM, about 70 mM to about 130 mM, about 70 mM to about 110 mM, about 70 mM to about 90 mM, about 90 mM to about 290 mM, about 90 mM to about 270 mM, about 90 mM to about 250 mM, about 90 mM to about 230 mM, about 90 mM to about 210 mM, about 90 mM to about 190 mM, about 90 mM to about 170 mM, about 90 mM to about 150 mM, about 90 mM to about 130 mM, about 90 mM to about 110 mM, about 110 mM to about 290 mM, about 110 mM to about 270 mM, about 110 mM to about 250 mM, about 110 mM to about 230 mM, about 110 mM to

about 210 mM, about 110 mM to about 190 mM, about 110 mM to about 170 mM, about 110 mM to about 150 mM, about 110 mM to about 130 mM, about 130 mM to about 290 mM, about 130 mM to about 270 mM, about 130 mM to about 250 mM, about 130 mM to about 230 mM, about 130 mM to about 210 mM, about 130 mM to about 190 mM, about 130 mM to about 170 mM, about 130 mM to about 150 mM, about 150 mM to about 290 mM, about 150 mM to about 270 mM, about 150 mM to about 250 mM, about 150 mM to about 230 mM, about 150 mM to about 210 mM, about 150 mM to about 190 mM, about 150 mM to about 170 mM, about 170 mM to about 290 mM, about 170 mM to about 270 mM, about 170 mM to about 250 mM, about 170 mM to about 230 mM, about 170 mM to about 210 mM, about 170 mM to about 190 mM, about 190 mM to about 290 mM, about 190 mM to about 270 mM, about 190 mM to about 250 mM, about 190 mM to about 230 mM, about 190 mM to about 210 mM, about 210 mM to about 290 mM, about 210 mM to about 270 mM, about 210 mM to about 250 mM, about 210 mM to about 230 mM, about 230 mM to about 290 mM, about 230 mM to about 270 mM, about 230 mM to about 250 mM, about 250 mM to about 290 mM, about 250 mM to about 270 mM, or about 270 mM to about 290 mM.

[00114] Optionally, the FCP and/or FDS can include pharmaceutically acceptable excipients, such as, but not limited to, one or more amino acids (e.g., histidine, proline), a stabilizer (e.g., a sugar, such as sucrose, or a sugar alcohol), and/or a surfactant (e.g., a polysorbate, e.g., polysorbate 80). Optionally, the amino acid can be in the concentration of about 5 mM to about 20 mM; the surfactant can be in the concentration of about 0.01% (w/v) – about 0.4% (w/v); and/or the stabilizer can be in the concentration of about 1% (w/v) to about 10% (w/v).

What is claimed is

1. A method of producing a concentrated formulated drug substance comprising Fc-containing proteins, wherein the method comprises the steps of:

(a) providing a concentrated Fc-containing protein pool;

(b) mixing the concentrated Fc-containing protein pool with an arginine salt solution to form a concentrated Fc-containing protein pool that has reduced viscosity;

(c) purifying the concentrated Fc-containing protein pool from step (b) to form a concentrated formulated drug substance; and

(d) mixing the concentrated formulated drug substance with an arginine salt solution to reduce viscosity,

wherein the molarity of arginine after mixing in step (b) is less than about 80% of the molarity of arginine after the mixing in step (d).

2. The method according to claim 1, wherein the arginine salt is arginine-HCl.

3. The method according to claim 1 or 2, wherein the concentrated formulated drug substance has an Fc-containing protein concentration of at least about 150 g/l, 151 g/l, 152 g/l, 153 g/l, 154 g/l, 155 g/l, 156 g/l, 157 g/l, 158 g/l, 159 g/l, 160 g/l, 161 g/l, 162 g/l, 163 g/l, 164 g/l, 165 g/l, 166 g/l, 167 g/l, 168 g/l, 169 g/l, 170 g/l, 171 g/l, 172 g/l, 173 g/l, 174 g/l, 175 g/l, 176 g/l, 177 g/l, 178 g/l, 179 g/l, 180 g/l, 181 g/l, 182 g/l, 183 g/l, 184 g/l, 185 g/l, 186 g/l, 187 g/l, 188 g/l, 189 g/l, 190 g/l, 191 g/l, 192 g/l, 193 g/l, 194 g/l, 195 g/l, 196 g/l, 197 g/l, 198 g/l, 199 g/l, 200 g/l, 201

