

METHOD OF PURIFICATION

RELATED APPLICATION DATA

This application claims priority from European Patent Application No
5 23183376.5 filed on 4 July 2023 and entitled "Method of Purification", the entire
contents of which are hereby incorporated by reference.

SEQUENCE LISTING

The present application is filed together with a Sequence Listing in electronic
10 form. The entire contents of the Sequence Listing are hereby incorporated by reference.

FIELD

The present disclosure relates to methods of isolating a protein-of-interest
enriched solution, such as an immunoglobulin G (IgG), and a plurality of protein-of-
15 interest depleted fractions from a protein mixture, such as plasma or a fraction thereof,
using continuous chromatography. The present disclosure also relates to a continuous
chromatography system for separating a protein-of-interest from a protein mixture,
formulations and uses of the purified products thereof.

20 BACKGROUND

Protein purification is one of the most costly aspects of therapeutic protein
production. Existing methods of protein purification include chromatography (e.g.
affinity chromatography, anion exchange chromatography, hydrophobic interaction
chromatography, SE-HPLC) and non-chromatography (e.g. precipitation and liquid
25 extraction) purification methods. Major obstacles of existing methods are the high cost
and time involved in purification and the need to ensure that the product is of a suitable
quality (e.g. purity and stability) for therapeutic use. For example, affinity resins used in
affinity chromatography can have relatively low binding capacity and chromatography
purification from an average size batch can reach volumes of several hundred litres, being
30 a huge capital investment in the amount of resin used, the infrastructure to handle and
pack the chromatography columns, along with the running costs.

In some instances, co-purification of one or more proteins from the same protein
mixture is required. For example, during plasma fractionation, blood plasma fractions
containing one or more plasma proteins (e.g., immunoglobulin G (IgG), albumin and
35 coagulation factors) are obtained from the same blood plasma sample. Each of these
plasma fractions can then be processed for therapeutic use.

To date, several methods of plasma fractionation have been developed. Current methods require extremely high volumes of raw material and can often result in poor yield of some plasma protein fractions due to additional processing steps required to remove protein aggregates. At present, up to 70-75% of the IgG present in plasma may
5 be recovered from plasma using existing technologies.

It will therefore be apparent to the skilled person that there is a need in the art for improved methods of fractionating proteins from protein mixtures, e.g., purifying IgG from plasma or fractions thereof.

10 SUMMARY

The present disclosure is based on the inventors' identification of a method of purifying immunoglobulin G (IgG) from plasma or a fraction thereof with efficient isolation of undiluted IgG depleted fractions. The method allows IgG to be recovered from plasma or a fraction thereof at high yields (e.g., $\geq 75\%$) and high purity (e.g., $\geq$
15 95%), whilst also permitting a high recovery rate (e.g., $>90\%$) of IgG depleted fractions with low dilution (e.g., $< 10\%$) for further downstream purification. In particular, the inventors have found that use of continuous affinity chromatography (e.g., simulated moving bed (SMB) chromatography) comprising at least four columns and two feedback loops permits separation of two outlet streams (i.e., feed/load flow-through and wash
20 flow-through) in parallel, enabling the efficient fractionation of IgG depleted undiluted plasma material from the diluted wash buffer fraction. The inventors found that directing the load flow-through onto another column over the first loopback enables recovery of the IgG product at high yield and high purity and also permits collection of the flow through undiluted for the isolation of further proteins of interest. Additionally, the
25 inventors found that directing the wash flow-through over the second loopback permits fractionation of a low diluted IgG-depleted flow-through fraction. The low-diluted IgG-depleted flow-through fraction can then be pooled with the main IgG-depleted flow-through fraction, increasing the recovery rate. The method advantageously enables the protein-of-interest to be isolated as well as the isolated protein-of-interest depleted
30 plasma (i.e., the undiluted IgG depleted fractions) to be further processed for isolation and processing of further plasma products with high efficiency and yields.

Accordingly, the findings by the inventors provide the basis for a method of isolating a protein-of-interest enriched solution and a plurality of protein-of-interest depleted fractions from a protein mixture using continuous chromatography.

35 It will be apparent to the skilled person from the disclosure herein that the methods of the disclosure permit isolating a protein-of-interest from a complex protein mixture

whilst also recovering the protein-of-interest depleted fractions from the protein mixture, wherein the protein-of-interest depleted fraction can be subjected to further processing.

According to one aspect of the present disclosure, there is provided a method of
5 isolating a protein-of-interest enriched solution and a plurality of protein-of-interest
depleted fractions from a protein mixture comprising a protein-of-interest using a
continuous chromatography system comprising at least four separation units, each
separation unit comprising a respective chromatography medium capable of separating
and retaining a portion of a protein-of-interest from a protein mixture, wherein the
10 method comprises:

performing at least one chromatography cycle comprising a series of cycle
segments, wherein a cycle segment comprises:

- a. loading a feed comprising the protein mixture comprising the protein-of-
15 interest onto a feed separation unit, wherein a portion of a protein-of-interest
content of the feed is retained in the feed separation unit, thereby producing
a feed flow through;
- b. directing the feed flow through from the feed separation unit onto a feed
loopback separation unit via a feed flow through feedback loop, wherein at
20 least a portion of a protein-of-interest content of the feed flow through is
retained in the feed loopback separation unit, thereby producing a protein-of-
interest depleted feed flow through fraction;
- c. loading a wash buffer onto a wash separation unit containing retained
protein-of-interest from a previous cycle segment, thereby producing a wash
flow through;
- 25 d. directing the wash flow through from the wash separation unit onto a wash
loopback separation unit via a wash flow through feedback loop, wherein a
portion of a protein-of-interest content of the wash flow through is retained
in the wash loopback separation unit, thereby producing a protein-of-interest
depleted wash flow through fraction; and
- 30 e. loading an elution buffer onto the wash separation unit and/or onto an elution
separation unit to elute retained protein-of-interest from the respective
chromatography medium, thereby producing a protein-of-interest enriched
solution,

wherein the feed flow through and the wash flow through are maintained separate
35 from each other.

Each separation unit may comprise an inlet, an outlet and a fluid flow path extending through the separation unit from the inlet to the outlet and in contact with the chromatography medium. Each inlet and/or each outlet is connected to a switching valve arrangement. In some examples, the continuous chromatography system comprises four
5 separation units. In some examples, the continuous chromatography system comprises five separation units. In some examples, the continuous chromatography system comprises more than five separation units.

A function of one or more of the at least four separation units may vary between
10 successive cycle segments. For example, within one chromatography cycle, the functions of the at least four separation units may vary such that each separation unit performs phases of the chromatography cycle, the phases including: loading, washing, eluting, equilibrating, feed loopback, wash loopback, and/or combinations thereof. The phases may optionally include a stripping phase and a re-equilibrating phase. The phases may
15 optionally include a regenerating phase. The phases may optionally include a sanitising phase.

Performing the chromatography cycle may comprise loading the feed comprising the protein mixture onto each of the at least four separation units successively, wherein the
20 loading of the feed onto each successive separation unit demarks a respective cycle segment.

Loading of the feed may be performed substantially continuously across successive cycle segments (and/or across successive chromatography cycles) such that a flow rate of the
25 feed during the chromatography cycle is substantially constant. Substantially continuous loading may comprise no idle time, or minimised idle time, when switching feeding the load from one separation unit to the subsequent separation unit. In some examples, substantially continuous loading may include interruptions to the flow of the feed a time taken to allow connection of the feed source to the next separation unit (for example, the
30 time taken to operate a switching valve). In other embodiments, the loading and/or flow rate may be discontinuous. For example, there may be an idle time between successive cycles and/or cycle segments.

The number of cycle segments in one chromatography cycle may be equal to the number
35 of separation units. In some examples, a load time taken to perform step a) may be equal to a cycle time of one chromatography cycle divided by the number of separation units.

- In some examples, the function of each of the separation units may vary based on the function performed by the respective separation unit in one or more preceding cycle segments. In some examples, in at least one cycle segment, the wash separation unit may be selected as the separation unit that functioned as the feed separation unit in an immediately preceding cycle segment. In some examples, the wash separation unit may also perform the elution and/or equilibration functions in a cycle segment. Alternatively, or additionally, in some examples, at least one cycle segment, an elution separation unit may be selected as the separation unit that functioned as the wash separation unit in the immediately preceding cycle segment. The elution separation unit may perform the equilibration function. In some examples, in at least one cycle segment, the feed separation unit may be selected as the separation unit that functioned as the wash loopback separation unit or the feed loopback separation unit in the immediately preceding cycle segment.
- 15 In some examples, the functions of each separation unit vary such that each separation unit performs each of the phases in a predetermined sequence. For example, each separation unit may perform the phases in the following sequence: loading, washing, eluting, equilibrating, feed loopback, wash loopback. each separation unit may perform the phases in the following sequence: loading, washing, eluting, equilibrating, wash loopback, feed loopback. The sequence may be continuous, that is, the loading phase occurs following the wash loopback or feed loopback.

According to one example, the present disclosure provides a method of isolating a protein-of-interest enriched solution and a plurality of protein-of-interest depleted fractions from a protein mixture using continuous chromatography comprising at least four separation units, wherein the method comprises:

- (i) a first chromatography cycle between a first separation unit and a third separation unit comprising:
 - a. loading a protein mixture comprising a protein-of-interest through an inlet onto the first separation unit comprising a first chromatography medium capable of separating the protein-of-interest from the protein mixture, wherein at least some of the protein-of-interest from the protein mixture is retained by the first chromatography medium, thereby producing a first chromatography cycle post-load flow through from the first separation unit through an outlet;

- 5 b. feeding the first chromatography cycle post-load flow through from an outlet of the first separation unit through an inlet onto a third separation unit via a first chromatography cycle first feedback loop in fluid communication between the outlet of the first separation unit and the inlet of the third separation unit, wherein the third separation unit comprises a third chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the third chromatography medium, thereby producing a first chromatography cycle first protein-of-interest depleted flow through fraction from the third separation unit through an outlet;
- 10 c. loading a wash buffer through the inlet onto the first separation unit, wherein loading the wash buffer onto the first separation unit produces a first chromatography cycle post-wash flow through from the first separation unit through the outlet;
- 15 d. feeding the first chromatography cycle post-wash flow through from the outlet of the first separation unit through the inlet onto the third separation unit via a first chromatography cycle second feedback loop; wherein at least some of the protein-of-interest from the post-wash flow through is retained by the third chromatography medium, thereby producing a first chromatography cycle second protein-of-interest depleted flow through fraction from the third separation unit through the outlet;
- 20 e. loading an elution buffer through the inlet onto the first separation unit, eluting the retained protein-of-interest from the first chromatography medium thereby producing a first chromatography cycle first protein-of-interest enriched solution from the first separation unit through the outlet; and
- 25 f. optionally, loading an elution buffer through the inlet onto the third separation unit, eluting the retained protein-of-interest from the second chromatography medium thereby producing a first chromatography cycle second protein-of-interest enriched solution from the third separation unit through the outlet; and
- 30 (ii) a second chromatography cycle between a second separation unit and a fourth separation unit comprising:
- 35 a. loading the protein mixture comprising the protein-of-interest through an inlet onto the second separation unit comprising a second chromatography

medium capable of separating the protein-of-interest from the protein mixture, wherein at least some of the protein-of-interest from the protein mixture is retained by the second chromatography medium, thereby producing a second chromatography cycle post-load flow through from the second separation unit through an outlet;

- b. feeding the second chromatography cycle post-load flow through from an outlet of the second separation unit through an inlet onto a fourth separation unit via a second chromatography cycle first feedback loop in fluid communication between the outlet of the second separation unit and the inlet of the fourth separation unit, wherein the fourth separation unit comprises a fourth chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the fourth chromatography medium, thereby producing a second chromatography cycle first protein-of-interest depleted flow through fraction from the fourth separation unit through an outlet;
- c. loading a wash buffer through the inlet onto the second separation unit, wherein loading the wash buffer onto the second separation unit produces a second chromatography cycle post-wash flow through from the second separation unit through the outlet;
- d. feeding the second chromatography cycle post-wash flow through from the outlet of the second separation unit through the inlet onto the fourth separation unit via a second chromatography cycle second feedback loop; wherein at least some of the protein-of-interest from the post-wash flow through is retained by the fourth chromatography medium, thereby producing a second chromatography cycle second protein-of-interest depleted flow through fraction from the fourth separation unit through the outlet;
- e. loading an elution buffer through the inlet onto the second separation unit, eluting the retained protein-of-interest from the second chromatography medium thereby producing a second chromatography cycle first protein-of-interest enriched solution from the second separation unit through the outlet; and
- f. loading an elution buffer through the inlet onto the fourth separation unit, eluting the retained protein-of-interest from the fourth chromatography medium thereby producing a second chromatography cycle second protein-

of-interest enriched solution from the fourth separation unit through the outlet;

wherein the method comprises repeating steps (i) and (ii) one or more times; and wherein the first and second chromatography cycles operate independently and are discordant
5 such that the first chromatography cycle first feedback loop and the second chromatography cycle second feedback loop operate in parallel.

As used herein:

“post-load flow through” may be considered equivalent to “feed flow through”;
10 “post-wash flow through” may be considered equivalent to “wash flow through”;
“first protein-of-interest depleted flow through fraction” may be considered equivalent to “protein-of-interest depleted feed flow through fraction”; and
“second protein-of-interest depleted flow through fraction” may be considered equivalent to “protein-of-interest depleted wash flow through fraction”.
15 “first protein-of-interest enriched solution” and “second protein-of-interest enriched solution” may each be considered equivalent to “protein-of-interest enriched solution”.

In the above example:

20 in step (i)(a), the first separation unit is functioning as the feed separation unit;
in step (i)(b), the first feedback loop is functioning as the feed flow through feedback loop, and the third separation unit is functioning as a feed loopback separation unit;
in step (i)(c), the first separation unit is functioning as the wash separation unit;
25 in step (i)(d), the second feedback loop is functioning as the wash flow through feedback loop, and the third separation unit is functioning as the wash loopback separation unit;
in step (i)(e), the first separation unit may be considered to be functioning as the wash separation unit or an elution separation unit;
30 in step (ii)(a), the second separation unit is functioning as the feed separation unit;
in step (ii)(b), the first feedback loop is functioning as the feed flow through feedback loop, and the fourth separation unit is functioning as a feed loopback separation unit;
in step (ii)(c), the second separation unit is functioning as the wash separation
35 unit;

in step (ii)(d), the second feedback loop is functioning as the wash flow through feedback loop, and the fourth separation unit is functioning as the wash loopback separation unit; and

in step (ii)(e), the second separation unit may be considered to be functioning as
5 the wash separation unit or the elution separation unit.

In one example, the method further comprises

- (i) a third chromatography cycle between the third separation unit and the first separation unit comprising:
- 10 a. loading a protein mixture comprising a protein-of-interest through an inlet onto the third separation unit comprising the third chromatography medium capable of separating the protein-of-interest from the protein mixture, wherein at least some of the protein-of-interest from the protein mixture is retained by the third chromatography medium, thereby producing a third
15 chromatography cycle post-load flow through from the third separation unit through an outlet;
- b. feeding the third chromatography cycle post-load flow through from an outlet of the third separation unit through an inlet onto the first separation unit via a third chromatography cycle first feedback loop in fluid
20 communication between the outlet of the third separation unit and the inlet of the first separation unit, wherein the first separation unit comprises a first chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the first chromatography medium, thereby producing a third chromatography cycle first protein-of-
25 interest depleted flow through fraction from the first separation unit through an outlet;
- c. loading a wash buffer through the inlet onto the third separation unit, wherein loading the wash buffer onto the third separation unit produces a third
30 chromatography cycle post-wash flow through from the third separation unit through the outlet;
- d. feeding the third chromatography cycle post-wash flow through from the outlet of the third separation unit through the inlet onto the first separation unit via a third chromatography cycle second feedback loop; wherein at least
35 some of the protein-of-interest from the post-wash flow through is retained by the first chromatography medium, thereby producing a third

- chromatography cycle second protein-of-interest depleted flow through fraction from the first separation unit through the outlet;
- e. loading an elution buffer through the inlet onto the third separation unit, eluting the retained protein-of-interest from the third chromatography medium thereby producing a third chromatography cycle first protein-of-interest enriched solution from the third separation unit through the outlet; and
- f. loading an elution buffer through the inlet onto the first separation unit, eluting the retained protein-of-interest from the first chromatography medium thereby producing a third chromatography cycle second protein-of-interest enriched solution from the first separation unit through the outlet; and
- (ii) a fourth chromatography cycle between a fourth separation unit and a second separation unit comprising:
- a. loading the protein mixture comprising the protein-of-interest through an inlet onto the fourth separation unit comprising a fourth chromatography medium capable of separating the protein-of-interest from the protein mixture, wherein at least some of the protein-of-interest from the protein mixture is retained by the fourth chromatography medium, thereby producing a fourth chromatography cycle post-load flow through from the fourth separation unit through an outlet;
- b. feeding the fourth chromatography cycle post-load flow through from an outlet of the fourth separation unit through an inlet onto a second separation unit via a fourth chromatography cycle first feedback loop in fluid communication between the outlet of the fourth separation unit and the inlet of the second separation unit, wherein the second separation unit comprises a second chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the second chromatography medium, thereby producing a fourth chromatography cycle first protein-of-interest depleted flow through fraction from the second separation unit through an outlet;
- c. loading a wash buffer through the inlet onto the fourth separation unit, wherein loading the wash buffer onto the fourth separation unit produces a fourth chromatography cycle post-wash flow through from the fourth separation unit through the outlet;

- d. feeding the fourth chromatography cycle post-wash flow through from the outlet of the fourth separation unit through the inlet onto the second separation unit via a fourth chromatography cycle second feedback loop; wherein at least some of the protein-of-interest from the post-wash flow through is retained by the second chromatography medium, thereby producing a fourth chromatography cycle second protein-of-interest depleted flow through fraction from the second separation unit through the outlet;
- e. loading an elution buffer through the inlet onto the fourth separation unit, eluting the retained protein-of-interest from the fourth chromatography medium thereby producing a fourth chromatography cycle first protein-of-interest enriched solution from the fourth separation unit through the outlet; and
- f. optionally, loading an elution buffer through the inlet onto the second separation unit, eluting the retained protein-of-interest from the second chromatography medium thereby producing a fourth chromatography cycle second protein-of-interest enriched solution from the second separation unit through the outlet;
- optionally wherein the method comprises repeating steps (i) and (ii) one or more times; and wherein optionally, the third and fourth chromatography cycles operate independently and are discordant such that the third chromatography cycle first feedback loop and the fourth chromatography cycle second feedback loop operate in parallel.

In the above example:

- in step (i)(a), the third separation unit is functioning as the feed separation unit;
- in step (i)(b), the first feedback loop is functioning as the feed flow through feedback loop, and the first separation unit is functioning as the feed loopback separation unit;
- in step (i)(c), the third separation unit is functioning as the wash separation unit;
- in step (i)(d), the second feedback loop is functioning as the wash flow through feedback loop, and the first separation unit is functioning as the wash loopback separation unit;
- in step (i)(e), the third separation unit may be considered to be functioning as the wash separation unit or the elution separation unit;
- in step (ii)(a), the fourth separation unit is functioning as the feed separation unit;

in step (ii)(b), the first feedback loop is functioning as the feed flow through feedback loop, and the second separation unit is functioning as the feed loopback separation unit;

in step (ii)(c), the fourth separation unit is functioning as the wash separation unit;

5 in step (ii)(d), the second feedback loop is functioning as the wash flow through feedback loop, and the second separation unit is functioning as the wash loopback separation unit; and

in step (ii)(e), the fourth separation unit may be considered to be functioning as the wash separation unit or the elution separation unit.

10

In one example, the method comprises:

(a) sequentially cycling through the first chromatography cycle between the first separation unit and the third separation unit; and the third chromatography cycle between the third separation unit and the first separation unit; and

15 (b) sequentially cycling through the second chromatography cycle between the second separation unit and the fourth separation unit; and the fourth chromatography cycle between the fourth separation unit and the second separation unit;

wherein the method comprises repeating steps (a) and (b) one or more times.

20

In one example, an inlet of each separation unit is connected to a switching valve arrangement operable to selectively direct flow of one or more of the protein mixture comprising the protein, the wash buffer, and/or the elution buffer to the inlet of any selected one of the separation units, for example to the first, second, third or fourth separation units, optionally to a fifth separation unit and optionally to further separation units.

In another example, an outlet of each separation unit is connected to the switching valve arrangement configured for selectively directing flow from the outlets. For example, flow from the outlets may be selectively directed an inlets of one or more of the other separation units. In another example, flow from the outlets may be selectively directed for collection, for recycling (e.g. via a feedback loop to another separation unit) and/or for further processing.

30 For example, the continuous chromatography system may comprise four separation units, wherein the switching valve arrangement is operable in any given cycle segment, to direct flow of the feed of the protein mixture to an inlet of a separation unit functioning as the load separation unit, from the outlet of the load separation unit to the

inlet of a separation unit functioning as the feed loopback separation unit, to direct flow of the wash buffer to an inlet of a separation unit functioning as the wash separation unit, and flow from the outlet of the wash separation unit to the inlet of a separation unit functioning as the wash loopback separation unit.

5 In one example, the continuous chromatography system comprises four separation units, wherein the switching valve arrangement is operable to selectively direct flow from the outlet of the first separation unit to the inlet of the third separation unit and flow from the outlet of the fourth separation unit to the inlet of the second separation unit. In this example, the switching valve arrangement is operable to
10 selectively direct flow from the outlet of the second separation unit to the inlet of the fourth separation unit and from the outlet of the first separation unit to the inlet of the third separation unit. In this example, the switching valve arrangement is operable to selectively direct flow from the outlet of the third separation unit to the inlet of the first separation unit, and from the outlet of the second separation unit to the inlet of the fourth
15 separation unit. In this example, the switching arrangement is operable to selectively direct flow from the outlet of the fourth separation unit to the inlet of the second separation unit and from the outlet of the third separation unit to the inlet of the first separation unit.

20 In some examples, the switching valve arrangement permits switching between different phases on the separation unit.

 In one example, the different phases are selected from the group consisting of loading, washing, eluting, equilibration, first loopback (e.g. feed loopback), second loopback (e.g. wash loopback) and combinations thereof. For example, the phase is a loading phase. In another example, the phase is a wash phase. In a further example, the
25 phase is an elution phase. In one example, the phase is a first loopback phase (e.g. feed loopback phase). In another example, the phase is a second loopback phase (e.g. wash loopback phase).

