

~~[00098]~~ All references cited in this specification, including without limitation, all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, and/or periodicals are hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

~~[00099]~~[00098] These and other modifications and variations to the present invention may be practiced by those of ordinary skill in the art, without departing from the spirit and scope of the present invention, which is more particularly set forth in the appended claims. In addition, it should be understood that aspects of the various embodiments may be interchanged in whole or in part. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is by way of example only, and is not intended to limit the invention so further described in such appended claims. Therefore, the spirit and scope of the appended claims should not be limited to the description of the versions contained therein.

- g. processing the second eluate through an additional chromatography resin to provide a third eluate comprising the protein;
- h. subjecting the third eluate to nanofiltration to provide a nanofiltered eluate comprising the protein; and
- i. subjecting the nanofiltered eluate to ultrafiltration and diafiltration.

25. The method of claim 24 wherein the additional chromatography resin comprises a mixed-mode chromatography resin.

26. The method of claim 25, wherein the processing of the second eluate through the additional mixed-mode chromatography resin comprises one or more chromatography techniques selected from the group consisting of anion exchange, cation exchange, hydrophobic interaction, hydrophilic interaction, hydrogen bonding, pi-pi bonding, and metal affinity.

27. The method of claim 26 wherein the processing of the second eluate through the additional mixed-mode chromatography resin comprises a combination of anion exchange and hydrophobic interaction chromatography mechanisms.

28. The method of claim 26 wherein the mixed-mode chromatography column can be operated in flow-through or bind-elute mode.

29. The method of claim 24 wherein the additional chromatography resin comprises a cation exchange resin.

30. The method of claim ~~29~~25, wherein the processing of the second eluate through the additional mixed-mode chromatography resin comprises one or more chromatography techniques selected from the group consisting of anion exchange, cation exchange, hydrophobic interaction, hydrophilic interaction, hydrogen bonding, pi-pi bonding, and metal affinity.