g/l, 202 g/l, 203 g/l, 204 g/l, 205 g/l, 206 g/l, 207 g/l, 208 g/l, 209 g/l, 210 g/l, 211 g/l, 212 g/l, 213 g/l, 214 g/l, 215 g/l, 216 g/l, 217 g/l, 218 g/l, 219 g/l, 220 g/l, 221 g/l, 222 g/l, 223 g/l, 224 g/l, 225 g/l, 226 g/l, 227 g/l, 228 g/l, 229 g/l, 230 g/l, 231 g/l, 232 g/l, 233 g/l, 234 g/l, 235 g/l, 236 g/l, 237 g/l, 238 g/l, 239 g/l, 240 g/l, 241 g/l, 242 g/l, 243 g/l, 244 g/l, 245 g/l, 246 g/l, 247 g/l, 248 g/l, 249 g/l, 250 g/l, 251 g/l, 252 g/l, 253 g/l, 254 g/l, 255 g/l, 256 g/l, 257 g/l, 258 g/l, 259 g/l or 260 g/l.

4. The method according to any one of claims 1-3, wherein the molarity of arginine after mixing in step (b) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 79.1%, 79.2%, 79.3%, 79.4%, 79.5%, 79.6%, 79.7%, 79.8% or 79.9% of the molarity of arginine after the mixing in step (d).

5. The method according to any one of claims 1-4, wherein the Fc-containing proteins are antibodies or fragments thereof.

6. The method according to claim 5, wherein the antibodies are monoclonal antibodies or fragments thereof.

7. The method according to claim 6, wherein the antibodies are bispecific antibodies or fragments thereof.

8. The method according to any one of claims 1-4, wherein the Fc-containing proteins are Fc-fusion proteins.

9. The method according to claim 8, wherein the Fc-fusion proteins are receptor Fc-fusion proteins.

10. The method according to claim 9, wherein the receptor Fc-fusion proteins are trap proteins.

11. The method according to any one of claims 1-10, wherein the purifying comprises ultrafiltration and diafiltration.

12. The method according to any one of claims 1-11, wherein the molarity of arginine after mixing in step (b) is about 40 mM to about 80 mM, about 40 mM to about 60 mM, about 40 mM to about 70 mM, about 20 mM to about 80 mM, about 20 mM to about 60 mM, about 20 mM to about 70 mM, about 30 mM to about 80 mM, about 30 mM to about 60 mM, or about 30 mM to about 70 mM.

13. The method according to claim 12, wherein the molarity of arginine after mixing in step (b) is about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, or about 65 mM.

14. The method according to claim 12 or 13, wherein the molarity of arginine after mixing in step (b) is about 40 mM, about 50 mM, or about 60 mM.

15. The method according to any one of claims 1-14, wherein the molarity of arginine after the mixing in step (d) is about 100 mM to about 150 mM, about 100 mM to about 105 mM, about 100 mM to about 125 mM, about 55 mM to about 125

mM, about 55 mM to about 105 mM, about 55 mM to about 125 mM, about 75 mM to about 150 mM, about 75 mM to about 105 mM, or about 75 mM to about 125 mM.

16. The method according to claim 15, wherein the molarity of arginine after the mixing in step (d) is about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, or about 125 mM.

17. The method according to claim 15 or 16, wherein the molarity of arginine after the mixing in step (d) is about 80 mM, about 100 mM, or about 125 mM.

18. The method according to any one of claims 1-17, wherein the Fc-containing protein is a human bispecific antibody that binds to different epitopes on MET.

19. The method according to claim 18, wherein the molarity of arginine after mixing in step (b) is about 60 mM; and wherein the molarity of arginine after the mixing in step (d) is about 125 mM.