 In any of the above aspects, the method may further comprise determining a protein concentration, for example determining a protein concentration of an output of at
30 least one separation unit. The method may comprise determining a protein concentration of an output of each of the separation units. In some examples, a protein sensor may be connected to an outlet of at least one of the separation units, for example, a protein sensor may be connected to an outlet of each of the separation units. For example, a protein sensor may be connected to the outlet of the first separation unit. In another example, a
35 protein sensor may be connected to the outlet of the second separation unit. In a further example, a protein sensor may be connected to the outlet of the third separation unit. In

one example, a protein sensor may be connected to the outlet of the fourth separation unit. In one example, a protein sensor may be connected to the outlet of a fifth separation unit.

5 In one example, the protein sensor detects the protein concentration in one or more of: the feed flow-through, the -wash flow-through, the protein-of-interest depleted feed flow through fraction, the protein-of-interest depleted wash flow through fraction and/or the protein-of-interest enriched solution. For example, the protein sensor detects the protein concentration in one or more of: the post-load flow-through. In another example, the protein sensor detects the protein concentration in the post-wash flow through. In a
10 further example, the protein sensor detects the protein concentration in the first protein-of-interest depleted flow through fraction. In one example, the protein sensor detects the protein concentration in the second protein-of-interest depleted flow through fraction. In another example, the protein sensor detects the protein concentration in the protein-of-interest enriched solution.

15 In some examples, determining a protein concentration may comprise using one or more spectroscopic analysis techniques. For example, the protein concentration may be determined using one or more reflection spectroscopy and/or one or more absorption spectroscopy techniques. The protein sensor may comprise one or more spectrometers and/or one or more detectors.

20 In some examples, determining the protein concentration may comprise detecting an optical density (OD), for example using optical absorbance detection. In such examples, the protein sensor may comprise an optical absorbance detector. For example, the optical absorbance detector is selected from the group consisting of an ultraviolet (UV) detector, a visible light (VIS) detector, a UV-VIS detector, a photodiode array
25 (PDA) detector and combinations thereof. In one example, the optical absorbance detector is an UV detector. In another example, the optical absorbance detector is a VIS detector. In a further example, the optical absorbance detector is a UV-VIS detector. In one example, the optical absorbance detector is a PDA detector.

In one example, the optical absorbance detector detects UV absorption. In one
30 example, UV absorption is measured at one or more of 300 nm, 280 nm, 245 nm, 224 nm, and 214 nm. In one example, UV absorption is measured at 300 nm. For example, UV absorption is measured at 280 nm. In another example, UV absorption is measured at 245 nm. In a further example, UV absorption is measured at 224 nm. In one example, UV absorption is measured at 214 nm. In one example, UV absorption is measured at
35 280-300 nm. For example, UV absorption is measured at 280 nm and 300 nm.

In one example, determining the protein concentration comprises detecting the optical density (OD), wherein the protein sensor comprises an optical absorbance detector.

In one example, determining a protein concentration may comprise performing
5 Raman spectroscopy, wherein the protein sensor comprises a Raman spectrometer.

In some examples, determining a protein concentration may comprise performing near-infrared spectroscopy, wherein the protein sensor comprises a near-infrared spectrometer. Performing NIR spectroscopy may comprise performing reflection NIR spectroscopy (NIRS) and/or performing absorption NIR spectroscopy.

10 For example, the method may further comprise detecting the protein concentration in one or more of the feed flow-through, the wash flow through, the protein-of-interest depleted feed flow through fraction, the protein-of-interest depleted wash flow through fraction and/or the protein-of-interest enriched solution by one or more of OD detection; Raman spectroscopy; and/or NIR spectroscopy.

15 In some examples, one or more protein-of-interest depleted flow through fractions may be subjected to further fractionation. For example, the protein-of-interest depleted wash flow through fraction may be further fractionated into a low protein concentration fraction and a high protein concentration fraction. For example, the first chromatography cycle second protein-of-interest depleted flow through fraction is further fractionated into
20 a low protein concentration fraction and a high protein concentration fraction. In another example, the second chromatography cycle second protein-of-interest depleted flow through fraction is further fractionated into a low protein concentration fraction and a high protein concentration fraction. In a further example, both the first and second chromatography cycle second protein-of-interest depleted flow through fractions are
25 further fractionated into a low protein concentration fraction and a high protein concentration fraction.

In one example, the protein-of-interest depleted wash flow through fraction is fractionated into the low protein concentration fraction and the high protein concentration fraction based on a determined protein concentration and/or a volume of
30 the wash buffer passed over the chromatography medium. For example, where the separation units comprise columns, the volume of the wash buffer may be determined based on a number of column volumes of the wash buffer passed over the chromatography media.

In one example, the protein-of-interest depleted wash flow through fraction is
35 fractionated into the low protein concentration fraction and the high protein concentration fraction based on a determined protein concentration, for example a

determined protein concentration of the protein-of-interest depleted wash flow through fraction.

In some examples, the valve control system may be communicatively coupled with the protein sensor and configured to control the switching valve arrangement to direct flow of the protein-of-interest depleted wash flow through fraction responsive to a protein concentration determined by the protein sensor. For example, the valve control system may be configured to operate the switching valve arrangement to selectively direct flow from the outlet of the wash loopback separation unit based on a determined protein concentration, for example, a determined protein concentration of the protein-of-interest depleted wash flow through fraction. For example, the protein concentration of the protein-of-interest depleted wash flow through fraction may decrease over time and the switching valve arrangement may be configured to direct the protein-of-interest depleted wash flow through fraction to a collection flow path until the protein sensor in connection with the wash loopback separation unit detects a protein concentration equal to or less than a predetermined further fractionation protein concentration threshold value, thereby to fractionate the protein-of-interest depleted wash flow through fraction into the low protein concentration fraction and the high protein concentration fraction. In some examples, the switching valve arrangement may be configured to selectively direct the protein-of-interest depleted wash flow through fraction to a waste flow path when the protein sensor in connection with the wash loopback separation unit detects a protein concentration equal to or less than the predetermined further fractionation protein concentration threshold value.

In another example, the second protein depleted flow through fraction is fractionated into the low protein concentration fraction and the high protein concentration fraction based on the volume of the wash buffer (e.g. the number of column volumes) passed over the chromatography media.

In one example, the method further comprises pooling the protein-of-interest depleted feed flow through fraction(s) and the high protein concentration fraction(s) from the protein-of-interest depleted wash flow through fraction(s) to produce a protein-of-interest depleted preparation. For example, the method further comprises pooling the first chromatography cycle first protein-of-interest depleted flow through fraction, the second chromatography cycle first protein-of-interest depleted flow through fraction, the high protein concentration fraction from the first chromatography cycle second protein-of-interest depleted flow through fraction and the high protein concentration fraction from the second chromatography cycle second protein-of-interest depleted flow through fraction to produce a protein-of-interest depleted preparation. It will be apparent to the

skilled person from the disclosure herein that additional protein-of-interest depleted flow through fraction(s) and/or additional high protein concentration fraction(s) may be pooled to produce the protein-of-interest depleted preparation.

In one example, the method further comprises pooling the protein-of-interest enriched solution(s) to produce a protein-of-interest enriched preparation. For example, the method further comprises pooling the first chromatography cycle protein-of-interest enriched solution and the second chromatography cycle protein-of-interest enriched solution to produce a protein-of-interest enriched preparation. It will be apparent to the skilled person from the disclosure herein that additional protein-of-interest enriched solution(s) may be pooled to produce the protein-of-interest enriched preparation.

In one example, the protein-of-interest depleted preparation has a dilution factor of less than 25%. For example, the protein-of-interest depleted preparation has a dilution factor of 25% or less. In one example, the protein-of-interest depleted preparation has a dilution factor of between 25% and 20%, or between 25% and 10%, or between 25% and 5%, or between 20%. For example, between 20% and 5%, or between 20% and 10%, or between 15% and 5%, or between 10% and 5%. In one example, the protein-of-interest depleted preparation has a dilution factor of 25% or less. For example, the protein-of-interest depleted preparation has a dilution factor of about 25%, or about 24%, or about 23%, or about 22%, or about 21%, or about 20%. In one example, the protein-of-interest depleted preparation has a dilution factor of 20% or less. In one example, the protein-of-interest depleted preparation has a dilution factor of between 20% and 10%. For example, the protein-of-interest depleted preparation has a dilution factor of about 20%, or about 19%, or about 18%, or about 17%, or about 16%, or about 15%. In one example, the protein-of-interest depleted preparation has a dilution factor of 15% or less. For example, the protein-of-interest depleted preparation has a dilution factor of between 15% and 5%. For example, the protein-of-interest depleted preparation has a dilution factor of between 15% and 10%. In one example, the protein-of-interest depleted preparation has a dilution factor of about 15%, or about 14%, or about 13%, or about 12%, or about 11%, or about 10%. In one example, the protein-of-interest depleted preparation has a dilution factor of about 10% or less. In one example, the protein-of-interest depleted preparation has a dilution factor of about 9%, or about 8%, or about 7%, or about 6%, or about 5%.

In one example, at least 75% of a protein-of-interest depleted fraction (such as the protein-of-interest depleted feed flow through fraction) is recovered from the protein mixture. For example, at least about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80% of the protein-of-interest depleted feed flow through fraction

is recovered from the protein mixture. In one example, at least about 80% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture. For example, at least about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture. In one example, at least about 85% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture. For example, at least about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture. In one example, at least about 90% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture. For example, at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture. In one example, at least about 95% of the protein depleted fraction is recovered from the protein mixture.

In one example of any method described herein, at least about 75% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture, wherein the protein-of-interest depleted feed flow through fraction has a dilution factor of 25% or less. For example, at least about 80% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture, wherein the protein-of-interest depleted feed flow through fraction has a dilution factor of 25% or less. In another example, at least about 85% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture, wherein the protein-of-interest depleted feed flow through fraction has a dilution factor of 25% or less. In a further example, at least about 90% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture, wherein the protein-of-interest depleted feed flow through fraction has a dilution factor of 25% or less. For example, at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95% of the protein-of-interest depleted feed flow through fraction is recovered from the protein mixture, wherein the protein-of-interest depleted feed flow through fraction has a dilution factor of 25% or less.

In one example, the continuous chromatography is selected from the group consisting of simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC). In one example, the continuous chromatography is simulated moving bed (SMB) chromatography. In another example, the continuous chromatography is periodic counter-current chromatography (PCC).

In one example, one or more of the chromatography media is selected from the group consisting of an ion exchange chromatography medium, an affinity chromatography medium, a hydrophobic interaction chromatography medium, a mixed mode chromatography medium, an adsorption chromatography medium and a partition chromatography medium.

In one example, one or more of the chromatography media is an ion exchange chromatography medium. For example, the ion exchange chromatography medium is an anion exchange chromatography medium or a cation exchange chromatography medium.

In one example, one or more of the chromatography media is an affinity chromatography medium.

In one example, one or more of the chromatography media is a hydrophobic interaction medium.

In one example, one or more of the chromatography media is a mixed mode chromatography medium.

In one example, one or more of the chromatography media is an adsorption chromatography medium.

In one example, one or more of the chromatography media is a partition chromatography medium.

In some examples, each of the chromatography media need not be the same. In one example, each separation unit comprises a different chromatography media. In one example, two separation units comprise the same chromatography media. In another example, three separation units comprise the same chromatography media. In a further example, four separation units comprise the same chromatography media. In one example, all separation units comprise the same chromatography media. In one example, the first and third separation units comprise the same chromatography media and the second and fourth separation units comprise the same chromatography media (but different from the first and third). In one example, the first and third separation units comprise the same chromatography media and each of the second and fourth separation units comprise different chromatography media.

In one example, one or more of the chromatography media is selected from the group consisting of a membrane, a monolith and a resin.

In one example, one or more of the chromatography media is a membrane. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography membrane. In one example, one separation unit comprises a chromatography membrane. In one example, two separation units comprise a chromatography membrane. In another example, three separation units comprise a

chromatography membrane. In a further example, four separation units comprise a chromatography membrane. In one example, all separation units comprise a chromatography membrane. In one example, the first separation unit comprises a chromatography membrane. In another example, the second separation unit comprises a chromatography membrane. In a further example, the third separation unit comprises a chromatography membrane. In one example, the fourth separation unit comprises a chromatography membrane.

In one example, one or more of the chromatography media is a monolith. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography monolith. In one example, one separation unit comprises a chromatography monolith. In one example, two separation units comprise a chromatography monolith. In another example, three separation units comprise a chromatography monolith. In a further example, four separation units comprise a chromatography monolith. In one example, all separation units comprise a chromatography monolith. In one example, the first separation unit comprises a chromatography monolith. In another example, the second separation unit comprises a chromatography monolith. In a further example, the third separation unit comprises a chromatography monolith. In one example, the fourth separation unit comprises a chromatography monolith.

In one example, one or more of the chromatography media is a resin. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography resin. In one example, one separation unit comprises a chromatography resin. In one example, two separation units comprise a chromatography resin. In another example, three separation units comprise a chromatography resin. In a further example, four separation units comprise a chromatography resin. In one example, all separation units comprise a chromatography resin. In one example, the first separation unit comprises a chromatography resin. In another example, the second separation unit comprises a chromatography resin. In a further example, the third separation unit comprises a chromatography resin. In one example, the fourth separation unit comprises a chromatography resin.

In one example, one or more of the chromatography media comprise a ligand capable of specifically binding the protein-of-interest. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In one example, one separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In another example, two separation units

comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In a further example, three separation units comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In one example, four separation units comprise a chromatography media
5 comprising a ligand capable of specifically binding the protein-of-interest. In one example, the first separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In another example, the second separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In a further example, the third separation
10 unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In one example, the fourth separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media,
15 comprise a ligand capable of specifically binding the protein-of-interest.

In one example, one or more of the chromatography media comprise a ligand capable of specifically binding human IgG. For example, one or more of the chromatography media may comprise a ligand capable of specifically binding to a constant domain of human IgG. For example, the ligand may be capable of specifically
20 binding to a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, or combinations thereof. For example, the first, second, third and/or fourth separation unit(s) may comprise a chromatography media comprising a ligand capable of specifically binding to human IgG, for example to a constant domain of human IgG, for example CH1, CH2, CH3, CH4 or combinations thereof. In one example, one separation unit comprises a
25 chromatography medium comprising a ligand capable of specifically binding to human IgG. In another example, two separation units comprise a chromatography media comprising a ligand capable of specifically binding to human IgG. In a further example, three separation units comprise a chromatography media comprising a ligand capable of specifically binding to a human IgG. In one example, four separation units comprise a
30 chromatography media comprising a ligand capable of specifically binding to human IgG. In one example, the first separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In another example, the second separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In a further example, the third separation
35 unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In one example, the fourth separation unit comprises a

chromatography medium comprising a ligand capable of specifically binding to human IgG. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand capable of specifically binding to human IgG. In any of the above
 5 examples, the ligand capable of specifically binding to human IgG may be capable of specifically binding to a constant domain of human IgG. For example, the ligand may be capable of specifically binding to a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, or combinations thereof.

In one example, the ligand comprises a camelid-derived single domain [VHH]
 10 antibody fragment. For example, the ligand is a VHH antibody fragment. In one example, the ligand does not comprise a CH1 domain.

In one example, the ligand comprises a VHH antigen-binding protein comprising an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1. In one example, the VHH
 15 antigen-binding protein comprises an amino acid sequence set forth in SEQ ID NO: 1. In one example, the VHH antigen-binding protein comprises a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1.

In one example, the ligand comprises a VHH antigen-binding protein comprising a framework region comprising an amino acid sequence set forth in SEQ ID NO: 1 or a
 20 sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1. In one example, the framework region comprises an amino acid sequence set forth in SEQ ID NO: 1. In another example, the framework region comprises a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1.

In one example, the ligand comprises an VHH antigen-binding protein comprising
 25 an amino acid sequence that comprises 4 framework regions, FR1, FR2, FR3 and FR4, and 3 complementarity determining regions, CDR1, CDR2 and CDR3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

- a) the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or
 30 two of the amino acid residues;
- b) the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
- c) the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the framework
 35 regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1; and

wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1.

In one example, the ligand comprises a VHH antigen-binding protein comprising an amino acid sequence that comprises 4 framework regions, FR1, FR2, FR3 and FR4, and 3 complementarity determining regions, CDR1, CDR2 and CDR3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

- a) the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
- 10 b) the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
- c) the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1, and
- 15

wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1 and wherein the antigen binding protein specifically binds to the Fc domain of a human IgG molecule and does not bind to an IgG molecule of murine origin or bovine origin.

20 In one example, the ligand comprises a VHH antigen-binding protein comprising a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues.

In one example, ligand comprises a VHH antigen-binding protein comprising a CDR2 comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3.

In one example, the ligand comprises a VHH antigen-binding protein comprising a CDR3 comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4.

30 In one example, one or more of the chromatography media comprise a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, one separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked

35

poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In another example, two separation units comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In a further example, three separation units comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, four separation units comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, the first separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In another example, the second separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In a further example, the third separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, the fourth separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix.

In one example, one or more of the chromatography media comprise a cross-linked poly(styrene-divinylbenzene) matrix. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, one separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In another example, two separation units comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In a further example, three separation units comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, four separation units comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the first separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In another example, the second separation unit comprises a chromatography medium comprising a cross-linked

poly(styrene-divinylbenzene) matrix. In a further example, the third separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the fourth separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a cross-linked poly(styrene-divinylbenzene) matrix.

In one example, one or more of the chromatography media comprise an agarose-based matrix. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography media comprising an agarose-based matrix. In one example, one separation unit comprises a chromatography medium comprising an agarose-based matrix. In another example, two separation units comprise a chromatography media comprising an agarose-based matrix. In a further example, three separation units comprise a chromatography media comprising an agarose-based matrix. In one example, four separation units comprise a chromatography media comprising an agarose-based matrix. In one example, the first separation unit comprises a chromatography medium comprising an agarose-based matrix. In another example, the second separation unit comprises a chromatography medium comprising an agarose-based matrix. In a further example, the third separation unit comprises a chromatography medium comprising an agarose-based matrix. In one example, the fourth separation unit comprises a chromatography medium comprising an agarose-based matrix. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise an agarose-based matrix.

In one example, one or more of the chromatography media comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography media comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In one example, one separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In another example, two separation units comprise a chromatography media comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In a further example, three separation units comprise a chromatography media comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In one example, four separation units comprise a chromatography media

comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the first separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In another example, the second separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In a further example, the third separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the fourth separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix.

In some examples, one or more of the separation units comprises an affinity chromatography medium and one or more of the separation units comprises an ion exchange chromatography medium. The affinity chromatography medium may comprise a ligand capable of specifically binding to human IgG, for example to a constant domain of human IgG. For example the ligand may be capable of specifically binding to a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, or combinations thereof.

In one example, the first separation unit comprises an affinity chromatography medium and the third separation unit comprises an ion exchange chromatography medium.

In one example, the first and second separation units each comprise an affinity chromatography medium, and the third and fourth separation units each comprise an ion exchange chromatography media.

In some examples, one or more of the at least four separation units is loaded with the feed comprising the protein-of-interest at a concentration above a dynamic binding capacity (DBC) of the chromatography medium of the respective separation unit.

In one example, the first separation unit and/or second separation unit is loaded with the protein mixture at a concentration above a dynamic binding capacity (DBC) of the first and/or second chromatography media. For example, the first separation unit is loaded with the protein mixture at a concentration above a dynamic binding capacity (DBC) of the first chromatography medium. In another example, the second separation unit is loaded with the protein mixture at a concentration above a dynamic binding

capacity (DBC) of the first chromatography medium. In a further example, the first separation unit and second separation unit are loaded with the protein mixture at a concentration above a dynamic binding capacity (DBC) of the first and second chromatography media.

5 Determining the DBC of a chromatography medium will be apparent to a skilled person and/or described herein. For example, the DBC of a resin may be determined by loading the protein mixture on the column and monitoring the concentration at which unbound protein-of-interest flows through the column, for example, by determining a protein concentration of the feed flow-through using a protein sensor as described herein
10 in connection with the outlet of the feed separation unit (e.g. by UV trace of the chromatography system and/or by other suitable method of determining the protein concentration). For example, the DBC of the media is 5 mg, or 10 mg, or 20 mg, or 30 mg, or 40 mg, or 50 mg, or 60 mg, or 70 mg protein-of-interest per mL of medium.

 In one example, the DBC of at least one chromatography medium (for example,
15 the first chromatography medium) is at least 5 mg protein-of-interest per mL of the chromatography medium. In another example, the DBC of the chromatography medium is at least 10 mg protein-of-interest per mL of chromatography medium. In a further example, the DBC of the chromatography medium is at least 20 mg protein-of-interest per mL of chromatography medium. In one example, the DBC of the chromatography
20 medium is at least 40 mg protein-of-interest per mL of chromatography medium.

 In one example, at least one separation unit (for example, the first separation unit) is loaded with the feed comprising the protein-of-interest at a concentration of more than 5 mg, or 10 mg, or 20 mg, or 30 mg, or 40 mg, or 50 mg, or 60 mg, or 70 mg protein-of-interest per mL of chromatography medium.

25 In one example, the at least one separation unit is loaded with the feed comprising the protein-of-interest at a concentration of more than 5 mg protein-of-interest per mL of chromatography medium.

 In one example, the at least one separation unit is loaded with the feed comprising the protein-of-interest at a concentration of more than 10 mg protein-of-interest per mL
30 of the chromatography medium.

 In one example, the at least one separation unit is loaded with the feed comprising the protein-of-interest at a concentration of more than 20 mg protein-of-interest per mL of the chromatography medium.

 In one example, the at least one separation unit is loaded with the feed comprising
35 the protein-of-interest at a concentration of more than 30 mg protein-of-interest per mL of the chromatography medium.

In one example, the at least one separation unit is loaded with the feed comprising the protein-of-interest at a concentration of more than 40 mg protein-of-interest per mL of the chromatography medium.

5 In one example, the at least one separation unit is loaded with the feed comprising the protein-of-interest at a concentration of more than 50 mg protein-of-interest per mL of the chromatography medium.

In one example, the at least one separation unit is loaded with the feed comprising the protein-of-interest at a concentration of more than 60 mg protein-of-interest per mL of the chromatography medium.

10 In one example, the at least one separation unit is loaded with the feed comprising the protein-of-interest at a concentration of more than 70 mg protein-of-interest per mL of the chromatography medium.

