

What is claimed is:

1. A method for preparing a composition comprising a protein, the method comprises:
providing a preparation comprising a protein of interest, the preparation is obtained after
subjecting a sample comprising the protein and one or more impurities to a purification
process,
adjusting the pH of the preparation to a target pH prior to a final ultrafiltration and diafiltration
operation, and
obtaining a composition comprising the protein from the final ultrafiltration and diafiltration
operation, wherein the target pH is at or about the pH of the composition comprising the
protein obtained from the final ultrafiltration and diafiltration operation.
2. The method of claim 1, wherein the pH is adjusted using a pH adjusting agent.
3. The method of claim 2, wherein the pH adjusting agent is an acid, a base, or a buffer.
4. The method of claim 3, wherein the acid is selected from the group consisting of acetic acid,
adipic acid, alginic acid, ascorbic acid, aspartic acid, benzene sulfonic acid, benzoic acid,
boric acid, camphorsulfonic acid, citric acid, caprylic acid, formic acid, glutamic acid,
hydrochloride acid, hydrobromic acid, hydroxy acids, hyaluronic acid, lactic acid, malic acid,
methanesulfonic acid, nitric acid, phosphoric acid, propionic acid, sulfuric acid, sulfonic acid,
transexamic acid, and tartaric acid.
5. The method of claim 4, wherein the acid is selected from the group consisting of lactic acid,
hydroxy acids and hyaluronic acid.
6. The method of claim 3, wherein the base is selected from the group consisting of ammonia
solution, ammonium carbonate, diethanolamine, calcium hydroxide, ethanolamine, lysine,
meglumine, poly-lysine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium
carbonate, sodium hydroxide, and triethanolamine.
7. The method of claim 3, wherein the buffer is selected from the group consisting of acetate
buffer, aspartate buffer, ascorbate buffer, borate buffer, benzoate buffer, carbonate buffer,
citrate buffer, glycine buffer, HEPES buffer, MOPS buffer, MES buffer, N-(2-
acetamido)iminodiacetic acid (ADA) buffer, histidine buffer, lactate buffer, phosphate

buffer, succinate buffer, Tris buffer, Bis-tris buffer, and tartrate buffer.

8. The method of any one of claims 1 to 7, wherein the target pH is from about pH 4.0 to about pH 7.0.
9. The method of any one of claims 1 to 7, wherein the target pH is from about pH 4.0 to about pH 6.0.
10. The method of any one of claims 1 to 9, wherein the method further comprises concentrating the protein in the preparation to a target concentration.
11. The method of claim 10, wherein the concentrating step is carried out before the pH adjusting step.
12. The method of claim 10, wherein the concentrating step is carried out after the pH adjusting step and before the final UF DF operation.
13. The method of any one of claims 1 to 12, wherein the final ultrafiltration and diafiltration operation is carried out using a medium that comprises essentially no buffer.
14. The method of claim 13, wherein the composition obtained from the final ultrafiltration and diafiltration operation comprises the protein at a concentration of from about 40 mg/mL to about 200 mg/mL.
15. The method of any one of claims 1 to 12, wherein the final ultrafiltration and diafiltration operation is carried out using a medium that comprises a buffer.
16. The method of claim 15, wherein the medium comprises a buffer at a concentration of from 2 mM to about 300 mM.
17. The method of claim 15 or claim 16, wherein the composition obtained from the final ultrafiltration and diafiltration operation comprises the protein at a concentration of from about 1 mg/mL to about 200 mg/mL.
18. The method of claim 15, wherein the medium comprises a buffer at a concentration of from 2 mM to about 10 mM, and wherein the composition obtained from the ultrafiltration and diafiltration operation comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL.
19. The method of claim 15, wherein the medium comprises a buffer at a pH outside the buffer capacity range of the buffer, and wherein the composition obtained from the ultrafiltration

and diafiltration operation comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL.

20. The method of any one of claim 1-19, wherein the purification process comprises one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, depth filtration, and mixed-mode chromatography.
21. The method of claim 20, wherein the purification process comprises a virus filtration step and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, and depth filtration, and
wherein the preparation is obtained from the virus filtration step.
22. The method of claim 20, wherein the purification process comprises a cation exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and
wherein the preparation is obtained from the cation exchange chromatography step.
23. The method of claim 20, wherein the purification process comprises an anion exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and
wherein the preparation is obtained from the anion exchange chromatography step.
24. The method of claim 20, wherein the purification process comprises hydrophobic interaction chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and

wherein the preparation is obtained from the hydrophobic interaction chromatography step.