20. The method according to any one of claims 1-17, wherein the Fc-containing protein is an anti-EGFR x anti-CD28 bispecific antibody.

21. The method according to claim 20, wherein the molarity of arginine after mixing in step (b) is about 40 mM; and wherein the molarity of arginine after the mixing in step (d) is about 80 mM.

22. The method according to any one of claims 1-17, wherein the Fc-containing protein is an anti-factor XI antibody.

23. The method according to claim 22, wherein the molarity of arginine after mixing in step (b) is about 50 mM; and wherein the molarity of arginine after the mixing in step (d) is about 100 mM.

24. A concentrated formulated drug substance comprising Fc-containing proteins, wherein the concentrated formulated drug substance is made by a method comprising the steps of:

(a) providing a concentrated Fc-containing protein pool;

(b) mixing the concentrated Fc-containing protein pool with an arginine salt solution to form a concentrated Fc-containing protein pool that has reduced viscosity;

(c) purifying the concentrated Fc-containing protein pool from step (b) to form a concentrated formulated drug substance; and

(d) mixing the concentrated formulated drug substance with an arginine salt solution to reduce viscosity,

wherein the molarity of arginine after mixing in step (b) is less than about 80% of the molarity of arginine after the mixing in step (d).

25. The concentrated formulated drug substance to claim 24, wherein the arginine salt is arginine-HCl.

26. The concentrated formulated drug substance according to claim 24 or 25, wherein the concentrated formulated drug substance has an Fc-containing protein concentration of at least about 150 g/l, 151 g/l, 152 g/l, 153 g/l, 154 g/l, 155 g/l, 156 g/l, 157 g/l, 158 g/l, 159 g/l, 160 g/l, 161 g/l, 162 g/l, 163 g/l, 164 g/l, 165 g/l,

166 g/l, 167 g/l, 168 g/l, 169 g/l, 170 g/l, 171 g/l, 172 g/l, 173 g/l, 174 g/l, 175 g/l, 176 g/l, 177 g/l, 178 g/l, 179 g/l, 180 g/l, 181 g/l, 182 g/l, 183 g/l, 184 g/l, 185 g/l, 186 g/l, 187 g/l, 188 g/l, 189 g/l, 190 g/l, 191 g/l, 192 g/l, 193 g/l, 194 g/l, 195 g/l, 196 g/l, 197 g/l, 198 g/l, 199 g/l, 200 g/l, 201 g/l, 202 g/l, 203 g/l, 204 g/l, 205 g/l, 206 g/l, 207 g/l, 208 g/l, 209 g/l, 210 g/l, 211 g/l, 212 g/l, 213 g/l, 214 g/l, 215 g/l, 216 g/l, 217 g/l, 218 g/l, 219 g/l, 220 g/l, 221 g/l, 222 g/l, 223 g/l, 224 g/l, 225 g/l, 226 g/l, 227 g/l, 228 g/l, 229 g/l, 230 g/l, 231 g/l, 232 g/l, 233 g/l, 234 g/l, 235 g/l, 236 g/l, 237 g/l, 238 g/l, 239 g/l, 240 g/l, 241 g/l, 242 g/l, 243 g/l, 244 g/l, 245 g/l, 246 g/l, 247 g/l, 248 g/l, 249 g/l, 250 g/l, 251 g/l, 252 g/l, 253 g/l, 254 g/l, 255 g/l, 256 g/l, 257 g/l, 258 g/l, 259 g/l or 260 g/l.

27. The concentrated formulated drug substance according to any one of claims 24-26, wherein the molarity of arginine after mixing in step (b) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 79.1%, 79.2%, 79.3%, 79.4%, 79.5%, 79.6%, 79.7%, 79.8% or 79.9% of the molarity of arginine after the mixing in step (d).

28. The concentrated formulated drug substance according to any one of claims 24-27, wherein the Fc-containing proteins are antibodies or fragments thereof.

29. The concentrated formulated drug substance according to claim 28, wherein the antibodies are monoclonal antibodies or fragments thereof.

30. The concentrated formulated drug substance according to claim 29, wherein the antibodies are bispecific antibodies or fragments thereof.