In one example, the at least one separation unit and one or more additional separation unit(s) are loaded with the the feed comprising the protein-of-interest at a concentration up to the DBC of the chromatography medium.

15 In one example, the at least one separation unit and the one or more additional separation unit(s) are loaded with the feed comprising the protein-of-interest at a concentration of up to 5 mg, or 10 mg, or 20 mg, or 30 mg, or 40 mg IgG per mL of the chromatography medium.

20 In one example, the at least one separation unit and the one or more additional separation unit(s) are loaded with the protein-of-interest at a concentration of up to 20 mg IgG per mL of the chromatography medium.

In one example, the at least one separation unit and the one or more additional separation unit(s) are loaded with the feed comprising the protein-of-interest at a concentration of up to 30 mg IgG per mL of the chromatography medium.

25 In one example, the at least one separation unit and the one or more additional separation unit(s) are loaded with the feed comprising the protein-of-interest at a concentration of up to 40 mg IgG per mL of the chromatography medium.

30 In one example, the chromatography media have a total bed height of between 2 cm and 30 cm.

In some examples, the valve control system may be communicatively coupled with the protein sensor and configured to control the switching valve arrangement to direct flow of the feed responsive to a protein concentration determined by the protein sensor. For example, the valve control system may be configured to operate the switching valve arrangement to selectively direct flow to the inlet of the feed separation unit based on a determined protein concentration, for example, a determined protein concentration

of the feed flow through. For example, the protein concentration of the feed flow through may increase over time and the switching valve arrangement may be configured to direct flow of the feed to the inlet of the feed separation unit until the protein sensor in connection with the outlet of the determines that a protein concentration of the feed flow through is equal to or greater than a predetermined feed flow through protein concentration threshold value.

In one example, the chromatography media have a total bed height of at least 2 cm. For example, the chromatography media have a total bed height of between 2 cm to 30 cm. For example, the chromatography media have a total bed height of between 10 cm and 30 cm. For example, the chromatography media have a total bed height of between 30 cm and 70 cm. For example, the chromatography media have a total bed height of 2cm, or 6 cm, or 10 cm, or 15cm, or 20 cm, or 25 cm, or 30 cm, or 35 cm, or 40 cm, or 45 cm, or 50 cm, or 55 cm, or 60 cm, or 65 cm, or 70 cm.

In one example, the chromatography media have a total bed height of at least 2 cm.

In one example, the chromatography media have a total bed height of 6 cm.

In one example, the chromatography media have a total bed height of 20 cm.

In one example, the chromatography media have a total bed height of 30 cm.

In one example, the chromatography media have a total bed height of 50 cm.

In one example, the chromatography media have a total bed height of 70 cm.

In one example, one or more of the separation units are selected from the group consisting of a column, a cassette, a capsule and a filter holder.

In one example, one or more of the at least four separation units is a column. In one example, one of the at least four separation units is a column. For example, the first separation unit is a column. In another example, the second separation unit is a column. In another example, the third separation unit is a column. In a further example, the fourth separation unit is a column. In a further example, a fifth separation unit is a column. In one example, two of the separation units are columns. In another example, three of the separation units are columns. In a further example, four of the separation units are columns. In another example, five of the separation units are columns. In one example, each separation unit is a column.

In one example, one or more of the separation units is a cassette. For example, the first separation unit is a cassette. In another example, the second separation unit is a cassette. In another example, the third separation unit is a cassette. In a further example, the fourth separation unit is a cassette. In a further example, a fifth separation unit is a cassette. In one example, two of the separation units are cassettes. In another example,

three of the separation units are cassettes. In a further example, four of the separation units are cassettes. In another example, five of the separation units are cassettes. In one example, each separation unit is a cassette.

In one example, one or more of the separation units is a capsule. For example, the first separation unit is a capsule. In another example, the second separation unit is a capsule. In another example, the third separation unit is a capsule. In a further example, the fourth separation unit is a capsule. In a further example, a fifth separation unit is a capsule. In one example, two of the separation units are capsules. In another example, three of the separation units are capsules. In a further example, four of the separation units are capsules. In another example, five of the separation units are capsules. In one example, each separation unit is a capsule.

In one example, one or more of the separation units is a filter holder. For example, the first separation unit is a filter holder. In another example, the second separation unit is a filter holder. In another example, the third separation unit is a filter holder. In a further example, the fourth separation unit is a filter holder. In a further example, a fifth separation unit is a filter holder. In one example, two of the separation units are filter holders. In another example, three of the separation units are filter holders. In a further example, four of the separation units are filter holders. In another example, five of the separation units are filter holders. In one example, each separation unit is a filter holder.

In one example, one or more of the separation units are a column, wherein the column has a diameter of between 5 cm and 200 cm. For example, the column has a diameter of 5 cm, or 10 cm, or 20 cm, or 30 cm, or 40 cm, or 50 cm, or 60 cm, or 70 cm, or 80 cm, or 90 cm, or 100 cm, or 110 cm, or 120 cm, or 130 cm, or 140 cm, or 150 cm, or 160 cm, or 170 cm, or 180 cm, or 190 cm, or 200 cm. In one example, the column has a diameter of 5 cm. In one example, the column has a diameter of 20 cm. In one example, the column has a diameter of 50 cm. In one example, the column has a diameter of 100 cm. In one example, the column has a diameter of 200 cm. In one example, the column has a diameter of less than 5 cm, such as 1 cm, 2 cm, 3 cm or 4 cm.

In one example, the wash buffer has a pH of between 5 and 10 and a dissociation constant (pKa) between 6.8 and 8.5 at 25°C.

In one example, the wash buffer has a pH of between 5 and 10. In one example, the wash buffer has a pH of between 5 and 9. For example, the wash buffer is at a pH of 5, or 5.1, or 5.2, or 5.3, or 5.4, or 5.5, or 5.6, or 5.7, or 5.8, or 5.9, or 6.0, or 6.1, or 6.2, or 6.3, or 6.4, or 6.5, or 6.6, or 6.7, or 6.8, or 6.9, or 7, or 7.1, or 7.2, or 7.3, or 7.4, or 7.5, or 7.6 or 7.7 or 7.8, or 7.9, or 8.0, or 8.1, or 8.2, or 8.3, or 8.4, or 8.5, or 8.6, or 8.7,

or 8.8, or 8.9, or 9.0, or 9.1 or 9.2, or 9.3, or 9.4, or 9.5, or 9.6, or 9.7, or 9.8, or 9.9, or 10.0.

In one example, the wash buffer has a pH of between 7 and 10 and a dissociation constant (pKa) between 6.8 and 8.5 at 25°C.

- 5 In one example, the wash buffer has a pH of between 7 and 8 and a dissociation constant (pKa) between 6.8 and 8.5 at 25°C.

In one example, the wash buffer has a pH of between 7 and 8. For example, the wash buffer has a pH of 7, or 7.1, or 7.2, or 7.3, or 7.4, or 7.5, or 7.6 or 7.7 or 7.8, or 7.9 or 8.0. In one example, the wash buffer has a pH of 7.4.

- 10 In one example, the wash buffer has a pH of between 7.4 and 7.8. For example, the wash buffer has a pH of 7.4, or 7.5, or 7.6 or 7.7 or 7.8.

In one example, the wash buffer has a pKa of between 6.8 and 8.5 at 25°C. For example, the wash buffer has a pKa of 6.8, or 6.9, or 7.0, or 7.1, or 7.2, or 7.3, or 7.4, or 7.5, or 7.6, or 7.7, or 7.8, or 7.9, or 8.0, or 8.1, or 8.2, or 8.3, or 8.4, or 8.5 at 25°C.

- 15 In one example, the wash buffer has a pKa of 7.21 at 25°C.

In one example, the wash buffer has a pH of 7.4 and dissociation constant (pKa) of 7.21 at 25°C.

- In one example, the wash buffer comprises a buffering agent selected from a group consisting of sodium dihydrogen phosphate, sodium citrate, imidazole, Tris, glycyglycine, 3-morpholinopropane-1-sulfonic acid (MOPS), piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES), bis[(2-hydroxyethyl)amino]acetic acid (Bicine), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sulfurous acid, 4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS), N-(Hydroxyethyl)piperazine-N'-2-hydroxypropanesulfonic acid (HEPPSO), 4-(N-Morpholino)butanesulfonic acid (MOBS), Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) (POPSO), N-[Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid (TAPSO), Tricine, triethanolamine (TEA) and combinations thereof. For example, the wash buffer is a sodium dihydrogen phosphate buffer. For example, the wash buffer is an imidazole buffer. In another example, the wash buffer is a Tris buffer. In a further example, the wash buffer is a glycyglycine buffer. In one example, the wash buffer is a MOPS buffer. In another example, the wash buffer is a PIPES buffer. In a further example, the wash buffer is a TES buffer. In one example, the wash buffer is a Bicine buffer. In another example, the wash buffer is a sulfurous acid buffer. In a further example, the wash buffer is an EPPS buffer. In one example, the wash buffer is a HEPPSO buffer. In another
- 20
- 25
- 30
- 35

example, the wash buffer is a MOBS buffer. In a further example, the wash buffer is a POPSO buffer. In one example, the wash buffer is a TAPSO buffer. In another example, the wash buffer is a Tricine buffer. In a further example, the wash buffer is a TEA buffer. In one example, the wash buffer is a sodium citrate buffer.

5 In one example, the buffering agent of the wash buffer is at a concentration of between 5mM to 200mM. For example, the buffering agent of the wash buffer is at a concentration of between 5mM to 10mM, or 5mM to 20mM, or 5mM to 50mM, or 50mM to 100mM, or 100mM to 150mM, or 150mM to 200mM. In another example, the buffering agent of the wash buffer is at a concentration of 5mM, or 10mM, or 15mM, or
10 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM, or 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM, or 105mM, or 110mM, or 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM.

15 In one example, the buffering agent of the wash buffer is at a concentration of 5mM. In one example, the buffering agent of the wash buffer is at a concentration of 20mM. In one example, the buffering agent of the wash buffer is at a concentration of 50mM. In one example, the buffering agent of the wash buffer is at a concentration of 100mM. In one example, the buffering agent of the wash buffer is at a concentration of
20 150mM. In one example, the buffering agent of the wash buffer is at a concentration of 200mM.

In one example, the wash buffer further comprises sodium chloride. For example, the wash buffer further comprises sodium chloride at a concentration of up to 1000 mM. In one example, the sodium chloride is at a concentration of between 5mM and 50mM.
25 For example, the sodium chloride is at a concentration of 5mM, or 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM. In another example, the sodium chloride is at a concentration of between 50mM and 100mM. For example, the sodium chloride is at a concentration of 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM. In one example,
30 the sodium chloride is at a concentration of between 100 and 200 mM. For example, the sodium chloride is at a concentration of 105mM, or 110mM, 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In another example, the sodium chloride is at a concentration of
35 between 200 and 300 mM. For example, the sodium chloride is at a concentration of 200mM, or 225mM, or 250mM, or 275mM, or 300mM. In a further example, the sodium

chloride is at a concentration of between 300 and 400 mM. For example, the sodium chloride is at a concentration of 300mM, or 325mM, or 350mM, or 375mM, or 400mM. In one example, the sodium chloride is at a concentration of between 400mM and 500mM. For example, the sodium chloride is at a concentration of 400mM, or 425mM, or 450mM, or 475mM, or 500mM. In another example, the sodium chloride is at a concentration of between 500mM and 1000mM. For example, the sodium chloride is at a concentration of 500mM, or 550mM, or 600mM, or 650mM, or 700mM, or 750mM, or 800mM, or 850mM, or 900mM, or 950mM, or 1000mM. In one example, the sodium chloride is at a concentration of less than 1000mM. For example, the sodium chloride is at a concentration of 500mM. In one example, the wash buffer further comprises sodium chloride, wherein the sodium chloride is at a concentration of 145 mM. In one example, the wash buffer further comprises sodium chloride, wherein the sodium chloride is at a concentration of 500 mM.

In one example, the wash buffer comprises 20 mM sodium dihydrogen phosphate, 145 mM sodium chloride and is at a pH of 7.4.

In one example, the wash buffer comprises 20 mM sodium dihydrogen phosphate, 500 mM sodium chloride and is at a pH of 7.4.

In one example, the method comprises one or more additional wash steps. For example, a washing phase according to the method may comprise a first wash and a second wash. In some examples the washing phase may comprise a third and optionally further (e.g. fourth, fifth or more) washes. The first wash may be performed with a first wash buffer. The second wash may be performed with a second wash buffer. The third and optionally further washes may be performed with respective third and further wash buffers. In such examples, loading the wash buffer onto the wash separation unit may comprise loading the first wash buffer, the second wash buffer and optionally the third or further wash buffers onto the wash separation unit in sequence.

In some examples, the one or more additional washes are performed with the same wash buffer. For example, the first wash buffer and the second wash buffer may be the same wash buffer. The further wash buffers may be the same as the first wash buffer and/or the second wash buffer. In one example, the one or more additional washes are performed with a different wash buffer. For example, the second wash may be different to the first wash buffer. In some examples, any of the third or further wash buffers may be different to the first wash buffer and/or the second wash buffer. For example, the one or more additional washes comprise a buffer comprising phosphoric acid. In some examples, one or more of the additional washes may be performed with low conductivity buffer. For example, one or more of the additional washes may be performed with a wash

buffer having a different salt content to the first wash buffer, for example a reduced salt content, for example no salt content. For example, the second wash buffer may have a lower conductivity than the first wash buffer. The second wash buffer may have a reduced salt content compared to the first wash buffer, for example the second wash
5 buffer may not contain salt. In some examples, one or more of the additional washes may be performed with a wash buffer having a different pH to the first wash buffer. For example, the second wash buffer may have a lower pH than the first wash buffer.

In one example, the one or more additional wash buffers comprises 10 mM phosphoric acid (H_3PO_4) at a pH of 6.8. In one example, the one or more additional wash
10 buffers comprises 10 mM phosphoric acid (H_3PO_4) at a pH of 6.8 with no salt included.

In one example, the wash buffer further comprises a divalent salt. For example, the wash buffer further comprises a divalent salt at a concentration of up to 1000 mM. In one example, the divalent salt is at a concentration of between 5mM and 50mM. For example, the divalent salt is at a concentration of 5mM, or 10mM, or 15mM, or 20mM,
15 or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM. In another example, the divalent salt is at a concentration of between 50mM and 100mM. For example, the divalent salt is at a concentration of 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM. In one example, the divalent salt is at a concentration of between 100 and 200 mM. For example, the divalent salt is at a
20 concentration of 105mM, or 110mM, 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In another example, the divalent salt is at a concentration of between 200 and 300 mM. For example, the divalent salt is at a concentration of 200mM, or 225mM, or 250mM, or
25 275mM, or 300mM. In a further example, the divalent salt is at a concentration of between 300 and 400 mM. For example, the divalent salt is at a concentration of 300mM, or 325mM, or 350mM, or 375mM, or 400mM. In one example, the divalent salt is at a concentration of between 400mM and 500mM. For example, the divalent salt is at a concentration of 400mM, or 425mM, or 450mM, or 475mM, or 500mM. In another
30 example, the divalent salt is at a concentration of between 500mM and 1000mM. For example, the divalent salt is at a concentration of 500mM, or 550mM, or 600mM, or 650mM, or 700mM, or 750mM, or 800mM, or 850mM, or 900mM, or 950mM, or 1000mM. In one example, the divalent salt is at a concentration of 500mM. In one example, the divalent salt is at a concentration of less than 1000mM.

In one example, the wash buffer comprises sodium chloride and/or a divalent salt at a concentration of up to 1000 mM. For example, the wash buffer comprises sodium chloride and/or a divalent salt at a concentration of about 500 mM.

In one example, the divalent salt is selected from a group consisting of magnesium chloride, calcium chloride, barium chloride, copper (II) chloride, nickel chloride, manganese chloride, and a combination thereof. For example, the divalent salt is magnesium chloride. In one example, the divalent salt is calcium chloride. In another example, the divalent salt is barium chloride. In a further example, the divalent salt is copper chloride. In one example, the divalent salt is nickel chloride. In another example, the divalent salt is manganese chloride.

In one example, the elution buffer has a pH of between 3 and 5. For example, the elution buffer has a pH of 3, or 3.1, or 3.2, or 3.3, or 3.4, or 3.5, or 3.6, or 3.7, or 3.8, or 3.9, or 4, or 4.1, or 4.2, or 4.3, or 4.4, or 4.5, or 4.6, or 4.7, or 4.8, or 4.9, or 5.

In one example, the elution buffer has a pH of 4.

In one example, the elution buffer comprises a buffering agent selected from the group consisting of sodium acetate, acetic acid and sodium citrate. In one example, the elution buffer comprises sodium acetate, acetic acid, sodium citrate and sodium dihydrogen phosphate. In one example, the elution buffer is or comprises a sodium phosphate buffer and/or an acetate buffer. For example, the elution buffer comprises sodium acetate. For example, the elution buffer comprises acetic acid. For example, the elution buffer comprises sodium citrate. For example, the elution buffer comprises sodium dihydrogen phosphate.

In one example, the buffering agent of the elution buffer is at a concentration of between 5mM to 200mM. For example, the buffering agent of the elution buffer is at a concentration of between 5mM to 10mM, or 5mM to 20mM, or 5mM to 50mM, or 50mM to 100mM, or 100mM to 150mM, or 150mM to 200mM. For example, the buffering agent of the wash buffer is at a concentration of 5mM, or 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM, or 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM, or 105mM, or 110mM, or 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In one example, the buffering agent of the elution buffer is at a concentration of 5mM. In one example, the buffering agent of the elution buffer is at a concentration of 20mM. In one example, the buffering agent of the elution buffer is at a concentration of 50mM. In one example, the buffering agent of the elution buffer is at a concentration of 100mM. In one example, the

buffering agent of the elution buffer is at a concentration of 150mM. In one example, the buffering agent of the elution buffer is at a concentration of 200mM.

In one example, the elution buffer is or comprises an acetate buffer. For example, a sodium acetate buffer.

5 In one example, the elution buffer is or comprises a phosphate buffer, an acetic acid buffer and/or an acetate buffer. For example, the elution buffer is or comprises a sodium dihydrogen phosphate and a sodium acetate buffer. In one example, the elution buffer is or comprises a phosphate buffer. In one example, the elution buffer is or comprises an acetic acid buffer.

10 In one example, the elution buffer is or comprises a phosphate, an acetic acid and/or an acetate buffer at a pH of between 3 and 5. For example, the elution buffer is or comprises a phosphate, acetic acid and/or an acetate buffer at a pH of 3, or 3.1, or 3.2, or 3.3, or 3.4, or 3.5, or 3.6, or 3.7, or 3.8, or 3.9, or 4, or 4.1, or 4.2, or 4.3, or 4.4, or 4.5, or 4.6, or 4.7, or 4.8, or 4.9, or 5. In one example, the elution buffer is or comprises
15 a phosphate, an acetic acid and/or an acetate buffer at a pH of 4.

In one example, the elution buffer is or comprises an acetate buffer at a pH of between 3 and 5. For example, the elution buffer is or comprises an acetate buffer at a pH of 3, or 3.1, or 3.2, or 3.3, or 3.4, or 3.5, or 3.6, or 3.7, or 3.8, or 3.9, or 4, or 4.1, or 4.2, or 4.3, or 4.4, or 4.5, or 4.6, or 4.7, or 4.8, or 4.9, or 5. In one example, the elution
20 buffer is or comprises an acetate buffer at a pH of 4. For example, the elution buffer is or comprises a sodium acetate buffer at a pH of 4.

In one example, the elution buffer is or comprises an acetic acid buffer at a pH of between 3 and 5. For example, the elution buffer is or comprises an acetic acid buffer at a pH of 3, or 3.1, or 3.2, or 3.3, or 3.4, or 3.5, or 3.6, or 3.7, or 3.8, or 3.9, or 4, or 4.1, or
25 4.2, or 4.3, or 4.4, or 4.5, or 4.6, or 4.7, or 4.8, or 4.9, or 5. In one example, the elution buffer is or comprises an acetic acid buffer at a pH of 4.

In one example, the elution buffer is or comprises a phosphate buffer at a pH of between 3 and 5. For example, the elution buffer is or comprises a phosphate buffer at a pH of 3, or 3.1, or 3.2, or 3.3, or 3.4, or 3.5, or 3.6, or 3.7, or 3.8, or 3.9, or 4, or 4.1, or
30 4.2, or 4.3, or 4.4, or 4.5, or 4.6, or 4.7, or 4.8, or 4.9, or 5. In one example, the elution buffer is or comprises a phosphate buffer at a pH of 4.

In one example, the elution buffer comprises 10 mM, or 11 mM, or 12 mM, or 13 mM, or 14 mM, or 15 mM, or 16 mM, or 17 mM, or 18 mM, or 19 mM, or 20 mM of a phosphate, acetic acid and/or an acetate buffer.

35 In one example, the elution buffer comprises 10 mM, or 11 mM, or 12 mM, or 13 mM, or 14 mM, or 15 mM, or 16 mM, or 17 mM, or 18 mM, or 19 mM, or 20 mM of an

acetate buffer. For example, the elution buffer comprises 20mM acetate buffer. In one example, the elution buffer comprises 20mM sodium acetate buffer. In one example, the elution buffer comprises 20mM acetic acid buffer.

5 In one example, the elution buffer comprises 10 mM, or 11 mM, or 12 mM, or 13 mM, or 14 mM, or 15 mM, or 16 mM, or 17 mM, or 18 mM, or 19 mM, or 20 mM of a phosphate buffer. For example, the elution buffer comprises 20mM phosphate buffer. In one example, the elution buffer comprises 20mM sodium phosphate buffer.

In one example, the elution buffer comprises 20 mM sodium acetate at a pH of 4.

In one example, the elution buffer comprises 20 mM acetic acid at a pH of 4.

10 In one example, the elution buffer further comprises sodium chloride. For example, the elution buffer further comprises sodium chloride at a concentration of up to 150 mM. In one example, the sodium chloride is at a concentration of between 50 to 100 mM. For example, the sodium chloride is at a concentration of 50mM, 55mM, 60mM, 65mM, 70mM, 75mM, 80mM, 85mM, 90mM, 95mM or 100mM. In another example, 15 the sodium chloride is at a concentration of between 100 to 150 mM. For example, the sodium chloride is at a concentration of 105mM, 110mM, 115mM, 120mM, 125mM, 130mM, 135mM, 140mM, 145mM, or 150mM.