25. The method of claim 20, wherein the purification process comprises a mixed-mode chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and
- wherein the preparation is obtained from the mixed-mode chromatography step.
26. The method of any one of claims 1 to 25, wherein the method further comprises adding a stabilizer to the preparation.
27. The method of claim 26, wherein the stabilizer is one or more selected from: an amino acid, a sugar, a polyol, an anti-oxidant, a chelating agent, a lipid or lipid derivative, a salt, a polymer, an inert protein, a surfactant, and a water-miscible co-solvent.
28. The method of claim 27, wherein the amino acid is one or more selected from: histidine, arginine, glycine, methionine, alanine, aspartic acid, lysine hydrochloride, proline, lysine, sarcosine, gamma-aminobutyric acid, and glutamic acid.
29. The method of claim 27, wherein the anti-oxidant is one or more selected from: ascorbic acid, glutathione, vitamin E, and poly(ethylenimine).
30. The method of claim 27, wherein the sugar is one or more selected from: sucrose, trehalose, xylitol, maltose, dextrose, glucose, raffinose, and lactose.
31. The method of claim 27, wherein the polyol is one or more of selected from: sugar alcohol, glycerol, erythritol, caprylate, tryptophanate, and sarcosine.
32. The method of claim 27, wherein the polymer is one or more selected from: gelatin, hyaluronic acid, polyvinylpyrrolidone (PVP), poly(lactic-co-glycolic acid) (PLGA), polyacrylic acid (PAA), Amphipol A8-35, PAA5-25C8-40C3, polyethylene glycol (PEG), hydroxyethyl (heta) starch, sulfated polysaccharides, polyamino acids, dextran, diethylaminoethyl-dextran, hydroxypropyl-beta-cyclodextrin, sulfobutylether-beta-cyclodextrin, polyethyleneimine (PEI), and carboxymethyl cellulose.
33. The method of claim 27, wherein the inert protein is one or more selected from: HSA, BSA and recombinant HA.

34. The method of claim 27, wherein the chelating agent is one or more selected from: EDTA, DPTA, citric acid, hexaphosphate, and thioglycolic acid.
35. The method of claim 27, wherein the salt is one or more selected from: sodium chloride, sodium sulfate, sodium thiocyanate, potassium chloride, potassium phosphate, calcium lactate, and guanidine hydrochloride.
36. The method of claim 27, wherein the surfactant is one or more selected from: polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer, PEG dodecyl ethers, and PEG tertoctylphenyl ether.
37. The method of claim 27, wherein the water-miscible co-solvent is one or more selected from: (DMSO), DMF, DMA and CSA.
38. The method of claim 27, wherein the lipid or lipid derivative is one or more of fatty acids (e.g., oleic acid), phospholipids and phospholipid derivatives, DEA phosphopate, DEA cetyl phosphate, oleth 10 phosphate, oleth-10, oleth-10 phosphate, DEA cetyl phosphate, mannosylglycerate, polidocanol, sulfobetaine cholate, phospholipids, and C12-15 alcohols benzoate.
39. The method of any one of claims 1 to 38, wherein the protein is any one of the following proteins: etanercept, aflibercept, adalimumab, epoetin alfa, darbepoetin alfa, filgrastim, pegfilgrastim, bevacizumab, cetuximab, infliximab, rituximab, eculizumab, trastuzumab, evolocumab, denosumab, romosozumab, erenumab, blinatumomab, and a BiTE antibody construct.
40. The method of claim 39, wherein the BiTE antibody construct is blinatumomab, anti-CD33 and anti-CD3 BiTE antibody construct, anti-EGFRvIII and anti-CD3 BiTE antibody construct, anti-DLL3 and anti-CD3 BiTE antibody construct, anti-CD19 and anti-CD3 BiTE antibody construct, anti-MSLN and anti-CD3 BiTE antibody construct, anti-CDH19 and anti-CD3 BiTE antibody construct, anti-FLT3 and anti-CD3 BiTE antibody construct, anti-DLL3 and anti-CD3 BiTE antibody construct, anti-CDH3 and anti-CD3 BiTE antibody construct, anti-CD70 and anti-CD3 BiTE antibody construct, anti-PSMA and anti-CD3 BiTE antibody construct, and anti-BCMA and anti-CD3 BiTE antibody construct.
41. The method of any one of claims 1-40, wherein the ultrafiltration and diafiltration operation is carried out at a temperature of from about 25 °C to about 50 °C.

42. The method of any one of claims 1-40, wherein the ultrafiltration and diafiltration operation is carried out at a temperature from about 25 °C to about 40 °C.
43. The method of any one of claims 1-40, wherein the ultrafiltration and diafiltration operation is carried out at a temperature from about 30 °C to about 40 °C.
44. The method of any one of claims 1 to 43, wherein the composition obtained from the final ultrafiltration and diafiltration operation is a pharmaceutical formulation comprising the protein.
45. The method of any one of claims 1 to 43, wherein the composition obtained from the final ultrafiltration and diafiltration operation is a drug substance comprising the protein.
46. The method of any one of claims 1 to 45, wherein the composition obtained from the final ultrafiltration and diafiltration operation is more stable compared to a composition prepared by the same method without the pH adjusting step.