31. The concentrated formulated drug substance according to any one of claims 24-27, wherein the Fc-containing proteins are Fc-fusion proteins.

32. The concentrated formulated drug substance according to claim 31, wherein the Fc-fusion proteins are receptor Fc-fusion proteins.

33. The concentrated formulated drug substance according to claim 32, wherein the receptor Fc-fusion proteins are trap proteins.

34. The concentrated formulated drug substance according to any one of claims 24-33, wherein the purifying comprises ultrafiltration and diafiltration.

35. The concentrated formulated drug substance to any one of claims 24-34, wherein the molarity of arginine after mixing in step (b) is about 40 mM to about 80 mM, about 40 mM to about 60 mM, about 40 mM to about 70 mM, about 20 mM to about 80 mM, about 20 mM to about 60 mM, about 20 mM to about 70 mM, about 30 mM to about 80 mM, about 30 mM to about 60 mM, or about 30 mM to about 70 mM.

36. The concentrated formulated drug substance to claim 35, wherein the molarity of arginine after mixing in step (b) is about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, or about 65 mM.

37. The concentrated formulated drug substance according to claim 35 or 36, wherein the molarity of arginine after mixing in step (b) is about 40 mM, about 50 mM, or about 60 mM.

38. The concentrated formulated drug substance to any one of claims 24-37, wherein the molarity of arginine after the mixing in step (d) is about 100 mM to about 150 mM, about 100 mM to about 105 mM, about 100 mM to about 125 mM, about 55 mM to about 125 mM, about 55 mM to about 105 mM, about 55 mM to about 125 mM, about 75 mM to about 150 mM, about 75 mM to about 105 mM, or about 75 mM to about 125 mM.

39. The concentrated formulated drug substance according to claim 38, wherein the molarity of arginine after the mixing in step (d) is about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, or about 125 mM.

40. The concentrated formulated drug substance according to claim 38 or 39 wherein the molarity of arginine after the mixing in step (d) is about 80 mM, about 100 mM, or about 125 mM.

41. The concentrated formulated drug substance according to any one of claims 24-40, wherein the Fc-containing protein is a human bispecific antibody that binds to different epitopes on MET.

42. The concentrated formulated drug substance according to claim 41, wherein the molarity of arginine after mixing in step (b) is about 60 mM; and wherein the molarity of arginine after the mixing in step (d) is about 125 mM.

43. The concentrated formulated drug substance according to any one of claims 24-40, wherein the Fc-containing protein is an anti-EGFR x anti-CD28 bispecific antibody.

44. The concentrated formulated drug substance according to claim 43, wherein the molarity of arginine after mixing in step (b) is about 40 mM; and wherein the molarity of arginine after the mixing in step (d) is about 80 mM.

45. The concentrated formulated drug substance according to any one of claims 24-40, wherein the Fc-containing protein is an anti-factor XI antibody.

46. The concentrated formulated drug substance according to claim 45, wherein the molarity of arginine after mixing in step (b) is about 50 mM; and wherein the molarity of arginine after the mixing in step (d) is about 100 mM.

47. A method of producing a concentrated formulated drug substance comprising Fc-containing proteins, wherein the method comprises the steps of:

(a) providing a concentrated Fc-containing protein pool;

(b) mixing the concentrated Fc-containing protein pool with an arginine salt solution to form a concentrated Fc-containing protein pool that has reduced viscosity;

(c) purifying the concentrated Fc-containing protein pool from step (b) to form a concentrated formulated drug substance; and

(d) mixing the concentrated formulated drug substance with an arginine salt solution to reduce viscosity,

wherein the molarity of arginine after mixing in step (b) is less than about 95% of the molarity of arginine after the mixing in step (d).

48. The method according to claim 47, wherein the arginine salt is arginine-HCl.

49. The method according to claim 47 or 48, wherein the molarity of arginine after mixing in step (b) is about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 94.1%, 94.2%, 94.3%, 94.5%, 94.6%, 94.7%, 94.8%, or 94.9% of the molarity of arginine after the mixing in step (d).