In one example, the elution buffer further comprises a divalent salt. For example, the elution buffer further comprises a divalent salt at a concentration of up to 3M. In one 20 example, the divalent salt is at a concentration of between 50 mM and 3M. In one example, the divalent salt is at a concentration of between 1M and 3M. For example, the divalent salt is at a concentration of 1M, or 1.1M, or 1.2M, or 1.3M, or 1.4M, or 1.5M, or 1.6M, or 1.7M, or 1.8M, or 1.9M, or 2M. In another example, the divalent salt is at a concentration of 2M, or 2.1M, or 2.2M, or 2.3M, or 2.4M, or 2.5M, or 2.6M, or 2.7M, or 25 2.8M, or 2.9M, or 3M. In one example, the divalent salt is at a concentration of 1M. In another example, the divalent salt is at a concentration of 1.5M. In a further example, the divalent salt is at a concentration of 2M. In one example, the divalent salt is at a concentration of 2.5M. In a further example, the divalent salt is at a concentration of 3M. In one example, the divalent salt is at a concentration of between 50 mM and 1M. In a 30 further example, the divalent salt is at a concentration of between 500 mM and 1M. For example, the divalent salt is at a concentration of 500 mM, or 550 mM, or 600 mM, or 650 mM, or 700 mM, or 750 mM, or 800 mM, or 850 mM, or 900 mM, or 950 mM or 1M. In one example, the divalent salt is at a concentration of about 500 mM. In a further example, the divalent salt is at a concentration of about 600 mM. In another example, 35 the divalent salt is at a concentration of about 700 mM. In one example, the divalent salt is at a concentration of about 800 mM. In a further example, the divalent salt is at a

concentration of about 900 mM. In another example, the divalent salt is at a concentration of about 1000 mM. In one example, the divalent salt is at a concentration of between 50 mM and 500 mM. For example, the divalent salt is at a concentration of between 100 mM and 500 mM. In one example, the divalent salt is at a concentration of 100 mM, 150 mM, 200 mM, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM or 500 mM. In one example, the divalent salt is at a concentration of between 50 to 100 mM. For example, the divalent salt is at a concentration of 50mM, 55mM, 60mM, 65mM, 70mM, 75mM, 80mM, 85mM, 90mM, 95mM, or 100mM. In another example, the divalent salt is at a concentration of between 100 to 150 mM. For example, the divalent salt is at a concentration of 100mM, 105mM, 110mM, 115mM, 120mM, 125mM, 130mM, 135mM, 140mM, 145mM, or 150mM.

In one example, the divalent salt is selected from a group consisting of magnesium chloride, calcium chloride, barium chloride, copper (II) chloride, nickel chloride, manganese chloride, and a combination thereof. For example, the divalent salt is magnesium chloride. For example, the divalent salt is calcium chloride. In one example, the divalent salt is barium chloride. In another example, the divalent salt is copper chloride. In a further example, the divalent salt is nickel chloride. In one example, the divalent salt is manganese chloride.

In one example, the divalent salt is at magnesium chloride at a concentration of about 2M.

In one example, the method further comprises equilibrating at least one chromatography medium with an equilibration buffer. For example, the chromatography medium may be equilibrated before loading the feed comprising protein mixture comprising the protein-of interest on to the chromatography medium. For example, the method may comprise loading the equilibration buffer onto a separation unit after loading the elution buffer. For example, the equilibration buffer may be loaded onto the wash separation unit or onto the elution separation unit after loading the elution buffer. In one example, the equilibration buffer may be loaded onto the wash separation unit after loading the elution buffer, wherein the wash separation unit functions as the feed separation unit in the immediately following cycle segment. In another example, the equilibration buffer may be loaded onto the elution separation unit after loading the elution buffer, wherein the elution separation unit functions as the feed separation unit in the immediately following cycle segment.

In one example, the equilibration buffer may have a pH of between 5 and 9. In one example, the equilibration buffer may have a pH between 7 and 8. For example, the

equilibration buffer has a pH of 5.0, or 5.1, or 5.2, or 5.3, or 5.4, or 5.5, or 5.6, or 5.7, or 5.8, or 5.9, or 6.0, or 6.1, or 6.2, or 6.3, or 6.4, or 6.5, or 6.6, or 6.7, or 6.8, or 6.9, or 7.0, or 7.1, or 7.2, or 7.3, or 7.4, or 7.5, or 7.6 or 7.7 or 7.8, or 7.9, or 8.0, or 8.1, or 8.2, or 8.3, or 8.4, or 8.5, or 8.6, or 8.7, or 8.8, or 8.9, or 9.0.

5 In one example, the equilibration buffer comprises a buffering agent selected from a group consisting of sodium dihydrogen phosphate, sodium citrate, imidazole, Tris, glycylglycine, MOPS, PIPES, TES, Bicine, HEPES, EPPS, HEPPSO, MOBS, POPSO, TAPSO, Tricine, TEA and combinations thereof. For example, the equilibration buffer is a sodium dihydrogen phosphate buffer. In another example, the equilibration buffer is
10 a sodium citrate buffer. In a further example, the equilibration buffer is an imidazole buffer. In one example, the equilibration buffer is a Tris buffer. In another example, the equilibration buffer is a glycylglycine buffer. In a further example, the equilibration buffer is a MOPS buffer. In one example, the equilibration buffer is a PIPES buffer. In another example, the equilibration buffer is a TES buffer. In a further example, the
15 equilibration buffer is a Bicine buffer. In one example, the equilibration buffer is a sulfurous acid buffer. In another example, the equilibration buffer is an EPPS buffer. In a further example, the equilibration buffer is a HEPPSO buffer. In one example, the equilibration buffer is a MOBS buffer. In another example, the equilibration buffer is a POPSO buffer. In a further example, the equilibration buffer is a TAPSO buffer. In one
20 example, the equilibration buffer is a Tricine buffer. In another example, the equilibration buffer is a TEA buffer.

 In one example, the buffering agent of the equilibration buffer is at a concentration of between 5mM and 200mM. In one example, the buffering agent of the equilibration buffer is at a concentration of between 5mM and 50mM, for example at a
25 concentration of 5mM, or 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM. In another example, the buffering agent of the equilibration buffer is at a concentration of between 50mM and 100mM, for example, 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM. In a further example, the equilibration buffer is at a concentration of
30 between 100mM and 150mM, for example 105mM, or 110mM, or 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM. In one example, the equilibration buffer is at a concentration of between 150mM and 200mM, for example 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In one example, the buffering agent of the
35 equilibration buffer is at a concentration of 5mM. In one example, the buffering agent of the equilibration buffer is at a concentration of 20mM. In one example, the buffering

agent of the equilibration buffer is at a concentration of 50mM. In one example, the buffering agent of the equilibration buffer is at a concentration of 100mM. In one example, the buffering agent of the equilibration buffer is at a concentration of 150mM. In one example, the buffering agent of the equilibration buffer is at a concentration of 200mM.

In one example, the equilibration buffer further comprises sodium chloride. For example, the equilibration buffer further comprises sodium chloride at a concentration of up to 1000 mM. In one example, the sodium chloride is at a concentration of between 5mM and 50mM. For example, the sodium chloride is at a concentration of 5mM, or 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM. In another example, the sodium chloride is at a concentration of between 50mM and 100mM. For example, the sodium chloride is at a concentration of 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM. In one example, the sodium chloride is at a concentration of between 100 and 200 mM. For example, the sodium chloride is at a concentration of 105mM, or 110mM, 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In another example, the sodium chloride is at a concentration of between 200 and 300 mM. For example, the sodium chloride is at a concentration of 200mM, or 225mM, or 250mM, or 275mM, or 300mM. In a further example, the sodium chloride is at a concentration of between 300 and 400 mM. For example, the sodium chloride is at a concentration of 300mM, or 325mM, or 350mM, or 375mM, or 400mM. In one example, the sodium chloride is at a concentration of between 400mM and 500mM. For example, the sodium chloride is at a concentration of 400mM, or 425mM, or 450mM, or 475mM, or 500mM. In another example, the sodium chloride is at a concentration of between 500mM and 1000mM. For example, the sodium chloride is at a concentration of 500mM, or 550mM, or 600mM, or 650mM, or 700mM, or 750mM, or 800mM, or 850mM, or 900mM, or 950mM, or 1000mM. In one example, the sodium chloride is at a concentration of about 1000 mM. In one example, the sodium chloride is at a concentration of less than 1000mM. For example, the sodium chloride is at a concentration of 500mM. In one example, the equilibration buffer further comprises sodium chloride, wherein the sodium chloride is at a concentration of 145 mM. In one example, the equilibration buffer further comprises sodium chloride, wherein the sodium chloride is at a concentration of 500 mM. In one example, the equilibration buffer further comprises sodium chloride, wherein the sodium chloride is at a concentration of 1000 mM.

In one example, the equilibration buffer further comprises a divalent salt. For example, the equilibration buffer further comprises a divalent salt at a concentration of up to 1000 mM. In one example, the divalent salt is at a concentration of between 5mM and 50mM. For example, the divalent salt is at a concentration of 5mM, or 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM. In another example, the divalent salt is at a concentration of between 50mM and 100mM. For example, the divalent salt is at a concentration of 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM. In one example, the divalent salt is at a concentration of between 100 and 200 mM. For example, the divalent salt is at a concentration of 105mM, or 110mM, 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In another example, the divalent salt is at a concentration of between 200 and 300 mM. For example, the divalent salt is at a concentration of 200mM, or 225mM, or 250mM, or 275mM, or 300mM. In a further example, the divalent salt is at a concentration of between 300 and 400 mM. For example, the divalent salt is at a concentration of 300mM, or 325mM, or 350mM, or 375mM, or 400mM. In one example, the divalent salt is at a concentration of between 400mM and 500mM. For example, the divalent salt is at a concentration of 400mM, or 425mM, or 450mM, or 475mM, or 400mM. In another example, the divalent salt is at a concentration of between 500mM and 1000mM. For example, the divalent salt is at a concentration of 500mM, or 550mM, or 600mM, or 650mM, or 700mM, or 750mM, or 800mM, or 850mM, or 900mM, or 950mM, or 1000mM. In one example, the divalent salt is at a concentration of less than 1000mM. For example, the divalent salt is at a concentration of 500mM.

In one example, the divalent salt is selected from a group consisting of magnesium chloride, calcium chloride, barium chloride, copper (II) chloride, nickel chloride, manganese chloride, and a combination thereof. For example, the divalent salt is magnesium chloride. In one example, the divalent salt is calcium chloride. In another example, the divalent salt is barium chloride. In a further example, the divalent salt is copper chloride. In one example, the divalent salt is nickel chloride. In another example, the divalent salt is manganese chloride.

In one example, the composition of the equilibration buffer is the same as the wash buffer. For example, the equilibration buffer comprises 20 mM sodium dihydrogen phosphate, 145 mM sodium chloride and is at a pH of 7.4. In another example, the equilibration buffer comprises 20 mM sodium dihydrogen phosphate, 500 mM sodium

chloride and is at a pH of 7.4. In another example, the equilibration buffer comprises 20 mM sodium dihydrogen phosphate, 1000 mM sodium chloride and is at a pH of 7.4.

In one example, the chromatography medium is equilibrated i) after stripping the chromatography medium or ii) without stripping the chromatography medium. For example, the chromatography medium is equilibrated after stripping the chromatography medium. In another example, the chromatography medium is equilibrated without stripping the chromatography medium.

In one example, the method further comprises equilibrating the chromatography medium after stripping the chromatography medium with an equilibration buffer having a pH between 7 and 8.

In one example, the method optionally comprises stripping the chromatography medium with a stripping buffer after collecting the protein-of-interest from the chromatography medium. For example, the method further comprises stripping the chromatography medium with a stripping buffer after collecting the protein-of-interest from the chromatography medium. In another example, the method does not comprise stripping the chromatography medium with a stripping buffer after collecting the protein-of-interest from the chromatography medium. For example, the chromatography medium is not stripped after collecting the protein-of-interest from the chromatography medium.

In one example, the stripping buffer has a pH of between 2 to 3. For example, the stripping buffer has a pH of 2, or 2.1, or 2.2, or 2.3, 2.4, or 2.5, or 2.6, or 2.7, or 2.8, or 2.9, or 3. In one example, the stripping buffer is at a pH of 2.5.

In one example, the stripping buffer comprises a buffering agent selected from a group consisting of sodium dihydrogen phosphate, glycine and sodium citrate. For example, the stripping buffer comprises sodium dihydrogen phosphate. For example, the stripping buffer comprises glycine. For example, the stripping buffer comprises sodium citrate.

In one example, the buffering agent of the stripping buffer is at a concentration of between 10mM to 500mM. For example, the buffering agent of the stripping buffer is at a concentration of between 10mM to 20mM, or 10mM to 50mM, or 10mM to 100mM, or 10mM to 200mM, or 10mM to 300mM, or 10mM to 400mM. For example, the buffering agent of the stripping buffer is at a concentration of 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM, or 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM, or 105mM, or 110mM, or 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM, or

210mM, or 220mM, or 230mM, or 240mM, or 250mM, or 260mM, or 270mM, or 280mM, or 290mM, or 300mM, or 310mM, or 320mM, or 330mM, or 340mM, or 350mM, or 360mM, or 370mM, or 380mM, or 390mM, or 400mM, or 410mM, or 420mM, or 430mM, or 440mM, or 450mM, or 460mM, or 470mM, or 480mM, or 490mM, or 500mM. In one example, the buffering agent of the stripping buffer is at a concentration of 5mM. In one example, the buffering agent of the stripping buffer is at a concentration of 20mM. In one example, the buffering agent of the stripping buffer is at a concentration of 50mM. In one example, the buffering agent of the stripping buffer is at a concentration of 100mM. In one example, the buffering agent of the stripping buffer is at a concentration of 150mM. In one example, the buffering agent of the stripping buffer is at a concentration of 200mM. In one example, the buffering agent of the stripping buffer is at a concentration of 250mM. In one example, the buffering agent of the stripping buffer is at a concentration of 300mM. In one example, the buffering agent of the stripping buffer is at a concentration of 350mM. In one example, the buffering agent of the stripping buffer is at a concentration of 400mM. In one example, the buffering agent of the stripping buffer is at a concentration of 450mM. In one example, the buffering agent of the stripping buffer is at a concentration of 500mM. In one example, the stripping buffer comprises 20 mM sodium dihydrogen phosphate and is at a pH of 2.5.

In one example, the stripping buffer further comprises sodium chloride. For example, the stripping buffer further comprises sodium chloride at a concentration of up to 1000 mM. In one example, the sodium chloride is at a concentration of between 5mM and 50mM. For example, the sodium chloride is at a concentration of 5mM, or 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM. In another example, the sodium chloride is at a concentration of between 50mM and 100mM. For example, the sodium chloride is at a concentration of 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM. In one example, the sodium chloride is at a concentration of between 100 and 200 mM. For example, the sodium chloride is at a concentration of 105mM, or 110mM, 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In another example, the sodium chloride is at a concentration of between 200 and 300 mM. For example, the sodium chloride is at a concentration of 200mM, or 225mM, or 250mM, or 275mM, or 300mM. In a further example, the sodium chloride is at a concentration of between 300 and 400 mM. For example, the sodium chloride is at a concentration of 300mM, or 325mM, or 350mM, or

375mM, or 400mM. In one example, the sodium chloride is at a concentration of between 400mM and 500mM. For example, the sodium chloride is at a concentration of 400mM, or 425mM, or 450mM, or 475mM, or 500mM. In another example, the sodium chloride is at a concentration of between 500mM and 1000mM. For example, the sodium chloride is at a concentration of 500mM, or 550mM, or 600mM, or 650mM, or 700mM, or 750mM, or 800mM, or 850mM, or 900mM, or 950mM, or 1000mM. In one example, the sodium chloride is at a concentration of less than 1000mM.

In one example, the stripping buffer further comprises a divalent salt. For example, the stripping buffer further comprises a divalent salt at a concentration of up to 1000 mM. In one example, the divalent salt is at a concentration of between 5mM and 50mM. For example, the divalent salt is at a concentration of 5mM, or 10mM, or 15mM, or 20mM, or 25mM, or 30mM, or 35mM, or 40mM, or 45mM, or 50mM. In another example, the divalent salt is at a concentration of between 50mM and 100mM. For example, the divalent salt is at a concentration of 55mM, or 60mM, or 65mM, or 70mM, or 75mM, or 80mM, or 85mM, or 90mM, or 95mM, or 100mM. In one example, the divalent salt is at a concentration of between 100 and 200 mM. For example, the divalent salt is at a concentration of 105mM, or 110mM, 115mM, or 120mM, or 125mM, or 130mM, or 135mM, or 140mM, or 145mM, or 150mM, or 155mM, or 160mM, or 165mM, or 170mM, or 175mM, or 180mM, or 185mM, or 190mM, or 195mM, or 200mM. In another example, the divalent salt is at a concentration of between 200 and 300 mM. For example, the divalent salt is at a concentration of 200mM, or 225mM, or 250mM, or 275mM, or 300mM. In a further example, the divalent salt is at a concentration of between 300 and 400 mM. For example, the divalent salt is at a concentration of 300mM, or 325mM, or 350mM, or 375mM, or 400mM. In one example, the divalent salt is at a concentration of between 400mM and 500mM. For example, the divalent salt is at a concentration of 400mM, or 425mM, or 450mM, or 475mM, or 500mM. In another example, the divalent salt is at a concentration of between 500mM and 1000mM. For example, the divalent salt is at a concentration of 500mM, or 550mM, or 600mM, or 650mM, or 700mM, or 750mM, or 800mM, or 850mM, or 900mM, or 950mM, or 1000mM. In one example, the divalent salt is at a concentration of less than 1000mM.

In one example, the divalent salt is selected from a group consisting of magnesium chloride, calcium chloride, barium chloride, copper (II) chloride, nickel chloride, manganese chloride, and a combination thereof. For example, the divalent salt is magnesium chloride. For example, the divalent salt is calcium chloride. For example, the divalent salt is barium chloride. For example, the divalent salt is copper chloride.

For example, the divalent salt is nickel chloride. For example, the divalent salt is manganese chloride.

In one example, the method further comprises regenerating one or more of the chromatography media.

- 5 In one example, the method further comprises sanitising one or more of the chromatography media.

- In one example, the protein mixture contacts the chromatography medium for at least 0.1 minutes during loading of the feed comprising the protein mixture. For example, the protein mixture contacts the chromatography medium for at least 0.25
10 minutes, or 0.5 minutes, or 1 minute, or 1.5 minutes, or 2 minutes or 2.5 minutes, or 3 minutes, or 3.5 minutes, or 4 minutes, or 4.5 minutes, or 5 minutes. For example, the protein mixture contacts the chromatography medium for 0.1 minutes, 0.25 minutes, 0.3 minutes, 0.35 minutes, 0.4 minutes, 0.45 minutes, 0.5 minutes, or 1 minute, or 1.5 minutes, or 2 minutes, or 2.5 minutes, or 3 minutes, or 3.5 minutes, or 4 minutes, or 4.5
15 minutes, or 5 minutes.

In one example, the protein mixture contacts the chromatography medium for up to 5 minutes during loading of the protein mixture.

- In one example, the protein mixture contacts the chromatography medium for between 0.25 and 5 minutes during loading of the protein mixture. For example, during
20 loading the protein mixture contacts the chromatography medium for 0.25 minutes, 0.3 minutes, 0.35 minutes, 0.4 minutes, 0.45 minutes, 0.5 minutes, or 1 minute, or 1.5 minutes, or 2 minutes, or 2.5 minutes, or 3 minutes, or 3.5 minutes, or 4 minutes, or 4.5 minutes, or 5 minutes. In one example, during loading the protein mixture contacts the chromatography medium for at least 0.25 minutes.

- 25 In one example, a buffer contacts the chromatography medium for at least 0.1 minutes during one or more non-loading phase(s) of the method.

In one example, the buffer contacts the chromatography medium for up to 5 minutes during one or more non-loading phase(s) of the continuous chromatography method.

- 30 In one example, the buffer contacts the chromatography medium between 0.1 and 5 minutes during one or more non-loading phase(s) of the continuous chromatography method. For example, the buffer contacts the chromatography medium for at least 0.1 minutes, or 0.25 minutes, or 0.5 minutes, or 1 minute, or 1.5 minutes, or 2 minutes or 2.5 minutes, or 3 minutes, or 3.5 minutes, or 4 minutes, or 4.5 minutes, or 5 minutes.

In one example, the non-loading phase is selected from the group consisting of an equilibration phase, a wash phase, an elution phase, a strip phase, a re-equilibration phase and combinations thereof.

5 In one example, the equilibration buffer contacts the chromatography medium for up to 5 minutes. For example, the equilibration buffer contacts the chromatography medium for between 0.1 and 5 minutes.

In one example, the wash buffer contacts the chromatography medium for up to 5 minutes. For example, the wash buffer contacts the chromatography medium for between 0.1 and 5 minutes.

10 In one example, the elution buffer contacts the chromatography medium for up to 5 minutes. For example, the elution buffer contacts the chromatography medium for between 0.1 and 5 minutes.

In one example, the stripping buffer contacts the chromatography medium for up to 5 minutes. For example, the stripping buffer contacts the chromatography medium for
15 between 0.1 and 5 minutes.

In one example, wherein the separation units comprise columns, the method comprises contacting the chromatography medium with a volume of elution buffer of less than a column volume (CV) before collecting the bound protein-of-interest from the chromatography medium. For example, the method comprises a 'pre-elution' phase of
20 contacting the chromatography medium with a volume of elution buffer of less than a column volume (CV) before collecting the bound protein-of-interest from the chromatography medium. In one example, the method comprises washing the chromatography medium with a volume of elution buffer of less than a CV before collecting the bound protein-of-interest from the chromatography medium. For example,
25 the volume of elution buffer used to wash the chromatography medium before collecting the bound protein-of-interest from the chromatography medium is up to 0.5 CV. For example, the volume of elution buffer used to wash the chromatography medium before collecting the bound protein-of-interest from the chromatography medium is between 0.5 and 1.0 CV. For example, the volume of elution buffer used to wash the chromatography
30 medium before collecting the bound protein-of-interest from the chromatography medium is 0.1 CV, or 0.2 CV, or 0.3 CV, or 0.4 CV, or 0.5 CV, or 0.6 CV, or 0.7 CV, or 0.8 CV, or 0.9 CV. In one example, the volume of the elution buffer is 0.1 CV. In one example, the volume of the elution buffer is 0.2 CV. In one example, the volume of the elution buffer is 0.3 CV. In one example, the volume of the elution buffer is 0.4 CV. In
35 one example, the volume of the elution buffer is 0.5 CV. In one example, the volume of the elution buffer is 0.6 CV. In one example, the volume of the elution buffer is 0.7 CV.

In one example, the volume of the elution buffer is 0.8 CV. In one example, the volume of the elution buffer is 0.9 CV.

In one example, the method comprises eluting the bound protein-of-interest from the chromatography medium after performing the step of contacting the chromatography medium with a volume of elution buffer of less than a CV.

In one example, the protein-of-interest is a plasma protein.

In one example, the protein-of-interest is a plasma protein selected from the group consisting of immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a prekallikrein activator (PKA) , a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof.

In one example, the protein-of-interest is an immunoglobulin. For example, the immunoglobulin is IgG. In another example, the immunoglobulin is IgA. In a further example, the immunoglobulin is IgM.

In one example, the protein-of-interest is an apolipoprotein A1.

In one example, the protein-of-interest is an albumin. For example, α -globulins and/or β -globulins.

In one example, the protein-of-interest is a serine protease.

In one example, the protein-of-interest is a plasmin.

In one example, the protein-of-interest is plasminogen.

In one example, the protein-of-interest is an alpha-1- antitrypsin.

In one example, the protein-of-interest is a fibrinogen.

In one example, the protein-of-interest is a von Willebrand factor.

In one example, the protein-of-interest is an activated clotting factor. For example, the activated clotting factor is selected from a group consisting of FXa, FIXa, FVIIa and thrombin. For example, the activated clotting factor is FXa. For example, the activated clotting factor is FIXa. For example, the activated clotting factor is FVIIa. For example, the activated clotting factor is thrombin.

In one example, the protein-of-interest is a contact system factor. For example, the contact system factor protein is selected from a group consisting of FXIa, FXIIa and kallikrein. For example, the contact system factor protein is FXIa. For example, the

contact system factor protein is FXII. For example, the contact system factor protein is kallikrein.

In one example, the protein-of-interest is a Prekallikrein activator (PKA).

In one example, the protein-of-interest is a prothrombin complex.

5 In one example, the protein-of-interest is a C1 esterase inhibitor.

In one example, the protein-of-interest is a protein C.

In one example, the protein-of-interest is an anti-thrombin III.

In one example, the protein-of-interest is a RhD immunoglobulin protein product.

In one example, the protein-of-interest is alpha acid glycoprotein.

10 In one example, the protein-of-interest is haptoglobin.

In one example, the protein-of-interest is hemopexin.

In one example, the protein-of-interest is transferrin.

In one example, the protein-of-interest is Factor H.

In one example, the protein-of-interest is a coagulation factor. For example, the
15 coagulation factor is selected from the group consisting of factor VII, factor VIII, factor IX, factor XIII and factor IX. In one example, the protein-of-interest is a factor VII (FVII). In one example, the protein-of-interest is a factor VIII (FVIII). In one example, the protein-of-interest is factor IX (FIX). In one example, the protein-of-interest is factor XIII (FXIII). In one example, the protein-of-interest is a factor IX (FIX).

20 In one example, the protein-of-interest is a serine protease inhibitor. For example, the serine protease inhibitor is selected from the group consisting of a C1 inhibitor, an alpha-1- antitrypsin and an anti-thrombin.

In one example, the protein mixture is a complex mixture of two or more proteins.

The skilled person will recognise suitable protein mixtures for use in a method of
25 the disclosure. Exemplary protein mixtures include plasma or serum of human or animal origin, fermentation broth, cell culture, protein suspension, milk or other original sources. Immunoglobulin containing material or solutions may contain monoclonal or polyclonal immunoglobulin(s). In some embodiments, the immunoglobulin-containing starting material is a solution comprising polyclonal antibodies. In other embodiments the
30 starting material comprises a monoclonal antibody or a fragment thereof. It is therefore within the knowledge of a skilled person that the term "immunoglobulin" as used herein can also be identified as antibody including monoclonal antibody or polyclonal antibody, either natural or recombinant.

In one example, the protein mixture is plasma or a plasma fraction.

35 In one example, the protein mixture comprises plasma proteins, peptide hormones, growth factors, cytokines and polyclonal immunoglobulins proteins, plasma

proteins selected from human and animal blood clotting factors including fibrinogen, prothrombin, thrombin, prothrombin complex, FX, FXa, FIX, FIXa, FVII, FVIIa, FXI, FXIa, FXII, FXIIa, FXIII and FXIIIa, von Willebrand factor, transport proteins including albumin, transferrin, ceruloplasmin, haptoglobin, hemoglobin and hemopexin, 5 protease inhibitors including β -antithrombin, α -antithrombin, α -2-macroglobulin, C1-inhibitor, tissue factor pathway inhibitor (TFPI), heparin cofactor II, protein C inhibitor (PAI-3), Protein C and Protein S, α -1 esterase inhibitor proteins, α -1 antitrypsin, antiangiogenic proteins including latent-antithrombin, highly glycosylated proteins including α -1-acid glycoprotein, antichymotrypsin, inter- α -trypsin inhibitor, α -2-HS 10 glycoprotein and C-reactive protein and other proteins including histidine-rich glycoprotein, mannan binding lectin, C4-binding protein, fibronectin, GC-globulin, plasminogen, blood factors such as erythropoietin, interferon, tumor factors, tPA and/or γ CSF.

In one example, the method comprises isolating a plasma protein depleted 15 fraction from plasma or a plasma fraction. For example, the method comprises isolating an IgG depleted fraction from plasma or a plasma fraction.

In one example, the plasma fraction is selected from a group consisting of cryo-rich plasma, cryo-poor plasma, Supernatant I (SN I), Cohn Fraction II (Fr II), Cohn Fraction II+III (Fr II+III), Cohn Fraction I+II+III (FrI+II+III), Kistler/Nitschmann 20 Precipitate A (KN A), Kistler/Nitschmann Precipitate B (KN B), Kistler/Nitschmann Precipitate of Supernatant B (KN B+1), and combinations thereof. In one example, the plasma fraction is cryo-rich plasma. For example, the plasma fraction is cryo-poor plasma. For example, the plasma fraction is Supernatant I (SN I). For example, the plasma fraction is Cohn Fraction II (Fr II). For example, the plasma fraction is Cohn 25 Fraction II+III (Fr II+III). For example, the plasma fraction is Cohn Fraction I+II+III (FrI+II+III). For example, the plasma fraction is Kistler/Nitschmann Precipitate A (KN A). For example, the plasma fraction is Kistler/Nitschmann Precipitate B (KN B). For example, the plasma fraction is Kistler/Nitschmann Precipitate of Supernatant B (KN B+1).

30 In one example, the plasma fraction is a suspended paste. For example, the suspended paste is selected from a group consisting of Cohn Fraction II (Fr II), Cohn Fraction II+III (Fr II+III), Cohn Fraction I+II+III (FrI+II+III), Kistler/Nitschmann Precipitate A (KN A), Kistler/Nitschmann Precipitate B (KN B), Kistler/Nitschmann Precipitate of Supernatant B (KN B+1), and combinations thereof. For example, the 35 suspended paste is a Cohn Fraction II (Fr II) paste. In one example, the suspended paste is a Cohn Fraction II+III (Fr II+III) paste. In another example, the suspended paste is a

Cohn Fraction I+II+III (FrI+II+III) paste. In another example, the suspended paste is a Kistler/Nitschmann Precipitate A (KN A) paste. In another example, the suspended paste is a Kistler/Nitschmann Precipitate B (KN B) paste. In a further example, the suspended paste is a Kistler/Nitschmann Precipitate of Supernatant B (KN B+1) paste.

- 5 In one example, the plasma fraction is selected from the group consisting of a mammalian plasma fraction, a human plasma fraction, an equine plasma fraction, and a bovine plasma fraction. In one example, the plasma fraction is a mammalian plasma fraction. In one example, the plasma fraction is a human plasma fraction. In one example, the plasma fraction is an equine plasma fraction. In one example the plasma fraction is a
10 bovine plasma fraction. In one example the plasma fraction is a bovine plasma fraction comprising human polyclonal antibodies.

- In one example, the plasma or plasma fraction is clarified. Methods of clarification of the plasma or plasma fraction will be apparent to the skilled person and/or described herein. For example, the plasma or plasma fraction is clarified by passing the
15 plasma or fraction thereof through a filter. For example, a depth or membrane filter can be used. For example, the plasma or plasma fraction is passed through a combination of filters. For example, the combination may be a 1.2 and 0.45/0.22 μm membrane filter combination. For example, the plasma or plasma fraction is clarified by passing the plasma or fraction thereof through a depth filter (e.g. BECO® depth filter). In one
20 example, the plasma or plasma fraction is clarified by passing the plasma or plasma fraction through a filter press (e.g. BECO® integra plate or compact plate) comprising one or more depth filter(s). In one example, the filter press further comprises one or more filter aid(s) (e.g. cellulose-based filter aids such as Diacel® 150). In one example, the plasma or plasma fraction is clarified by passing the plasma or fraction thereof
25 through a lipid-specific filter (e.g. Zeta Plus™ DEL Series filter). For example, the plasma fraction is clarified Supernatant I (SN I). For example, the plasma fraction is clarified Cohn Fraction II (Fr II). For example, the plasma fraction is clarified Cohn Fraction II+III (Fr II+III). For example, plasma fraction is clarified Cohn Fraction I+II+III (FrI+II+III). For example, the plasma fraction is clarified Kistler/Nitschmann
30 Precipitate A (KN A). For example, the plasma fraction is clarified Kistler/Nitschmann Precipitate B (KN B). For example, the plasma fraction is clarified Kistler/Nitschmann Precipitate of Supernatant B (KN B+1).

In one example, the plasma is clarified cryo-rich plasma.

In one example, the plasma fraction is clarified cryo-poor plasma.

In one example, the plasma or plasma fraction is thawed at a temperature of at least 32°C. For example, the plasma or plasma fraction is thawed at a temperature of at least 32°C before the continuous chromatography.

In one example, the plasma or plasma fraction is at a temperature in the range of 2°C to 35°C before loading onto the separation unit. In one example, the plasma or plasma fraction is at a temperature in the range of 2°C to 28°C before loading onto the separation unit. For example, a temperature in the range of 10°C to 28°C, such as 10°C, or 11°C, or 12°C, or 13°C, or 14°C, 15°C, or 16°C, or 17°C, or 18°C, or 19°C, or 20°C, or 21°C, or 22°C, or 23°C, or 24°C, or 25°C, or 26°C, or 27°C, or 28°C. For example, a temperature in the range of 10°C to 28°C, such as 10°C, or 11°C, or 12°C, or 13°C, or 14°C, 15°C, or 16°C, or 17°C, or 18°C, or 19°C, or 20°C, or 21°C, or 22°C, or 23°C, or 24°C, or 25°C, or 26°C, or 27°C, or 28°C. In one example, the plasma or plasma fraction is at a temperature in the range of 2°C to 35°C before loading onto the separation unit. In one example, the plasma or plasma fraction is at a temperature in the range of 2°C to 28°C before loading onto the separation unit. For example, the plasma or plasma fraction is at a temperature in the range of 2°C to 28°C before loading onto the separation unit. For example, a temperature in the range of 10°C to 35°C. For example, the plasma or plasma fraction is at a temperature in the range of from 30°C to 35°C. For example, the plasma or plasma fraction is at a temperature of at least 32°C. For example, the plasma or plasma fraction is at a temperature in the range of from 32°C to 35°C. In one example, the plasma or plasma fraction is at a temperature of 32°C. For example, a temperature in the range of 10°C to 28°C. In one example, the plasma or plasma fraction is at a temperature in the range of from 2°C to 25°C. For example, a temperature in the range of 10°C to 25°C. In one example, the plasma or plasma fraction is at a temperature in the range of from 20°C to 25°C. For example, the plasma or plasma fraction is at a temperature of 21°C. In one example, the plasma or plasma fraction is at a temperature of 21°C before loading onto the separation unit. In one example, the plasma or plasma fraction is at a temperature in the range of from 2°C to 20°C. For example, a temperature in the range of 10°C to 20°C. In one example, the plasma or plasma fraction is at a temperature in the range of from 2°C to 18°C. For example, a temperature in the range of 10°C to 18°C. In one example, the plasma or plasma fraction is at a temperature in the range of from 2°C to 15°C. For example, a temperature in the range of 10°C to 15°C. In one example, the plasma or plasma fraction is at a temperature in the range of 2°C to 10°C. For example, the plasma or plasma fraction is at a temperature of 2°C, or 3°C, or 4°C, or 5°C, or 6°C, or 7°C, or 8°C, or 9°C, or 10°C. In one example, the plasma or plasma fraction is at a temperature of 2°C. In one example, the plasma or plasma fraction is at a temperature of 10°C. In one

example, the plasma or plasma fraction is at a temperature of 18°C. In one example, the plasma or plasma fraction is at a temperature of 21°C. In one example, the plasma or plasma fraction is at a temperature of 28°C. In one example, the plasma or plasma fraction is at a temperature of 32°C.

5 In one example, at least 75% of the protein-of-interest is recovered from the protein mixture. In another example, at least 75% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method. For example, at least 75% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method without further purification steps. For
10 example, at least 75% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method with further purification steps.

 In one example, at least 75% of IgG is recovered from the plasma or fraction thereof. In another example, at least 75% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method. For example, at least
15 75% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method without further purification steps. For example, at least 75% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method with further purification steps. In one example, at least 75% of the IgG is recovered from the plasma or fraction thereof, wherein the IgG is derived from
20 at least 500 kg of plasma or fractions thereof. For example, at least 75% of the IgG is recovered from large scale purification of the plasma or fraction thereof. For example, 75%, or 76%, or 77%, or 78%, or 79% of IgG is recovered from the plasma or fraction thereof. In one example, 75% of the IgG is recovered from the plasma or fraction thereof. In one example, 76% of the IgG is recovered from the plasma or fraction thereof. In one
25 example, 77% of the IgG is recovered from the plasma or fraction thereof. In one example, 78% of the IgG is recovered from the plasma or fraction thereof. In another example, 79% of the IgG is recovered from the plasma or fraction thereof.

 In one example, at least 80% of the protein-of-interest is recovered from the protein mixture. In another example, at least 80% of the protein-of-interest is recovered
30 from the protein mixture following the continuous chromatography method. For example, at least 80% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method without further purification steps. For example, at least 80% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method with further purification steps.

35 In one example, at least 80% of the IgG is recovered from the plasma or fraction thereof. In another example, at least 80% of the IgG is recovered from the plasma or

fraction thereof following the continuous chromatography method. For example, at least 80% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method without further purification steps. For example, at least 80% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method with further purification steps. For example, at least 80% of the IgG is recovered from the plasma or fraction thereof following an ion exchange chromatography step. In one example, at least 80% of the IgG is recovered from the plasma or fraction thereof following an anion exchange chromatography step. In one example, at least 80% of the IgG is recovered from the plasma or fraction thereof, wherein the IgG is derived from at least 500kg of plasma or fractions thereof. For example, at least 80% of the IgG is recovered from large scale purification of the plasma or fraction thereof. For example, 80%, or 81%, or 82%, or 83%, or 84% of IgG is recovered from the plasma or fraction thereof. In one example, 80% of the IgG is recovered from the plasma or fraction thereof. In one example, 81% of the IgG is recovered from the plasma or fraction thereof. In one example, 82% of the IgG is recovered from the plasma or fraction thereof. In one example, 83% of the IgG is recovered from the plasma or fraction thereof. In another example, 84% of the IgG is recovered from the plasma or fraction thereof.

In one example, at least 85% of the protein-of-interest is recovered from the protein mixture. In another example, at least 85% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method. For example, at least 85% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method without further purification steps. For example, at least 85% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method with further purification steps.

In one example, at least 85% of the IgG is recovered from the plasma or fraction thereof. In another example, at least 85% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method. For example, at least 85% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method without further purification steps. For example, at least 85% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method with further purification steps. For example, at least 85% of the IgG is recovered from the plasma or fraction thereof following an ion exchange chromatography step. In one example, at least 85% of the IgG is recovered from the plasma or fraction thereof following an anion exchange chromatography step. In one example, at least 85% of the IgG is recovered from the plasma or fraction thereof,

wherein the IgG is derived from at least 500kg of plasma or fractions thereof. For example, at least 85% of the IgG is recovered from large scale purification of the plasma or fraction thereof. For example, 85%, or 86%, or 87%, or 88%, or 89% of IgG is recovered from the plasma or fraction thereof. In one example, 85% of the IgG is recovered from the plasma or fraction thereof. In one example, 86% of the IgG is recovered from the plasma or fraction thereof. In one example, 87% of the IgG is recovered from the plasma or fraction thereof. In one example, 88% of the IgG is recovered from the plasma or fraction thereof. In another example, 89% of the IgG is recovered from the plasma or fraction thereof.

10 In one example, at least 90% of the protein-of-interest is recovered from the protein mixture. In another example, at least 90% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method. For example, at least 90% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method without further purification steps. For example, at least 90% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method with further purification steps.

15 In one example, at least 90% of the IgG is recovered from the plasma or fraction thereof. In another example, at least 90% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method. For example, at least 90% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method without further purification steps. For example, at least 90% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method with further purification steps. For example, at least 90% of the IgG is recovered from the plasma or fraction thereof following an ion exchange chromatography step. In one example, at least 90% of the IgG is recovered from the plasma or fraction thereof following an anion exchange chromatography step. In one example, at least 90% of the IgG is recovered from the plasma or fraction thereof, wherein the IgG is derived from at least 500kg of plasma or fractions thereof. For example, at least 90% of the IgG is recovered from large scale purification of the plasma or fraction thereof. For example, 90%, or 91%, or 92%, or 93%, or 94% of the IgG is recovered from the plasma or fraction thereof. In one example, 90% of the IgG is recovered from the plasma or fraction thereof. In one example, 91% of the IgG is recovered from the plasma or fraction thereof. In one example, 92% of the IgG is recovered from the plasma or fraction thereof. In one example, 93% of the IgG is recovered from the plasma or fraction thereof. In another example, 94% of the IgG is recovered from the plasma or fraction thereof.

In one example, at least 95% of the protein-of-interest is recovered from the protein mixture. In another example, at least 95% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method. For example, at least 95% of the protein-of-interest is recovered from the protein mixture
5 following the continuous chromatography method without further purification steps. For example, at least 95% of the protein-of-interest is recovered from the protein mixture following the continuous chromatography method with further purification steps.

In one example, at least 95% of the IgG is recovered from the plasma or fraction thereof. In another example, at least 95% of the IgG is recovered from the plasma or
10 fraction thereof following the continuous chromatography method. For example, at least 95% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method without further purification steps. For example, at least 95% of the IgG is recovered from the plasma or fraction thereof following the continuous chromatography method with further purification steps. For example, at least 95% of the
15 IgG is recovered from the plasma or fraction thereof following an ion exchange chromatography step. In one example, at least 95% of the IgG is recovered from the plasma or fraction thereof following an anion exchange chromatography step. In one example, at least 95% of the IgG is recovered from the plasma or fraction thereof, wherein the IgG is derived from at least 500kg of plasma or fractions thereof. For
20 example, at least 95% of the IgG is recovered from large scale purification of the plasma or fraction thereof. For example, 95%, or 96%, or 97%, or 98%, or 99% of IgG is recovered from the plasma or fraction thereof. In one example, 95% of the IgG is recovered from the plasma or fraction thereof. In one example, 96% of the IgG is recovered from the plasma or fraction thereof. In one example, 97% of the IgG is
25 recovered from the plasma or fraction thereof. In one example, 98% of the IgG is recovered from the plasma or fraction thereof. In another example, 99% of the IgG is recovered from the plasma or fraction thereof.

In one example, the protein-of-interest enriched solution comprises a purity of at least 95% IgG. For example, the protein-of-interest enriched solution comprises a purity
30 of 95%, or 96%, or 97%, or 98% or 99%. In one example, the protein-of-interest enriched solution comprises a purity of 95%. In one example, the protein-of-interest enriched solution comprises a purity of 96%. In one example, the protein-of-interest enriched solution comprises a purity of 97%. In one example, the protein-of-interest enriched solution comprises a purity of 98%. In one example, the protein-of-interest enriched
35 solution comprises a purity of 99%.

In one example, the method of the disclosure is performed at large scale. For example, the method is performed on an industrial or a commercial scale. Methods of performing on an industrial or a commercial scale will be apparent to a skilled person and/or described herein. For example, the method performed on an industrial scale
5 comprises large scale isolation of plasma protein depleted fractions (e.g., an IgG depleted fraction) from the plasma or plasma fraction.

In one example, large scale purification is performed using at least 500kg of the plasma or plasma fraction. For example, large scale purification is performed using between 500kg to 1000kg, or 1000kg to 2500kg, or 2500kg to 5000kg, or 5000kg to
10 7500kg, or 7500kg, or 10000kg, or 10000kg to 12500kg, or 12500kg to 15000kg of the plasma or plasma fraction. In one example, large scale purification is performed using at least 1000kg, or 2500kg, or 5000kg, or 7500kg, or 10000kg, or 12500kg, or 15000kg of the plasma or plasma fraction. In one example, large scale purification is performed using at least 1000kg of the plasma or plasma fraction. In one example, large scale
15 purification is performed using at least 2500kg of the plasma or plasma fraction. In one example, large scale purification is performed using at least 5000kg of the plasma or plasma fraction. In one example, large scale purification performed using at least 7500kg of the plasma or plasma fraction. In one example, large scale purification is performed using at least 10000kg of the plasma or plasma fraction. In one example, large scale
20 purification is performed using at least 12500kg of the plasma or plasma fraction. In one example, large scale is performed using at least 15000kg of the plasma or plasma fraction.

In one example, the method is repeated on the chromatography media for at least 10 chromatography cycles. It will be apparent to the skilled person from the disclosure
25 herein that the method is repeated on the chromatography medium in each of the separation units for at least 10 cycles. For example, the method is repeated on the media for at least 10 cycles per batch of plasma or plasma fraction. In one example, the method is repeated on the media for between 10 to 50 cycles, for 20 to 50 cycles or 30 to 50 cycles per batch of plasma or plasma fraction. In one example, the method is repeated on
30 the media for at least 50 cycles. For example, the method is repeated on the media for at least 50 cycles per batch of plasma or plasma fraction. In an example, the method is repeated on the media for between 50 to 80 cycles, 60 to 80 cycles, for 70 to 80 cycles per batch of plasma or plasma fraction. For example, the method is repeated on the media for at least 60, or 65, or 70, or 75, or 80 cycles per batch of plasma or plasma fraction. In
35 one example, the method is repeated on the media for 50 cycles per batch of plasma or plasma fraction. In one example, the method is repeated on the media for 60 cycles per

batch of plasma or plasma fraction. In one example, the method is repeated on the media for 70 cycles per batch of plasma or plasma fraction. In one example, the method is repeated on the media for 80 cycles per batch of plasma or plasma fraction.