50. The method according to any one of claims 47-49, wherein the Fc-containing proteins are antibodies or fragments thereof.

51. The method according to claim 5, wherein the antibodies are monoclonal antibodies or fragments thereof, or bispecific antibodies or fragments thereof.

52. The method according to any one of claims 47-49, wherein the Fc-containing proteins are Fc-fusion proteins.

53. The method according to claim 52, wherein the Fc-fusion proteins are receptor Fc-fusion proteins, optionally wherein the receptor Fc-fusion proteins are trap proteins.

54. The method according to any one of claims 47-53, wherein the purifying comprises ultrafiltration and diafiltration.

55. The method according to any one of claims 47-54, wherein the molarity of arginine after mixing in step (b) is about 1.5 mM to about 285 mM, 1.5 mM to about 275 mM, 1.5 mM to about 250 mM, 1.5 mM to about 225 mM, 1.5 mM to about 200 mM, 1.5 mM to about 190 mM, 1.5 mM to about 180 mM, 1.5 mM to about 170 mM, 1.5 mM to about 160 mM, 1.5 mM to about 150 mM, 1.5 mM to about 140 mM, 1.5 mM to about 130 mM, 1.5 mM to about 120 mM, 1.5 mM to about 110 mM, 1.5 mM to about 100 mM, 1.5 mM to about 90 mM, 1.5 mM to about 80 mM, 1.5 mM to about 70 mM, 1.5 mM to about 60 mM, 1.5 mM to about 50 mM, 1.5 mM to about 40 mM, 1.5 mM to about 30 mM, 1.5 mM to about 20 mM, 1.5 mM to about 10 mM, 1.5 mM to about 5 mM, 5 mM to about 285 mM, 5 mM to about 275 mM, 5 mM to about 250 mM, 5 mM to about 225 mM, 5 mM to about 200 mM, 5 mM to about 190 mM, 5 mM to about 180 mM, 5 mM to about 170 mM, 5 mM to about 160 mM, 5 mM to about 150 mM, 5 mM to about 140 mM, 5 mM to about 130 mM, 5 mM to about 120 mM, 5 mM to about 110 mM, 5 mM to about 100 mM, 5 mM to about 90 mM, 5 mM to about 80 mM, 5 mM to about 70 mM, 5 mM to about 60 mM, 5 mM to about 50 mM, 5 mM to about 40 mM, 5 mM to about 30 mM, 5 mM to about 20 mM, 5 mM to about 10 mM, 10 mM to about 285 mM, 10 mM to about 275 mM, 10 mM to about 250 mM, 10 mM to about 225 mM, 10 mM to about 200 mM, 10 mM to about 190 mM, 10 mM to about 180 mM, 10 mM to about 170 mM, 10 mM to about 160 mM, 10