5 It will be apparent to the skilled person from the disclosure herein that the number of cycles per batch of plasma or fraction thereof will be dependent on the volume and/or weight of plasma or fraction thereof.

10 In one example, the method is repeated on the media with multiple batches of plasma or plasma fractions. For example, the method is repeated on the chromatography medium in each of the separation units with multiple batches of plasma or plasma fractions. For example, the method is repeated on the media with at least two batches of plasma or plasma fractions. In one example, the method is repeated on the media with 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10 batches of plasma or plasma fractions. In one example, the method is repeated on the media with between 4 to 10 batches of plasma or plasma fractions. In one example, the method is repeated on the media with at least 15 batches of plasma or plasma fractions. For example, the method is repeated on the media with 10, or 20, or 30, or 40, or 50, or 60, or 70 or 80, or 90, or 100 batches of plasma or plasma fractions. In another example, the method is repeated on the media with at least 100 batches of plasma or plasma fractions. For example, the method is repeated on the v with 100, or 125, or 150, or 175, or 200, or 225, or 250 batches of plasma or plasma 20 fractions. In one example, the method is repeated on the media with between 10 and 250 batches of plasma or plasma fractions. For example, the method is repeated on the media with between 100 and 250 batches of plasma or plasma fractions. In one example, the method is repeated on the media with between 150 and 250 batches of plasma or plasma fractions.

25 In one example, the method is repeated on the media for up to a total of 800 cycles. For example, the method is repeated on the chromatography medium in each of the separation units for up to a total of 800 cycles per chromatography medium. For example, the media is reused for up to a total number of 800 cycles. In one example, the method is repeated on the media for up to a total of 100, or 200, or 300, or 400, or 500, or 600, or 700 cycles. For example, the method is repeated on the media for up to a total of 100 cycles. For example, the method is repeated on the media for up to a total of 200 cycles. For example, the method is repeated on the media for up to a total of 300 cycles. For example, the method is repeated on the media for up to a total of 400 cycles. For example, the method is repeated on the media for up to a total of 500 cycles. For example, the method is repeated on the media for up to a total of 600 cycles. For example, the method is repeated on the media for up to a total of 700 cycles. In one example, the method is 35

repeated on the media for between 100 to 200 cycles, or 200 to 300 cycles, or 200 to 500 cycles, or 500 to 800 cycles. In one example, the method is repeated on the media for 200 cycles. In one example, the method is repeated on the media for 300 cycles. In one example, the method is repeated on the media for 400 cycles. In one example, the method is repeated on the media for 500 cycles. In one example, the method is repeated on the media for 600 cycles. In one example, the method is repeated on the media for 700 cycles. In one example, the method is repeated on the media for 800 cycles. In one example, the method is repeated on the media for between 200 and 500 cycles. For example, the media is reused for up to a total number of 200 to 500 cycles. In one example, the media is reused up to a total of up to 500 cycles with up to 10 batches of plasma or fractions thereof.

In one example, the protein-of-interest enriched solution comprises polyvalent IgG.

In one example, the IgG is further purified from the protein-of-interest enriched solution.

In one example, the IgG is further purified from the protein-of-interest enriched solution by one or more purification steps selected from the group consisting of: precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation; membrane or resin chromatography (for example, ion exchange chromatography, hydrophobic interaction chromatography, isoagglutinin affinity chromatography); viral inactivation; viral filtration and ultrafiltration/diafiltration. For example, the method further comprises precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation. For example, the method further comprises octanoic acid fractionation. For example, the method further comprises membrane or resin chromatography. For example, the method further comprises ion exchange chromatography. For example, the method further comprises anion exchange chromatography. For example, the method further comprises cation exchange chromatography. For example, the method comprises hydrophobic interaction chromatography. For example, the method comprises isoagglutinin affinity chromatography. For example, the method further comprises viral inactivation. For example, the method further comprises nanofiltration. For example, the method further comprises ultrafiltration/diafiltration.

In one example, the method further comprises anion exchange chromatography and viral filtration.

In one example, the method further comprises low pH incubation, depth filtration, anion exchange chromatography and viral filtration.

In one example, the method further comprises formulating the protein-of-interest enriched solution into a pharmaceutical composition.

The present disclosure provides a pharmaceutical composition comprising a protein-of-interest purified or produced by a method of the present disclosure.

5 It will be apparent to the skilled person from the disclosure herein that reference to a pharmaceutical composition comprising a protein-of-interest purified or produced by a method of the present disclosure refers to a composition which meets the appropriate pharmacopoeia standards, such as Ph. Eur or USP or as otherwise herein defined. For example, a composition suitable for pharmaceutical use is a composition that meets the
10 pharmacopoeia standards, such as Ph. Eur, or USP and which is specifically formulated for administration in humans.

The present disclosure also provides a pharmaceutical composition comprising IgG purified or produced as a protein-of-interest by a method of the present disclosure.

In one example, the pharmaceutical composition comprises between 5 % and 25%
15 (w/v) IgG. For example, the pharmaceutical composition comprises 5% (w/v) or 7.5% (w/v), or 10% (w/v), or 12.5% (w/v), or 15% (w/v), or 16.5% (w/v), or 20% (w/v), or 22.5% (w/v) or 25% (w/v) IgG. In one example, the pharmaceutical composition comprises 5% (w/v) IgG. In another example, the pharmaceutical composition comprises 7.5% (w/v) IgG. In a further example, the pharmaceutical composition
20 comprises 10% (w/v) IgG. In one example, the pharmaceutical composition comprises 12.5% (w/v) IgG. In another example, the pharmaceutical composition comprises 16.5% (w/v) IgG. In a further example, the pharmaceutical composition comprises 20% (w/v) IgG. In one example, the pharmaceutical composition comprises 25% (w/v) IgG.

In one example, the pharmaceutical composition comprises:

- 25 (a) 10% (w/v) IgG; or
(b) 20% (w/v) IgG.

In one example, the pharmaceutical composition comprises 10% (w/v) IgG.

In one example, the pharmaceutical composition comprises 20% (w/v) IgG.

In one example, the IgG is polyvalent IgG.

30 In one example, the pharmaceutical composition comprises a purity of at least 95% IgG. For example, the pharmaceutical composition comprises a purity of 95%, or 96%, or 97%, or 98% or 99% IgG. In one example, the pharmaceutical composition comprises a purity of 95% IgG. In one example, the pharmaceutical composition comprises a purity of 96% IgG. In one example, the pharmaceutical composition
35 comprises a purity of 97% IgG. In one example, the pharmaceutical composition

comprises a purity of 98% IgG. In one example, the pharmaceutical composition comprises a purity of 99% IgG.

In one example, the pharmaceutical composition comprises an IgG1 subclass distribution of at least 45%. For example, the pharmaceutical composition comprises an IgG1 subclass distribution between 47.6% and 56.2%. In one example, the pharmaceutical composition comprises an IgG1 subclass distribution of at least 56%. In one example, the pharmaceutical composition comprises an IgG1 subclass distribution of at least 60%. In another example, the pharmaceutical composition comprises an IgG1 subclass distribution of at least 65%. For example, the pharmaceutical composition comprises an IgG1 subclass distribution of 66.6% or 69%.

In one example, the pharmaceutical composition comprises an IgG2 subclass distribution of less than 50%. In one example, the pharmaceutical composition comprises an IgG2 subclass distribution between about 41.5% and 49.5%. In one example, the pharmaceutical composition comprises an IgG2 subclass distribution of less than 40%. For example, the pharmaceutical composition comprises an IgG2 subclass distribution of about 32%. In one example, the pharmaceutical composition comprises an IgG2 subclass distribution of less than 30%. For example, the pharmaceutical composition comprises an IgG2 subclass distribution of about 28.5%. In one example, the pharmaceutical composition comprises an IgG2 subclass distribution of less than 28%. For example, the pharmaceutical composition comprises an IgG2 subclass distribution of 27.9%, 26.6%, 26%.

In one example, the pharmaceutical composition comprises an IgG3 subclass distribution of less than 10%. For example, the pharmaceutical composition comprises an IgG3 subclass distribution of 10%, or 9%, or 8%, or 7%. In one example, the pharmaceutical composition comprises an IgG3 subclass distribution of less than or equal to 7%. For example, the pharmaceutical composition comprises an IgG3 subclass distribution of 6%, or 5%, or 4%. For example, the pharmaceutical composition comprises an IgG3 subclass distribution of less than 4%. In one example, the pharmaceutical composition comprises an IgG3 subclass distribution of less than 3%. For example, the pharmaceutical composition comprises an IgG3 subclass distribution of 3%, or 2.7% or 1.6% or 1.3%.

In one example, the pharmaceutical composition comprises an IgG4 subclass distribution of less than 5%. For example, the pharmaceutical composition comprises an IgG4 subclass distribution of less than 3%. For example, the pharmaceutical composition comprises an IgG4 subclass distribution of 2.5%, 2.2%, 2%, 1.7%, 1.3% or 0.9%.

In one example, the pharmaceutical composition comprises an IgG subclass distribution that is similar to that of normal human plasma, for example 69% IgG₁, 26% IgG₂, 3% IgG₃ and 2% IgG₄.

The present disclosure also provides a pharmaceutical composition comprising
5 albumin purified or produced as a protein-of-interest by a method of the present disclosure.

In one example, the pharmaceutical composition comprises between 4 and 25% (w/v) albumin. For example, the pharmaceutical composition comprises 4% (w/v), 5% (w/v), 20% (w/v), or 25% (w/v) albumin. In one example, the pharmaceutical
10 composition comprises 4% (w/v) albumin. In another example, the pharmaceutical composition comprises 5% (w/v) albumin. In a further example, the pharmaceutical composition comprises 20% (w/v) albumin. In one example, the pharmaceutical composition comprises 25% (w/v) albumin.

In one example, the albumin protein content may be adjusted as required to
15 manufacture a 4%, 5%, 20% and 25% human albumin solution (hSA).

In one example, the pharmaceutical composition comprising albumin purified or produced as a protein-of-interest by a method of the present disclosure meets the pharmacopoeia standards. For example, Ph. Eur or USP. In one example, the pharmaceutical composition comprises appropriate amounts of albumin as the active
20 ingredient according to pharmacopoeia standards such as Ph. Eur or USP.

In one example, the pharmaceutical composition comprises a nominal osmolality of between about 200 mOsm/kg and 500 mOsm/kg. In another example, the pharmaceutical composition comprises a nominal osmolality of between about 300 mOsm/kg and 500 mOsm/kg. For example, the pharmaceutical composition comprises a
25 nominal osmolality of between 310 and 380 mOsm/kg. In one example, the pharmaceutical composition comprises a nominal osmolality of 380 mOsm/kg. For example, the pharmaceutical composition comprises a nominal osmolality of between about 300 mOsm/kg and 350 mOsm/kg. In one example, the pharmaceutical composition comprises a nominal osmolality of 320 mOsm/kg. In one example, the
30 pharmaceutical composition comprises a nominal osmolality of 325 mOsm/kg. In another example, the pharmaceutical composition comprises a nominal osmolality of 343 mOsm/kg. In a further example, the pharmaceutical composition comprises a nominal osmolality of between about 420 and 500 mOsm/kg. In one example, the pharmaceutical composition comprises a nominal osmolality of between 208 and 292 mOsm/kg. In
35 another example, the pharmaceutical composition comprises a nominal osmolality of between 240 and 440 mOsm/kg. For, example, the pharmaceutical composition

comprises a nominal osmolality of between 240 and 310 mOsm/kg. In another example, the pharmaceutical composition comprises a nominal osmolality of between 240 and 300 mOsm/kg. For example, the pharmaceutical composition comprises a nominal osmolality of about 258 mOsm/kg. In one example, the pharmaceutical composition comprises a
5 nominal osmolality of between 280 and 288 mOsm/kg.

In one example, the pharmaceutical composition comprises a pH of between 3.5 and 7.5. For example, the pharmaceutical composition comprises a pH of between 4.0 and 4.6. In one example, the pharmaceutical composition comprises a pH of between 4.0 and 4.5. In one example, the pharmaceutical composition comprises a pH of between 4
10 and 5.5. For example, the pharmaceutical composition comprises a pH of between 4.5 and 5.0. In one example, the pharmaceutical composition comprises a pH of between 4.6 and 5.0. For example, the pharmaceutical composition comprises a pH of 4.6. For example, the pharmaceutical composition comprises a pH of between 4.6 and 5.1. In another example, the pharmaceutical composition comprises a pH of between 4.6 and
15 5.2. In one example, the pharmaceutical composition comprises a pH of 4.7. In another example, the pharmaceutical composition comprises a pH of 4.8. In one example, the pharmaceutical composition comprises a pH of between 4.8 and 5.1. In a further example, the pharmaceutical composition comprises a pH of 4.9. In one example, the pharmaceutical composition comprises a pH of between 4.9 and 5.2. In one example, the
20 pharmaceutical composition comprises a pH of 5.0. In one example, the pharmaceutical composition comprises a pH of between 5.0 and 7.5. In another example, the pharmaceutical composition comprises a pH of between 5.0 and 5.5. For example, the pharmaceutical composition comprises a pH of 5.5. In another example, the pharmaceutical composition comprises a pH of 5.6. In one example, the pharmaceutical
25 composition comprises a pH of between 5.1 and 6.0. In another example, the pharmaceutical composition comprises a pH of between 6.0 and 7.5. For example, the pharmaceutical composition comprises a pH of between 6.4 and 7.2.

In one example, the pharmaceutical composition further comprises one or more stabilisers. In one example, the one or more stabilisers is selected from the group
30 consisting of an amino acid, a polyol, a surfactant, sodium N-acetyl-tryptophan, sodium caprylate and combinations thereof.

In one example, the one or more stabilisers is an amino acid stabiliser. For example, the amino acid stabiliser is selected from the group consisting of glycine, proline and combinations thereof.

35 In one example, the amino acid stabiliser is L-proline. In one example, the pharmaceutical composition further comprises 200 mmol/L to 300 mmol/L of L-proline.

For example, the pharmaceutical composition further comprises 225 mmol/L to 275 mmol/L of L-proline. In one example, the pharmaceutical composition further comprises 240 mmol/L to 260 mmol/L of L-proline. For example, the pharmaceutical composition further comprises 250 mmol/L of L-proline.

5 In one example, the amino acid stabiliser is glycine.

 In one example, the one or more stabilisers is a polyol. For example, the polyol is selected from the group consisting of sorbitol, maltose and combinations thereof. In one example, the stabiliser is sorbitol. In one example, the stabiliser is maltose.

 In one example, the one or more stabilisers is a surfactant. For example, the
10 surfactant is polysorbate. For example, the polysorbate is polysorbate 80.

 In one example, the stabiliser is glycine and sorbitol.

 In one example, the stabiliser is glycine and polysorbate 80.

 In one example, the stabiliser is sorbitol, glycine and polysorbate 80.

 In one example, the stabiliser is sodium N-acetyl-tryptophan.

15 In one example, the stabiliser is sodium caprylate.

 In one example, the stabiliser is sodium N-acetyl-tryptophan and sodium caprylate.

 In one example, the pharmaceutical composition further comprises a tonicity agent. For example, the tonicity agent is sodium chloride.

20 In one example, the pharmaceutical composition further comprises a solvent. For example, the solvent is water for injections.

 In one example, the pharmaceutical composition comprises IgG as the protein-of-interest and one or more of the following excipients: an amino acid stabiliser, a polyol and a surfactant.

25 In one example, the pharmaceutical composition comprises albumin as the protein-of-interest and one or more of the following excipients: sodium N-acetyl-tryptophan, sodium caprylate, sodium chloride and water for injections.

 In one example, the pharmaceutical composition comprises a sodium content of ≤ 1 mmol/L.

30 In one example, the pharmaceutical composition comprises an IgA content of ≤ 0.5 mg/mL. For example, the pharmaceutical composition comprises an IgA content of ≤ 0.4 mg/mL. In one example, the pharmaceutical composition comprises an IgA content of ≤ 0.3 mg/mL. In one example, the pharmaceutical composition comprises an IgA content of ≤ 0.2 mg/mL. For example, ≤ 0.14 mg/mL. In one example, the
35 pharmaceutical composition comprises an IgA content of ≤ 0.1 mg/mL. For example, ≤ 0.084 mg/mL. In one example, the pharmaceutical composition comprises an IgA content

of ≤ 0.05 mg/mL. For example, the pharmaceutical composition comprises an IgA content of ≤ 0.04 mg/mL, or ≤ 0.03 mg/mL. In one example, the pharmaceutical composition comprises an IgA content of ≤ 0.025 mg/mL. In one example, the pharmaceutical composition comprises an IgA content of ≤ 0.01 mg/mL. For example, the pharmaceutical composition comprises an IgA content of ≤ 0.009 mg/mL.

In one example, the pharmaceutical composition comprises an IgA content of ≤ 0.1 mg/g IgG. In one example, the pharmaceutical composition comprises an IgA content of ≤ 0.09 mg/g.

In one example, the pharmaceutical composition comprises an IgM content of ≤ 10 mg/L. For example, an IgM content of ≤ 10 mg/L, ≤ 9 mg/L, ≤ 8 mg/L, ≤ 7 mg/L, ≤ 6 mg/L, ≤ 5 mg/L, ≤ 4 mg/L, ≤ 3 mg/L, ≤ 2 mg/L. In one example, the pharmaceutical composition comprises an IgM content of ≤ 2 mg/L. In one example, the pharmaceutical composition comprises an IgM content of ≤ 1 mg/L. In one example, the pharmaceutical composition comprises an IgM content of ≤ 0.5 mg/L. For example, the pharmaceutical composition comprises an IgM content of < 0.17 mg/L.

In one example, the pharmaceutical composition comprises an IgM content of ≤ 2 μ g/g IgG. In one example, the pharmaceutical composition comprises an IgM content of ≤ 1.9 μ g/g IgG.

In one example, the pharmaceutical composition comprises an albumin content of ≤ 0.50 mg/mL. For example, the pharmaceutical composition comprises an albumin content of ≤ 0.40 mg/mL. In one example, the pharmaceutical composition comprises an albumin content of ≤ 0.30 mg/mL. In one example, the pharmaceutical composition comprises an albumin content of ≤ 0.20 mg/mL. In one example, the pharmaceutical composition comprises an albumin content of ≤ 0.10 mg/mL. For example, the pharmaceutical composition comprises an albumin content of ≤ 0.09 mg/mL. In one example, the pharmaceutical composition comprises an albumin content of ≤ 0.08 mg/mL. In one example, the pharmaceutical composition comprises an albumin content of ≤ 0.07 mg/mL.

In one example, the pharmaceutical composition comprising IgG comprises an albumin content of ≤ 1 mg/g IgG. In one example, the pharmaceutical composition comprising IgG comprises an albumin content of ≤ 0.80 mg/g IgG.

In one example, the pharmaceutical composition comprises a Prekallikrein activator (PKA) level of ≤ 35 IU/mL. In one example, the pharmaceutical composition comprises a Prekallikrein activator (PKA) level of ≤ 30 IU/mL. In one example, the pharmaceutical composition comprises a Prekallikrein activator (PKA) level of ≤ 50 IU/mL. In one example, the pharmaceutical composition comprises a Prekallikrein

activator (PKA) level of ≤ 20 IU/mL. For example, the pharmaceutical composition comprises a Prekallikrein activator (PKA) level of ≤ 15 IU/mL. In one example, the pharmaceutical composition comprises a Prekallikrein activator (PKA) level of ≤ 10 IU/mL.

5 In one example, the pharmaceutical composition comprises 5% (w/v) IgG, sorbitol, $<3.1\mu\text{g/mL}$ IgA, pH of 5.6 and a nominal osmolality of 325 mOsm/kg.

In one example, the pharmaceutical composition comprises 10% (w/v) IgG, sorbitol, $<3.1\mu\text{g/mL}$ IgA, pH of 5.5 and a nominal osmolality of 343 mOsm/kg.

10 In one example, the pharmaceutical composition comprises 5% (w/v) IgG, sorbitol, glycine, polysorbate 80, $<10\mu\text{g/mL}$ IgA, pH of 4.8-5.1 and a nominal osmolality of 420-500 mOsm/kg.

In one example, the pharmaceutical composition comprises 10% (w/v) IgG, glycine, polysorbate 80, $<20\mu\text{g/mL}$ IgA, pH of 4.9-5.2 and a nominal osmolality of 280-288 mOsm/kg.

15 In one example, the pharmaceutical composition comprises 5% (w/v) IgG, maltose, $<200\mu\text{g/mL}$ IgA, pH of 5.1-6.0 and a nominal osmolality of 310-380 mOsm/kg.

In one example, the pharmaceutical composition comprises 10% (w/v) IgG, maltose, $106\mu\text{g/mL}$ IgA, pH of 4.5-5.0 and a nominal osmolality of 310-380 mOsm/kg.

20 In one example, the pharmaceutical composition comprises 10% (w/v) IgG, glycine, $100\mu\text{g/mL}$ IgA, pH of 4.5-5.0 and a nominal osmolality of 240-310 mOsm/kg.

In one example, the pharmaceutical composition comprises 10% (w/v) IgG, proline, $<25\mu\text{g/mL}$ IgA, pH of 4.6-5.0 and a nominal osmolality of 240-440 mOsm/kg.

In one example, the pharmaceutical composition comprises 10% (w/v) IgG, glycine, $37\mu\text{g/mL}$ IgA, pH of 4.9-5.2 and a nominal osmolality of 240-300 mOsm/kg.

25 In one example, the pharmaceutical composition comprises 10% (w/v) IgG, glycine, $46\mu\text{g/mL}$ IgA, pH of 4.0-4.5 and a nominal osmolality of 258 mOsm/kg.

In one example, the pharmaceutical composition comprises 16.5% (w/v) IgG, maltose, $<600\mu\text{g/mL}$ IgA, pH of 5.0-5.5 and a nominal osmolality of 310-380 mOsm/kg.

30 In one example, the pharmaceutical composition comprises 20% (w/v) IgG, glycine, $80\mu\text{g/mL}$ IgA, pH of 4.6-5.1 and a nominal osmolality of 208-292 mOsm/kg.

In one example, the pharmaceutical composition comprises 20% (w/v) IgG, proline, $<50\mu\text{g/mL}$ IgA, pH of 4.6-5.2 and a nominal osmolality of 380 mOsm/kg.

In one example, the pharmaceutical composition comprises 10% (w/v) IgG, glycine, $37\mu\text{g/mL}$ IgA, pH of 4.6-5.1 and a nominal osmolality of 240-300 mOsm/kg.

35 In one example, the pharmaceutical composition comprises 10% (w/v) polyvalent IgG, 250 mM proline and a pH of 4.8.

In one example, the pharmaceutical composition comprises 20% (w/v) polyvalent IgG, 250 mM proline, 20 µg/mL PS80 and a pH of 4.8.

In one example, the pharmaceutical composition comprises:

- i) protein (4% w/v), sodium (140 mM) and caprylate (6.4 mM) for 4% w/v hSA;
- 5 ii) protein (5% w/v), sodium (140 mM) and caprylate (8 mM) for 5% w/v hSA;
- iii) protein (20% w/v) and caprylate (32 mM) for 20% w/v hSA; or
- iv) protein (25% w/v) and caprylate (40 mM) for 25% w/v hSA.

In one example, the pharmaceutical composition comprises:

- i) protein (4% w/v), 3.2 mM sodium N-acetyltryptophanate and 3.2 mM sodium caprylate for 4% w/v hSA;
- 10 ii) protein (5% w/v), 4 mM sodium N-acetyltryptophanate and 4 mM sodium caprylate for 5% w/v hSA;
- iii) protein (20% w/v) 0.016 M sodium N-acetyltryptophanate and 0.016 M sodium caprylate for 20% w/v hSA; or
- 15 iv) protein (25% w/v) 0.02 M sodium N-acetyltryptophanate and 0.02 M sodium caprylate for 25% w/v hSA.

In one example, the pharmaceutical composition does not comprise preservatives.

In one example, the hSA pharmaceutical composition according to the present disclosure will meet the appropriate pharmacopoeia standard. For example, following
 20 the test procedures as described for a human albumin solution in the European Pharmacopoeia version 10.6 the hSA preparation is sterile; pyrogen free; has endotoxin levels below 0.5 IU per mL for solutions less than 50 g/L, or less than 1.3 IU per mL for solutions from 50 g/L to 200 g/L, or less than 1.7 IU/mL for solutions greater than 200 g/L; an aluminium content of a maximum of 200 µg/L, a prekallikrein activator (PKA)
 25 maximum of 35 IU/mL; a haem content not greater than 0.15; a potassium maximum of 0.05 mmol per gram of protein; a sodium maximum of 160 mmol/L and 95 % to 105% of the content of Na stated on the label; a maximum of 10% polymers and aggregates; not more than 5% of protein has a mobility different from the principal band by zone electrophoresis; a pH of 6.7 to 7.3 and a total protein not less than 9% and not more than
 30 10% of the stated content.

In one example, the protein-of-interest depleted preparation comprises one or more plasma protein products.

In one example, the method further comprises purifying one or more of the plasma protein products from the protein-of-interest depleted preparation.

35 In one example, the one or more plasma protein products is further purified from the protein-of-interest depleted preparation by one or more purification steps selected

from the group consisting of: precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation; membrane or resin chromatography (for example, ion exchange chromatography, hydrophobic interaction chromatography, isoagglutinin affinity chromatography); viral inactivation; viral filtration; ultrafiltration/diafiltration and combinations thereof. For example, the method further comprises ethanol precipitation. For example, the method further comprises octanoic acid fractionation. For example, the method further comprises membrane or resin chromatography. For example, the method further comprises ion exchange chromatography. For example, the method further comprises anion exchange chromatography. For example, the method further comprises cation exchange chromatography. For example, the method comprises hydrophobic interaction chromatography. For example, the method comprises isoagglutinin affinity chromatography. For example, the method further comprises viral inactivation. For example, the method further comprises nanofiltration. For example, the method further comprises ultrafiltration/diafiltration.

In one example, the one or more plasma protein products is further purified from the protein-of-interest depleted preparation using a continuous chromatography system.

In one example, the one or more plasma protein products purified from the protein-of-interest depleted preparation is selected from the group consisting of immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a prekallikrein activator (PKA) , a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof.

In one example, the one or more plasma protein products is an immunoglobulin. For example, the immunoglobulin is IgG. In another example, the immunoglobulin is IgA. In a further example, the immunoglobulin is IgM.

In one example, the one or more plasma protein products is an apolipoprotein A1. In one example, the one or more plasma protein products is an albumin. For example, α -globulins and/or β -globulins.

In one example, the one or more plasma protein products is a serine protease.

In one example, the one or more plasma protein products is a plasmin.

In one example, the one or more plasma protein products is plasminogen.

In one example, the one or more plasma protein products is an alpha-1-antitrypsin.

In one example, the one or more plasma protein products is a fibrinogen.

In one example, the one or more plasma protein products is a von Willebrand factor.

In one example, the one or more plasma protein products is an activated clotting
 5 factor. For example, the activated clotting factor is selected from a group consisting of
 FXa, FIXa, FVIIa and thrombin. For example, the activated clotting factor is FXa. For
 example, the activated clotting factor is FIXa. For example, the activated clotting factor
 is FVIIa. For example, the activated clotting factor is thrombin.

In one example, the one or more plasma protein products is a contact system
 10 factor. For example, the contact system factor protein is selected from a group consisting
 of FXIa, FXIIa and kallikrein. For example, the contact system factor protein is FXIa.
 For example, the contact system factor protein is FXII. For example, the contact system
 factor protein is kallikrein.

In one example, the one or more plasma protein products is a Prekallikrein
 15 activator (PKA).

In one example, the one or more plasma protein products is a prothrombin
 complex.

In one example, the one or more plasma protein products is a C1 esterase inhibitor.

In one example, the one or more plasma protein products is a protein C.

20 In one example, the one or more plasma protein products is an anti-thrombin III.

In one example, the one or more plasma protein products is a RhD
 immunoglobulin protein product.

In one example, the one or more plasma protein products is alpha acid
 glycoprotein.

25 In one example, the one or more plasma protein products is haptoglobin.

In one example, the one or more plasma protein products is hemopexin.

In one example, the one or more plasma protein products is transferrin.

In one example, the one or more plasma protein products is Factor H.

In one example, the one or more plasma protein products is a coagulation factor.
 30 For example, the coagulation factor is selected from the group consisting of factor VII,
 factor VIII, factor IX and factor XIII. In one example, the one or more plasma protein
 products is a factor VII (FVII). In one example, the one or more plasma protein products
 is a factor VIII (FVIII). In one example, the one or more plasma protein products is factor
 IX (FIX). In one example, the one or more plasma protein products is factor XIII (FXIII).

35 In one example, the protein-of-interest is a factor IX (FIX).

In one example, the one or more plasma protein products is a serine protease inhibitor. For example, the serine protease inhibitor is selected from the group consisting of a C1 inhibitor, an alpha-1- antitrypsin and an anti-thrombin.

5 In one example, the method further comprises formulating the purified plasma protein products from the protein-of-interest depleted preparation into a pharmaceutical composition.

10 The present disclosure provides a pharmaceutical composition comprising one or more plasma protein products from the protein-of-interest depleted preparation purified or produced by a method of the present disclosure. For example, the pharmaceutical composition comprises one or more plasma protein products purified or produced by a method described herein and a pharmaceutically acceptable carrier.

15 The present disclosure also provides the pharmaceutical composition described herein for use in treating, preventing and/or delaying progression of a condition in a subject. For example, the present disclosure provides a pharmaceutical composition described herein for use in treating a condition in a subject. In another example, the present disclosure provides a pharmaceutical composition described herein for use in preventing a condition in a subject. In a further example, the present disclosure provides a pharmaceutical composition described herein for use in delaying progression of a condition in a subject.

20 In some examples, the pharmaceutical composition is present in a vial, a prefilled syringe or an autoinjector device.

The present disclosure also provides a prefilled syringe comprising the pharmaceutical composition described herein.

25 The present disclosure also provides an autoinjector device comprising the pharmaceutical composition described herein.

In one example, the composition of the disclosure is administered subcutaneously to the subject in need thereof. In another example, the composition of the disclosure is administered intravenously to the subject in need thereof.

30 In one example, the composition of the disclosure is self-administered. For example, the composition of the disclosure is self-administered subcutaneously.

In one example, the composition of the disclosure is provided in a pre-filled syringe.

In one example, the composition of the disclosure is self-administered subcutaneously, with a pre-filled syringe.

35 The present disclosure further provides use of IgG purified or produced by a method described herein or use of one or more plasma protein products purified or

produced by a method described herein in the manufacture of a medicament for treating, preventing and/or delaying progression of a condition in a subject. For example, the present disclosure provides use of the IgG purified or produced by a method described herein in the manufacture of a medicament for treating a condition in a subject. In another
5 example, the present disclosure provides use of the IgG purified or produced by a method described herein in the manufacture of a medicament for preventing a condition in a subject. In a further example, the present disclosure provides use of the IgG purified or produced by a method described herein in the manufacture of a medicament for delaying progression of a condition in a subject. For example, the present disclosure provides use
10 of the one or more plasma protein products purified or produced by a method described herein in the manufacture of a medicament for treating a condition in a subject. In another example, the present disclosure provides use of the one or more plasma protein products purified or produced by a method described herein in the manufacture of a medicament for preventing a condition in a subject. In a further example, the present disclosure
15 provides use of the one or more plasma protein products purified or produced by a method described herein in the manufacture of a medicament for delaying progression of a condition in a subject.

The present disclosure also provides a method of treating, preventing and/or delaying progression of a condition in a subject, the method comprising administering
20 the pharmaceutical composition of the present disclosure to the subject. For example, the present disclosure provides a method of treating a condition in a subject. In another example, the present disclosure provides a method of preventing a condition in a subject. In a further example, the present disclosure provides a method of delaying progression of a condition in a subject.

25 The present disclosure also provides a kit for use in treating or preventing or delaying progression of a condition in a subject, the kit comprising:

- (a) at least one pharmaceutical composition described herein;
- (b) instructions for using the kit in treating or preventing or delaying the condition in the subject; and
- 30 (c) optionally, at least one further therapeutically active compound or drug.

In one example, the condition is an immunodeficiency, autoimmune disease or acute infection. For example, the condition is allogenic bone marrow transplant, chronic lymphocytic leukaemia, idiopathic thrombocytopenic purpura (ITP), pediatric HIV, primary immunodeficiencies, Kawasaki disease, chronic inflammatory demyelinating
35 polyneuropathy (CIDP), kidney transplant with a high antibody recipient or with an ABO incompatible donor, chronic fatigue syndrome, Clostridium difficile colitis,

dermatomyositis and polymyositis, Graves' ophthalmopathy, Guillain-Barre syndrome, muscular dystrophy, inclusion body myositis, Lambert-Eaton syndrome, Lupus erythematosus, multifocal motor neuropathy, multiple sclerosis (MS), myasthenia gravis, neonatal alloimmune thrombocytopenia, Parvovirus B19 infection, pemphigus, post-transfusion purpura, renal transplant rejection, spontaneous Abortion Miscarriage, stiff person syndrome, opsoclonus Myoclonus, severe sepsis and septic shock in critically ill adults, toxic epidermal necrolysis, chronic lymphocytic leukemia, multiple myeloma, X-linked agammaglobulinemia, hypogammaglobulinemia, primary immune deficiency, RRMS, Alzheimer's disease, and Parkinson's disease.

10 In one example, the condition is selected from a group consisting of immune conditions, particular autoimmune diseases and certain neurological diseases. These conditions include Rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), Antiphospholipid syndrome, immune thrombocytopenia (ITP), Kawasaki disease, Guillain Barre syndrome (GBS), multiple sclerosis (MS), chronic inflammatory
15 demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), myasthenia gravis (MG), skin blistering diseases, scleroderma, Dermatomyositis, Polymyositis, Alzheimer's Disease, Parkinson's Disease, Alzheimer's Disease related to Downs Syndrome, cerebral amyloid angiopathy, Dementia with Lewy bodies, Fronto-temporal lobar degeneration, vascular dementia, cell and organ transplant and
20 combinations thereof.

In one example of any method described herein, the subject is a mammal, for example a primate such as a human.

According to another aspect of the present disclosure, there is provided a
25 continuous chromatography system for separating a protein-of-interest from a protein mixture, wherein the system comprises:

at least four separation units, each separation unit comprising:

an inlet;

an outlet;

30 a fluid flow path extending through the separation unit from the inlet to the outlet; and

a chromatography medium in contact with the fluid flow path and capable of separating at least a portion of the protein-of-interest from the protein mixture;

a switching valve arrangement configured to:

35 selectively direct flow of one or more of a feed comprising the protein mixture, a wash buffer and an eluting agent to the inlet of any one of the separation units;

selectively direct flow from the outlet of each separation unit to:
at least one feedback loop in fluid communication with the inlet of
another one of the separation units;
at least one collection flow path; and
5 a waste flow path; and
a valve control system operable to control the switching valve arrangement,
wherein the valve control system is configured to operate the switching valve
arrangement such that:
the feed is directed to the inlet of a first selected separation unit;
10 the wash buffer is directed to the inlet of a second selected separation unit;
a feed flow through from the outlet of the first separation unit and a wash
flow through from the outlet of the second separation unit are directed to respective
feedback loops, wherein the feed flow through and the wash flow through are maintained
separate from each other.

15 In some examples, the system may include a single feedback loop in fluid connection
with the outlet of each separation unit and the inlet of another one of the separation units,
wherein the switching valve arrangement is configured to selectively direct flow from
the outlet of each separation unit to the feedback loop. In some examples, the system
20 may include two feedback loops in fluid connection with the outlet of each separation
unit and the inlets of two other the separation units, wherein the switching valve
arrangement is configured to selectively direct flow from the outlet of each separation
unit via a selected one of the two feedback loops. In other examples, the system may
comprise feedback loops in fluid connection between the outlet of each separation unit
25 and the inlets each of the other separation units, wherein the switching valve arrangement
is configured to selectively direct flow from the outlet of each separation unit to a selected
any one of the other separation units via a selected one of the feedback loops.

30 In some examples, the connections between the various separation units are fixed. For
example, the system may comprise fixed piping for connection between the separation
units. The switching valve arrangement may be provided in connection with the fixed
piping for selectively directing flow between the separation units.

For example, the valve control system may be configured to operate the switching valve
35 arrangement such that: the feed flow through from the outlet of the first separation unit
is directed to the inlet of a third separation unit via a feed flow through feedback loop;

and the wash flow through from the outlet of the second separation unit is directed to the inlet of a fourth separation unit via a wash flow through feedback loop.

5 The valve control system may be configured to operate the switching valve arrangement to perform a series of chromatography cycle segments. The valve control system may be configured to operate the switching valve arrangement to vary the selections for the directing of the flows between successive cycle segments. In some examples, the function of each of the separation units may be determined by the flow directed to the inlets of each of the separation units. In some examples, the function of each of the
10 separation units may vary between successive cycle segments.

In some examples, the valve control system may be configured to operate the switching valve arrangement to direct the flow of the feed comprising the protein mixture onto each of the at least four separation units successively, wherein the loading of the feed onto
15 each successive separation unit demarks a respective cycle segment. In some examples, the valve control system may be configured to operate the switching valve arrangement such that loading the feed is performed substantially continuously across successive cycle segments such that a flow rate of the feed is substantially constant. Substantially continuous loading may comprise no idle time, or minimised idle time, when switching
20 feeding the load from one separation unit to the subsequent separation unit. In some examples, substantially continuous loading may include interruptions to the flow of the feed a time taken to allow connection of the feed source to the next separation unit (for example, the time taken to operate a switching valve). In other embodiments, the loading and/or flow rate may be discontinuous. For example, there may be an idle time between
25 successive cycles and/or cycle segments.

The functions of the feedback loops may vary depending on the outlet flow that is directed to the feedback loop. The feed flow through feedback loop may be defined as the selected feedback loop to which the feed flow through is selectively directed. The
30 wash flow through feedback loop may be defined as the selected feedback loop to which the wash flow through is directed. In some examples, a feedback loops may perform the functions of feed flow through feedback loop and wash flow through feedback loop, depending on flow through of the outlet to which the feedback loop is connected. In other examples, the system may comprise feedback loops designated a discrete function as
35 either a feed flow through feedback loop or a wash flow through feedback.

The at least one collection flow path may comprise one or more of: a protein-of-interest enriched solution collection flow path; a protein-of-interest depleted feed flow through collection flow path; and a protein-of-interest depleted wash flow through collection flow path. In some examples, one or more of the collection flow paths may be combined. For
5 example, one or more collection flow paths may function as the protein-of-interest enriched solution collection flow path, the protein-of-interest depleted feed flow through collection flow path, and/or the protein-of-interest depleted wash flow through collection flow path. The switching valve arrangement may be configured to selectively connect the collection flow path(s) with one or more collection receptacles.

10

In some examples, the valve control system may be configured to operate the switching valve arrangement such that a protein-of-interest depleted feed from the outlet of the third separation unit is directed to the at least one collection flow path. In some examples, the valve control system is configured to operate the switching valve arrangement such
15 that protein-of-interest depleted wash flow through is directed from the outlet of the fourth separation unit to the at least one collection flow path and/or to the waste flow path.

In some examples, the valve control system is configured to operate the switching valve
20 arrangement to direct flow of an elution buffer to the inlet of at least one selected separation unit, and direct flow of a protein-of-interest enriched solution from the outlet of the at least one separation unit selected to receive the elution buffer to the at least one collection flow path collection flow path. In some examples, the switching valve arrangement may be configured to direct flow of an elution buffer to the inlet of the
25 second separation unit after directing of the wash buffer to the second separation unit. In some examples, the switching valve arrangement may be configured to direct flow of an elution buffer to the inlet of a fifth separation unit.

In one example, the system comprises:

30 a first separation unit comprising: a first chromatography medium capable of separating the protein-of-interest from the protein mixture; and a first separation unit inlet and a first separation unit outlet defining a flowpath through the first separation unit and in contact with at least a portion of the first chromatography medium;

a second separation unit comprising: a second chromatography medium capable
35 of separating the protein-of-interest from the protein mixture; and a second separation unit inlet and a second separation unit outlet defining a flowpath through the second

separation unit and in contact with at least a portion of the second chromatography medium;

5 a third separation unit comprising: a third chromatography medium capable of separating the protein-of-interest from the protein mixture; and a third separation unit inlet and a third separation unit outlet defining a flowpath through the third separation unit and in contact with at least a portion of the third chromatography medium;

10 a fourth separation unit comprising: a fourth chromatography medium capable of separating the protein-of-interest from the protein mixture; and a fourth separation unit inlet and a fourth separation unit outlet defining a flowpath through the fourth separation unit and in contact with at least a portion of the fourth chromatography;

a switching valve arrangement operable to:

selectively direct flow of a feed of the protein mixture to be separated to the inlet of the first, second, third or fourth separation unit;

15 selectively direct flow of a wash buffer to the inlet of the first, second, third or fourth separation unit;

selectively direct flow from the first separation unit outlet to: the third separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath;

20 selectively direct flow from the second separation unit outlet to: the fourth separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath;

selectively direct flow from the third separation unit outlet to: the first separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath;

25 selectively direct flow from the fourth separation unit outlet to: the second separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath; and

a valve control system configured to operate the switching valve arrangement to sequentially cycle through:

a first chromatography cycle in which:

30 a first chromatography cycle first feedback loop is defined in which the feed flows through the first and third separation units in series and to the protein-of-interest depleted feed flowpath, and

a first chromatography cycle second feedback loop is defined in which the wash buffer flows through the fourth and second separation units in series and to the wash buffer output flowpath; and

35 a second chromatography cycle in which:

a second chromatography cycle first feedback loop is defined in which the feed flows through the second and fourth separation units in series and to the protein-of-interest depleted feed flowpath, and

5 a second chromatography cycle second feedback loop is defined in which the wash buffer flows through the first and third separation units in series and to the wash buffer output flowpath.

In one example, the valve control system is further configured to operate the switching valve arrangement to sequentially cycle through:

10 a third chromatography cycle in which:
a third chromatography cycle first feedback loop is defined in which the feed flows through the third and first separation units in series and to the protein-of-interest depleted feed flowpath, and

15 a third chromatography cycle second feedback loop is defined in which the wash buffer flows through the second and fourth separation units in series and to the wash buffer output flowpath; and

a fourth chromatography cycle in which:

a fourth chromatography cycle first feedback loop is defined in which the feed flows through the fourth and second separation units in series and to the protein-of-interest depleted feed flowpath, and

20 a fourth chromatography cycle second feedback loop is defined in which the wash buffer flows through the third and first separation units in series and to the wash buffer output flowpath.

In one example,

25 during the third chromatography cycle, the valve control system is further configured to operate the switching valve arrangement to direct flow of the elution buffer to the second separation unit inlet such that flow of the wash buffer can be discontinued to be replaced with flow of the elution buffer through the second and fourth separation units in series and to the protein-of-interest enriched solution flowpath; and

30 during the fourth chromatography cycle, the valve control system is further configured to operate the switching valve arrangement to direct flow of the elution buffer to the third separation unit inlet such that flow of the wash buffer can be discontinued to be replaced with flow of the elution buffer through the third and first separation units in series and to the protein-of-interest enriched solution flowpath.

35

In some examples, a protein sensor may be connected to each outlet of the separation units. For example, a protein sensor is connected to the outlet of the first separation unit. In another example, a protein sensor is connected to the outlet of the second separation unit. In a further example, a protein sensor is connected to the outlet of the third separation unit. In one example, a protein sensor is connected to the outlet of the fourth separation unit. In one example, a protein sensor is connected to the outlet of the fifth separation unit.

In one example, the protein sensor is configured to detect the protein concentration in the feed (post-load) flow-through, the wash (post-wash) flow through, the protein-of-interest depleted feed flow through fraction, the protein-of-interest depleted wash flow through fraction and/or the protein-of-interest enriched solution. For example, the protein sensor detects the protein concentration in the post-load flow-through. In another example, the protein sensor detects the protein concentration in the post-wash flow through. In a further example, the protein sensor detects the protein concentration in the first protein-of-interest depleted flow through fraction. In one example, the protein sensor detects the protein concentration in the second protein-of-interest depleted flow through fraction. In another example, the protein sensor detects the protein concentration in the protein-of-interest enriched solution.