mM to about 150 mM, 10 mM to about 140 mM, 10 mM to about 130 mM, 10 mM to about 120 mM, 10 mM to about 110 mM, 10 mM to about 100 mM, 10 mM to about 90 mM, 10 mM to about 80 mM, 10 mM to about 70 mM, 10 mM to about 60 mM, 10 mM to about 50 mM, 10 mM to about 40 mM, 10 mM to about 30 mM, 10 mM to about 20 mM, 20 mM to about 285 mM, 20 mM to about 275 mM, 20 mM to about 250 mM, 20 mM to about 225 mM, 20 mM to about 200 mM, 20 mM to about 190 mM, 20 mM to about 180 mM, 20 mM to about 170 mM, 20 mM to about 160 mM, 20 mM to about 150 mM, 20 mM to about 140 mM, 20 mM to about 130 mM, 20 mM to about 120 mM, 20 mM to about 1 20 mM, 20 mM to about 100 mM, 20 mM to about 90 mM, 20 mM to about 80 mM, 20 mM to about 70 mM, 20 mM to about 60 mM, 20 mM to about 50 mM, 20 mM to about 40 mM, 20 mM to about 30 mM, 30 mM to about 285 mM, 30 mM to about 275 mM, 30 mM to about 250 mM, 30 mM to about 225 mM, 30 mM to about 200 mM, 30 mM to about 190 mM, 30 mM to about 180 mM, 30 mM to about 170 mM, 30 mM to about 160 mM, 30 mM to about 150 mM, 30 mM to about 140 mM, 30 mM to about 130 mM, 30 mM to about 120 mM, 30 mM to about 110 mM, 30 mM to about 100 mM, 30 mM to about 90 mM, 30 mM to about 80 mM, 30 mM to about 70 mM, 30 mM to about 60 mM, 30 mM to about 50 mM, 30 mM to about 40 mM, 40 mM to about 285 mM, 40 mM to about 275 mM, 40 mM to about 250 mM, 40 mM to about 225 mM, 40 mM to about 200 mM, 40 mM to about 190 mM, 40 mM to about 180 mM, 40 mM to about 170 mM, 40 mM to about 160 mM, 40 mM to about 150 mM, 40 mM to about 140 mM, 40 mM to about 130 mM, 40 mM to about 120 mM, 40 mM to about 110 mM, 40 mM to about 100 mM, 40 mM to about 90 mM, 40 mM to about 80 mM, 40 mM to about 70 mM, 40 mM to about 60 mM, 40 mM to about 50 mM, 50 mM to about 285 mM, 50 mM to about 275 mM, 50 mM to about 250 mM, 50 mM to about 225 mM, 50 mM to about 200 mM, 50 mM to

about 190 mM, 50 mM to about 180 mM, 50 mM to about 170 mM, 50 mM to about 160 mM, 50 mM to about 150 mM, 50 mM to about 140 mM, 50 mM to about 130 mM, 50 mM to about 120 mM, 50 mM to about 110 mM, 50 mM to about 100 mM, 50 mM to about 90 mM, 50 mM to about 80 mM, 50 mM to about 70 mM, 50 mM to about 60 mM, 60 mM to about 285 mM, 60 mM to about 275 mM, 60 mM to about 250 mM, 60 mM to about 225 mM, 60 mM to about 200 mM, 60 mM to about 190 mM, 60 mM to about 180 mM, 60 mM to about 170 mM, 60 mM to about 160 mM, 60 mM to about 150 mM, 60 mM to about 140 mM, 60 mM to about 130 mM, 60 mM to about 120 mM, 60 mM to about 110 mM, 60 mM to about 100 mM, 60 mM to about 90 mM, 60 mM to about 80 mM, 60 mM to about 70 mM, 70 mM to about 285 mM, 70 mM to about 275 mM, 70 mM to about 250 mM, 70 mM to about 225 mM, 70 mM to about 200 mM, 70 mM to about 190 mM, 70 mM to about 180 mM, 70 mM to about 170 mM, 70 mM to about 160 mM, 70 mM to about 150 mM, 70 mM to about 140 mM, 70 mM to about 130 mM, 70 mM to about 120 mM, 70 mM to about 110 mM, 70 mM to about 100 mM, 70 mM to about 90 mM, 70 mM to about 80 mM, 80 mM to about 285 mM, 80 mM to about 275 mM, 80 mM to about 250 mM, 80 mM to about 225 mM, 80 mM to about 200 mM, 80 mM to about 190 mM, 80 mM to about 180 mM, 80 mM to about 170 mM, 80 mM to about 160 mM, 80 mM to about 150 mM, 80 mM to about 140 mM, 80 mM to about 130 mM, 80 mM to about 120 mM, 80 mM to about 110 mM, 80 mM to about 100 mM, 80 mM to about 90 mM, 90 mM to about 285 mM, 90 mM to about 275 mM, 90 mM to about 250 mM, 90 mM to about 225 mM, 90 mM to about 200 mM, 90 mM to about 190 mM, 90 mM to about 180 mM, 90 mM to about 170 mM, 90 mM to about 160 mM, 90 mM to about 150 mM, 90 mM to about 140 mM, 90 mM to about 130 mM, 90 mM to about 120 mM, 90 mM to about 110 mM, 90 mM to about 100 mM, 100 mM to about 285 mM, 100 mM to

about 275 mM, 100 mM to about 250 mM, 100 mM to about 225 mM, 100 mM to about 200 mM, 100 mM to about 190 mM, 100 mM to about 180 mM, 100 mM to about 170 mM, 100 mM to about 160 mM, 100 mM to about 150 mM, 100 mM to about 140 mM, 100 mM to about 130 mM, 100 mM to about 120 mM, 100 mM to about 110 mM, 110 mM to about 285 mM, 110 mM to about 275 mM, 110 mM to about 250 mM, 110 mM to about 225 mM, 110 mM to about 200 mM, 110 mM to about 190 mM, 110 mM to about 180 mM, 110 mM to about 170 mM, 110 mM to about 160 mM, 110 mM to about 150 mM, 110 mM to about 140 mM, 110 mM to about 130 mM, 110 mM to about 120 mM, 120 mM to about 285 mM, 120 mM to about 275 mM, 120 mM to about 250 mM, 120 mM to about 225 mM, 120 mM to about 200 mM, 120 mM to about 190 mM, 120 mM to about 180 mM, 120 mM to about 170 mM, 120 mM to about 160 mM, 120 mM to about 150 mM, 120 mM to about 140 mM, 110 mM to about 130 mM, 130 mM to about 285 mM, 130 mM to about 275 mM, 130 mM to about 250 mM, 130 mM to about 225 mM, 130 mM to about 200 mM, 130 mM to about 190 mM, 130 mM to about 180 mM, 130 mM to about 170 mM, 130 mM to about 160 mM, 130 mM to about 150 mM, 130 mM to about 140 mM, 140 mM to about 285 mM, 140 mM to about 275 mM, 140 mM to about 250 mM, 140 mM to about 225 mM, 140 mM to about 200 mM, 140 mM to about 190 mM, 140 mM to about 180 mM, 140 mM to about 170 mM, 140 mM to about 160 mM, 140 mM to about 150 mM, 150 mM to about 285 mM, 150 mM to about 275 mM, 150 mM to about 250 mM, 150 mM to about 225 mM, 150 mM to about 200 mM, 150 mM to about 190 mM, 150 mM to about 180 mM, 150 mM to about 170 mM, 150 mM to about 160 mM, 160 mM to about 285 mM, 160 mM to about 275 mM, 160 mM to about 250 mM, 160 mM to about 225 mM, 160 mM to about 200 mM, 160 mM to about 190 mM, 160 mM to about 180 mM, 160 mM to

about 170 mM, 170 mM to about 285 mM, 170 mM to about 275 mM, 170 mM to about 250 mM, 170 mM to about 225 mM, 170 mM to about 200 mM, 170 mM to about 190 mM, 170 mM to about 180 mM, 180 mM to about 285 mM, 180 mM to about 275 mM, 180 mM to about 250 mM, 180 mM to about 225 mM, 180 mM to about 200 mM, 180 mM to about 190 mM, 190 mM to about 285 mM, 190 mM to about 275 mM, 190 mM to about 250 mM, 190 mM to about 225 mM, 190 mM to about 200 mM, 200 mM to about 285 mM, 200 mM to about 275 mM, 200 mM to about 250 mM, 200 mM to about 225 mM, 225 mM to about 285 mM, 225 mM to about 275 mM, 225 mM to about 250 mM, 250 mM to about 285 mM, 250 mM to about 275 mM, or 275 mM to about 285 mM.

56. The method according to any one of claims 47-55, wherein the molarity of arginine after the mixing in step (d) is about 7.5 mM to about 297 mM, about 7.5 mM to about 290 mM, about 7.5 mM to about 270 mM, about 7.5 mM to about 250 mM, about 7.5 mM to about 230 mM, about 7.5 mM to about 210 mM, about 7.5 mM to about 190 mM, about 7.5 mM to about 170 mM, about 7.5 mM to about 150 mM, about 7.5 mM to about 130 mM, about 7.5 mM to about 110 mM, about 7.5 mM to about 90 mM, about 7.5 mM to about 70 mM, about 7.5 mM to about 50 mM, about 7.5 mM to about 30 mM, about 7.5 mM to about 10 mM, about 10 mM to about 290 mM, about 10 mM to about 270 mM, about 10 mM to about 250 mM, about 10 mM to about 230 mM, about 10 mM to about 210 mM, about 10 mM to about 190 mM, about 10 mM to about 170 mM, about 10 mM to about 150 mM, about 10 mM to about 130 mM, about 10 mM to about 110 mM, about 10 mM to about 90 mM, about 10 mM to about 70 mM, about 10 mM to about 50 mM, about 10 mM to about 30 mM, about 30 mM to about 290 mM, about 30 mM to about 270 mM, about 30 mM to

about 250 mM, about 30 mM to about 230 mM, about 30 mM to about 210 mM, about 30 mM to about 190 mM, about 30 mM to about 170 mM, about 30 mM to about 150 mM, about 30 mM to about 130 mM, about 30 mM to about 110 mM, about 30 mM to about 90 mM, about 30 mM to about 70 mM, about 30 mM to about 50 mM, about 50 mM to about 290 mM, about 50 mM to about 270 mM, about 50 mM to about 250 mM, about 50 mM to about 230 mM, about 50 mM to about 210 mM, about 50 mM to about 190 mM, about 50 mM to about 170 mM, about 50 mM to about 150 mM, about 50 mM to about 130 mM, about 50 mM to about 110 mM, about 50 mM to about 90 mM, about 50 mM to about 70 mM, about 70 mM to about 290 mM, about 70 mM to about 270 mM, about 70 mM to about 250 mM, about 70 mM to about 230 mM, about 70 mM to about 210 mM, about 70 mM to about 190 mM, about 70 mM to about 170 mM, about 70 mM to about 150 mM, about 70 mM to about 130 mM, about 70 mM to about 110 mM, about 70 mM to about 90 mM, about 90 mM to about 290 mM, about 90 mM to about 270 mM, about 90 mM to about 250 mM, about 90 mM to about 230 mM, about 90 mM to about 210 mM, about 90 mM to about 190 mM, about 90 mM to about 170 mM, about 90 mM to about 150 mM, about 90 mM to about 130 mM, about 90 mM to about 110 mM, about 110 mM to about 290 mM, about 110 mM to about 270 mM, about 110 mM to about 250 mM, about 110 mM to about 230 mM, about 110 mM to about 210 mM, about 110 mM to about 190 mM, about 110 mM to about 170 mM, about 110 mM to about 150 mM, about 110 mM to about 130 mM, about 130 mM to about 290 mM, about 130 mM to about 270 mM, about 130 mM to about 250 mM, about 130 mM to about 230 mM, about 130 mM to about 210 mM, about 130 mM to about 190 mM, about 130 mM to about 170 mM, about 130 mM to about 150 mM, about 150 mM to about 290 mM, about 150 mM to about 270 mM, about 150 mM to about 250 mM, about 150 mM to

about 230 mM, about 150 mM to about 210 mM, about 150 mM to about 190 mM, about 150 mM to about 170 mM, about 170 mM to about 290 mM, about 170 mM to about 270 mM, about 170 mM to about 250 mM, about 170 mM to about 230 mM, about 170 mM to about 210 mM, about 170 mM to about 190 mM, about 190 mM to about 290 mM, about 190 mM to about 270 mM, about 190 mM to about 250 mM, about 190 mM to about 230 mM, about 190 mM to about 210 mM, about 210 mM to about 290 mM, about 210 mM to about 270 mM, about 210 mM to about 250 mM, about 210 mM to about 230 mM, about 230 mM to about 290 mM, about 230 mM to about 270 mM, about 230 mM to about 250 mM, about 250 mM to about 290 mM, about 250 mM to about 270 mM, or about 270 mM to about 290 mM.

57. The method according to any one of claims 47-56, wherein the Fc-containing protein is a dupilumab.

58. A concentrated formulated drug substance comprising Fc-containing proteins, wherein the concentrated formulated drug substance is made by a method according to any one of claims 47-57.