In some examples, determining a protein concentration may comprise using one or more spectroscopic analysis techniques. For example, the protein concentration may be determined using one or more reflection spectroscopy and/or one or more absorption spectroscopy techniques. The protein sensor may comprise one or more spectrometers and/or one or more detectors.

In some examples, determining the protein concentration may comprise detecting an optical density (OD), for example using optical absorbance detection. In such examples, the protein sensor may comprise an optical absorbance detector. For example, the optical absorbance detector is selected from the group consisting of an ultraviolet (UV) detector, a visible light (VIS) detector, a UV-VIS detector, a photodiode array (PDA) detector and combinations thereof. In one example, the optical absorbance detector is an UV detector. In another example, the optical absorbance detector is a VIS detector. In a further example, the optical absorbance detector is a UV-VIS detector. In one example, the optical absorbance detector is a PDA detector.

In some examples, determining the protein concentration may comprise performing Raman spectroscopy. In such examples, the protein sensor may comprise a Raman spectrometer.

In some examples, determining the protein concentration may comprise performing near-infrared (NIR) spectroscopy. Performing NIR spectroscopy may comprise using reflection NIR spectroscopy (NIRS) and/or using absorption NIR spectroscopy. In such examples, the protein sensor may comprise a near-infrared
5 spectrometer.

In some examples, detecting the protein concentration comprises detecting the protein concentration in one or more of the feed flow through, the wash flow through, the protein-of-interest depleted feed flow through fraction, the protein-of-interest depleted wash flow through fraction and/or the protein-of-interest enriched solution by
10 one or more of an optical absorbance detector; a Raman spectrometer, and/or a near-infrared spectrometer.

In one example, a pressure sensor is connected to each inlet of the separation units. For example, a pressure sensor is connected to the inlet of the first separation unit. In another example, a pressure sensor is connected to the inlet of the second separation unit.
15 In a further example, a pressure sensor is connected to the inlet of the third separation unit. In one example, a pressure sensor is connected to the inlet of the fourth separation unit. In one example, a pressure sensor is connected to the inlet of the fifth separation unit.

In one example, the continuous chromatography is selected from the group
20 consisting of simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC). In one example, the continuous chromatography is simulated moving bed (SMB) chromatography. In another example, the continuous chromatography is periodic counter-current chromatography (PCC).

In one example, one or more of the chromatography media is selected from the
25 group consisting of an ion exchange chromatography medium, an affinity chromatography medium, a hydrophobic interaction chromatography medium, a mixed mode chromatography medium, an adsorption chromatography medium and a partition chromatography medium.

In one example, one or more of the chromatography media is an ion exchange
30 chromatography medium. For example, the ion exchange chromatography medium is an anion exchange chromatography medium or a cation exchange chromatography medium.

In one example, one or more of the chromatography media is an affinity chromatography medium.

In one example, one or more of the chromatography media is a hydrophobic
35 interaction medium.

In one example, one or more of the chromatography media is a mixed mode chromatography medium.

In one example, one or more of the chromatography media is an adsorption chromatography medium.

5 In one example, one or more of the chromatography media is a partition chromatography medium.

In some examples, each of the chromatography media need not be the same. In one example, each separation unit comprises a different chromatography media. In one example, two separation units comprise the same chromatography media. In another
10 example, three separation units comprise the same chromatography media. In a further example, four separation units comprise the same chromatography media. In one example, all separation units comprise the same chromatography media. In one example, the first and third separation units comprise the same chromatography media and the second and fourth separation units comprise the same chromatography media (but
15 different from the first and third). In one example, the first and third separation units comprise the same chromatography media and each of the second and fourth separation units comprise different chromatography media.

In one example, one or more of the chromatography media is selected from the group consisting of a membrane, a monolith and a resin.

20 In one example, one or more of the chromatography media is a membrane. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography membrane. In one example, one separation unit comprises a chromatography membrane. In one example, two separation units comprise a chromatography membrane. In another example, three separation units comprise a
25 chromatography membrane. In a further example, four separation units comprise a chromatography membrane. In one example, all separation units comprise a chromatography membrane. In one example, the first separation unit comprises a chromatography membrane. In another example, the second separation unit comprises a chromatography membrane. In a further example, the third separation unit comprises a
30 chromatography membrane. In one example, the fourth separation unit comprises a chromatography membrane. In one example, the fifth separation unit comprises a chromatography membrane.

In one example, one or more of the chromatography media is a monolith. For example, the first, second, third and/or fourth separation unit(s) comprise a
35 chromatography monolith. In one example, one separation unit comprises a chromatography monolith. In one example, two separation units comprise a

chromatography monolith. In another example, three separation units comprise a chromatography monolith. In a further example, four separation units comprise a chromatography monolith. In one example, all separation units comprise a chromatography monolith. In one example, the first separation unit comprises a chromatography monolith. In another example, the second separation unit comprises a chromatography monolith. In a further example, the third separation unit comprises a chromatography monolith. In one example, the fourth separation unit comprises a chromatography monolith. In one example, the fifth separation unit comprises a chromatography monolith.

10 In one example, one or more of the chromatography media is a resin. For example, the first, second, third and/or fourth separation unit(s) comprise a chromatography resin. In one example, one separation unit comprises a chromatography resin. In one example, two separation units comprise a chromatography resin. In another example, three separation units comprise a chromatography resin. In a further example, 15 four separation units comprise a chromatography resin. In one example, all separation units comprise a chromatography resin. In one example, the first separation unit comprises a chromatography resin. In another example, the second separation unit comprises a chromatography resin. In a further example, the third separation unit comprises a chromatography resin. In one example, the fourth separation unit comprises a chromatography resin. In one example, the fifth separation unit comprises a chromatography resin.

In one example, one or more of the chromatography media comprise a ligand capable of specifically binding the protein-of-interest. For example, the first, second, third, fourth and/or fifth separation unit(s) comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In one example, one separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In another example, two separation units comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In a further example, three separation units comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In one example, four separation units comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In one example, five or more separation units comprise a chromatography media comprising a ligand capable of specifically binding the protein-of-interest. In one example, the first 30 separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In another example, the second separation 35

unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In a further example, the third separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In one example, the fourth separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In one example, the fifth separation unit comprises a chromatography medium comprising a ligand capable of specifically binding the protein-of-interest. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand capable of specifically binding the protein-of-interest.

In one example, one or more of the chromatography media comprise a ligand capable of specifically binding to human IgG. The ligand may be capable of specifically binding to a constant domain of human IgG, for example CH1, CH2, CH3, CH4 or combinations thereof. For example, the first, second, third, fourth and/or fifth separation unit(s) comprise a chromatography media comprising a ligand capable of specifically binding to human IgG, such as a constant domain of human IgG, for example CH1, CH2, CH3, CH4 or combinations thereof. In one example, one separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In another example, two separation units comprise a chromatography media comprising a ligand capable of specifically binding to human IgG. In a further example, three separation units comprise a chromatography media comprising a ligand capable of specifically binding to human IgG. In one example, four separation units comprise a chromatography media comprising a ligand capable of specifically binding to human IgG. In one example, five or more separation units comprise a chromatography media comprising a ligand capable of specifically binding to human IgG. In one example, the first separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In another example, the second separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In a further example, the third separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In one example, the fourth separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In one example, the fifth separation unit comprises a chromatography medium comprising a ligand capable of specifically binding to human IgG. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand capable of specifically binding to human IgG.

In any of the above examples, the ligand capable of specifically binding to human IgG may be capable of binding to a constant domain of human IgG. For example, the ligand may be capable of specifically binding to a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, or combinations thereof.

5 In one example, the ligand comprises a camelid-derived single domain [VHH] antibody fragment. For example, the ligand is a VHH antibody fragment. In one example, the ligand does not comprise a CH1 domain.

In one example, the ligand comprises a VHH antigen-binding protein comprising an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1. In one example, the VHH antigen-binding protein comprises an amino acid sequence set forth in SEQ ID NO: 1. In one example, the VHH antigen-binding protein comprises a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1.

10 In one example, the ligand comprises a VHH antigen-binding protein comprising a framework region comprising an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1. In one example, the framework region comprises an amino acid sequence set forth in SEQ ID NO: 1. In another example, the framework region comprises a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1.

15 In one example, the ligand comprises an VHH antigen-binding protein comprising an amino acid sequence that comprises 4 framework regions, FR1, FR2, FR3 and FR4, and 3 complementarity determining regions, CDR1, CDR2 and CDR3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

- 25 a) the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
- b) the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
- 30 c) the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1; and

wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1.

35 In one example, the ligand comprises a VHH antigen-binding protein comprising an amino acid sequence that comprises 4 framework regions, FR1, FR2, FR3 and FR4,

and 3 complementarity determining regions, CDR1, CDR2 and CDR3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:

- a) the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
- b) the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
- c) the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1, and

wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1 and wherein the antigen binding protein specifically binds to the Fc domain of a human IgG molecule and does not bind to an IgG molecule of murine origin or bovine origin.

In one example, the ligand comprises a VHH antigen-binding protein comprising a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues.

In one example, ligand comprises a VHH antigen-binding protein comprising a CDR2 comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3.

In one example, the ligand comprises a VHH antigen-binding protein comprising a CDR3 comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4.

In one example, one or more of the chromatography media comprise a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. For example, the first, second, third, fourth and/or fifth separation unit(s) comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, one separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In another example, two separation units comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In a further example, three separation units comprise a

chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, four separation units comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, five or more separation units comprise a chromatography media comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, the first separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In another example, the second separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In a further example, the third separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, the fourth separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In one example, the fifth separation unit comprises a chromatography medium comprising a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix.

In one example, one or more of the chromatography media comprise a cross-linked poly(styrene-divinylbenzene) matrix. For example, the first, second, third, fourth and/or fifth separation unit(s) comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, one separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In another example, two separation units comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In a further example, three separation units comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, four separation units comprise a chromatography media comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, five or more separation units comprise a chromatography media comprising a cross-linked poly(styrene-

divinylbenzene) matrix. In one example, the first separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In another example, the second separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In a further
5 example, the third separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the fourth separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the fifth separation unit comprises a chromatography medium comprising a cross-linked poly(styrene-divinylbenzene)
10 matrix. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a cross-linked poly(styrene-divinylbenzene) matrix.

In one example, one or more of the chromatography media comprise an agarose-based matrix. For example, the first, second, third, fourth and/or fifth separation unit(s)
15 comprise a chromatography media comprising an agarose-based matrix. In one example, one separation unit comprises a chromatography medium comprising an agarose-based matrix. In another example, two separation units comprise a chromatography media comprising an agarose-based matrix. In a further example, three separation units comprise a chromatography media comprising an agarose-based matrix. In one example,
20 four separation units comprise a chromatography media comprising an agarose-based matrix. In one example, five or more separation units comprise a chromatography media comprising an agarose-based matrix. In one example, the first separation unit comprises a chromatography medium comprising an agarose-based matrix. In another example, the second separation unit comprises a chromatography medium comprising an agarose-
25 based matrix. In a further example, the third separation unit comprises a chromatography medium comprising an agarose-based matrix. In one example, the fourth separation unit comprises a chromatography medium comprising an agarose-based matrix. In one example, the fifth separation unit comprises a chromatography medium comprising an agarose-based matrix. In another example, the first chromatography medium and the
30 second chromatography medium, and optionally one or more additional chromatography media, comprise an agarose-based matrix.

In one example, one or more of the chromatography media comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. For example, the first, second, third and/or fourth separation
35 unit(s) comprise a chromatography media comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In

one example, one separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In another example, two separation units comprise a chromatography media comprising a ligand comprising a VHH antibody fragment
5 conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In a further example, three separation units comprise a chromatography media comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In one example, four separation units comprise a chromatography media comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked
10 poly(styrene-divinylbenzene) matrix. In one example, five or more separation units comprise a chromatography media comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the first separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-
15 divinylbenzene) matrix. In another example, the second separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In a further example, the third separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-
20 divinylbenzene) matrix. In one example, the fourth separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix. In one example, the fifth separation unit comprises a chromatography medium comprising a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-
25 divinylbenzene) matrix. In another example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix.

In one example, one or more of the separation units comprises an affinity
30 chromatography medium and one or more of the separation units comprises an ion exchange chromatography medium.

In one example, the first separation unit comprises an affinity chromatography medium and the third separation unit comprises an ion exchange chromatography medium.

In one example, the first and second separation units each comprise an affinity chromatography medium, and the third and fourth separation units each comprise an ion exchange chromatography media.

5 In one example, the chromatography media have a total bed height of between 2 cm and 30 cm.

In one example, the chromatography media has a total bed height of at least 2 cm. For example, the chromatography media has a total bed height of between 2 cm to 30 cm. For example, the chromatography media has a total bed height of between 10 cm and 30 cm. For example, the chromatography media has a total bed height of between 10 30 cm and 70 cm. For example, the chromatography media has a total bed height of 2cm, or 6 cm, or 10 cm, or 15cm, or 20 cm, or 25 cm, or 30 cm, or 35 cm, or 40 cm, or 45 cm, or 50 cm, or 55 cm, or 60 cm, or 65 cm, or 70 cm.

In one example, the chromatography media has a total bed height of at least 2 cm.

In one example, the chromatography media has a total bed height of 6 cm.

15 In one example, the chromatography media has a total bed height of 20 cm.

In one example, the chromatography media has a total bed height of 30 cm.

In one example, the chromatography media has a total bed height of 50 cm.

In one example, the chromatography media has a total bed height of 70 cm.

20 In one example, one or more of the separation units are selected from the group consisting of a column, a cassette, a capsule and a filter holder.

In one example, one or more of the separation units are a column. In one example, one of the separation units is a column. For example, the first separation unit is a column. In another example, the second separation unit is a column. In another example, the third separation unit is a column. In a further example, the fourth separation unit is a column. 25 In a further example, the fifth separation unit is a column. In one example, two of the separation units are columns. In another example, three of the separation units are columns. In a further example, four of the separation units are columns. In a further example, five or more of the separation units are columns. In one example, each separation unit is a column.

30 In one example, one or more of the separation units are a cassette. For example, the first separation unit is a cassette. In another example, the second separation unit is a cassette. In another example, the third separation unit is a cassette. In a further example, the fourth separation unit is a cassette. In a further example, the fifth separation unit is a cassette. In one example, two of the separation units are cassettes. In another example, 35 three of the separation units are cassettes. In a further example, four of the separation

units are cassettes. In a further example, five or more of the separation units are cassettes. In one example, each separation unit is a cassette.

In one example, one or more of the separation units are a capsule. For example, the first separation unit is a capsule. In another example, the second separation unit is a capsule. In another example, the third separation unit is a capsule. In a further example, the fourth separation unit is a capsule. In a further example, the fifth separation unit is a capsule. In one example, two of the separation units are capsules. In another example, three of the separation units are capsules. In a further example, four of the separation units are capsules. In a further example, five or more of the separation units are capsules.

10 In one example, each separation unit is a capsule.

In one example, one or more of the separation units are a column, wherein the column has a diameter of between 5 cm and 200 cm. For example, the column has a diameter of 5 cm, or 10 cm, or 20 cm, or 30 cm, or 40 cm, or 50 cm, or 60 cm, or 70 cm, or 80 cm, or 90 cm, or 100 cm, or 110 cm, or 120 cm, or 130 cm, or 140 cm, or 150 cm, or 160 cm, or 170 cm, or 180 cm, or 190 cm, or 200 cm. In one example, the column has a diameter of 5 cm. In one example, the column has a diameter of 20 cm. In one example, the column has a diameter of 50 cm. In one example, the column has a diameter of 100 cm. In one example, the column has a diameter of 200 cm.

20 BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments will now be described, by way of example only, with reference to the accompanying drawings in which:

Figure 1 is a schematic representation of a chromatography capture program with a feed flow-through loopback;

25 **Figure 2** is a schematic representation of a cycle segment of a continuous chromatography capture program with four columns and one loopback;

Figure 3 is an alternative schematic representation of the cycle segment of Figure 1;

30 **Figure 4** is a schematic representation of a cycle segment of a continuous chromatography capture program according to one embodiment of the present disclosure with four columns and two loopbacks;

Figure 5 is an alternative schematic representation of the cycle segment of Figure 4;

35 **Figure 6** is a schematic representation of a cycle segment of a continuous chromatography capture program according to another embodiment of the present disclosure with four columns and two loopbacks;

Figure 7 is an alternative schematic representation of the cycle segment of Figure 6;

Figure 8 is a schematic representation of another cycle segment of the continuous chromatography capture program of Figure 5;

5 **Figure 9** is an alternative schematic representation of the cycle segment of Figure 7;

Figure 10 is a schematic representation of a cycle segment of a continuous chromatography capture program according to another embodiment of the present disclosure with five columns and two loopbacks;

10 **Figure 11** is a schematic representation of a cycle segment of a continuous chromatography capture program according to another embodiment of the present disclosure with five columns and two loopbacks;

Figure 12 is a schematic chromatogram showing a continuous chromatography capture program according to the present disclosure with two loopbacks, shown on a
15 single column;

Figure 13 is another schematic chromatogram showing the continuous chromatography capture program of Figure 12 including the loopback phases applied to the column;

20 **KEY TO SEQUENCE LISTING**

- SEQ ID NO: 1 is an amino acid sequence of VHH fragment
- SEQ ID NO: 2 is an amino acid sequence of CDR1 of the VHH fragment
- SEQ ID NO: 3 is an amino acid sequence of CDR2 of the VHH fragment
- SEQ ID NO: 4 is an amino acid sequence of CDR3 of the VHH fragment

DETAILED DESCRIPTION

General

25 Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter.

30 Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The

disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present disclosure.

Any example of the present disclosure herein shall be taken to apply *mutatis mutandis* to any other example of the disclosure unless specifically stated otherwise.

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein the term “derived from” shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

Furthermore, as used herein the singular forms of “a”, “and” and “the” include plural references unless the context clearly dictates otherwise.

Selected Definitions

As used herein, the term “isolate”, “isolating” or “isolation” shall be taken to mean the separation of, whether completely or partially, a protein (e.g., a plasma protein such as IgG) present in a protein mixture (e.g., plasma or a plasma fraction).

As used herein, the term “protein-of-interest enriched solution” refers to a solution (e.g., an eluate or composition described herein) comprising the protein-of-interest at a greater purity compared to the purity in the protein mixture. It will be apparent from the disclosure that the protein-of-interest is not the only protein in the protein-of-interest enriched solution, rather the protein-of-interest need only be enriched or at a higher purity than in the original protein mixture.

The term “purity” shall refer to the portion of the protein-of-interest (e.g., IgG) relative to the total protein content expressed as a percentage.

As used herein, the term “protein-of-interest depleted fraction” refers to a fraction or part of the protein mixture (e.g., of the plasma or plasma fraction) that has had a protein-of-interest (e.g., a plasma protein, such as IgG) removed or reduced, whether completely or partially. It will be apparent from the disclosure that the protein-of-interest to be depleted need not be completely removed from the protein mixture, rather it need only be reduced or removed such that the protein-of-interest depleted fraction comprises a reduced amount (or reduced purity) of the protein-of-interest compared to the level of the protein-of-interest in the protein mixture.

As used herein, the term “protein mixture” shall be understood to refer to a solution containing two or more proteins or a complex mix of proteins, including the protein-of-interest.

The term “protein” shall be taken to include a single polypeptide chain, i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). For example, the series of polypeptide chains can be covalently linked using a suitable chemical or a disulfide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions.

The term “polypeptide” or “polypeptide chain” will be understood from the foregoing paragraph to mean a series of contiguous amino acids linked by peptide bonds.

The term “specifically binds”, “specifically binding” or “binds specifically” shall be taken to mean that a protein of the disclosure reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular antigen or cell expressing same than it does with alternative antigens or cells. For example, a ligand capable of specifically binding to a constant domain of human IgG, such as CH1, CH2, CH3, CH4 or combinations thereof, with materially greater affinity (e.g., 1.5 fold or 2 fold or 5 fold or 10 fold or 20 fold or 40 fold or 60 fold or 80 fold to 100 fold or 150 fold or 200 fold) than it does to other antigens. Generally, but not necessarily, reference to binding means specific binding, and each term shall be understood to provide explicit support for the other term.

The term “chromatography medium” shall be taken to mean a solid or semi solid phase for use in chromatography. In one example, a chromatography medium is made up of a porous or non-porous support to which a plurality of ligands are attached, examples of which are described herein. For example, the chromatography medium is a chromatography resin. Exemplary chromatography resins include MabSelect® SuRe®

(Cytiva), MabSelect® SuRe® LX (Cytiva), POROS® PI50 (ThermoFisher), CaptureSelect® FcXP affinity chromatography resins (Thermo Fisher), CaptureSelect® FcXL affinity resin (Thermo Fisher), CaptureSelect® IgG-CH1 affinity resin (Thermo Fisher), and CaptureSelect® FcXP agarose affinity resin (Thermo Fisher). Further
5 exemplary affinity chromatography resins include IgSelect® affinity resin (Cytiva), HiTrap® IgSelect® affinity resin (Cytiva), Pierce® Protein G agarose affinity resin (Thermo Fisher), and Protein G sepharose 4 fast flow affinity resin (Cytiva). Further exemplary affinity chromatography resins include Protein A ligand affinity resin, and IgG-CH1-XL affinity resin (Thermo Fisher). For example, the chromatography medium
10 is a chromatography membrane. Exemplary chromatography membranes include Purexa® A (Purilogs), Mustang Q (Pall Corporation).

The term “ion exchange medium” shall be taken to mean a chromatography medium comprising a negatively charged or positively charged functional group. For example, an ion exchange medium includes anion exchange chromatography medium
15 and cation exchange chromatography medium. Anion exchange chromatography medium is a positively charged medium with an affinity for molecules (e.g. proteins) having net negative surface charges. Exemplary anion exchange chromatography medium include POROS® PI50 anion resin (ThermoFisher), POROS® XQ anion resin (ThermoFisher), POROS® HQ 50 anion resin (ThermoFisher), and POROS® D50 anion
20 resin (ThermoFisher). Cation exchange chromatography medium is a negatively charged medium with an affinity for molecules (e.g. proteins) having net positive surface charges. Exemplary cation exchange chromatography medium include POROS® XS strong cation exchange resin (ThermoFisher) and POROS® HS strong cation exchange resin (ThermoFisher).

25 The term “affinity chromatography medium” shall be taken to mean a chromatography medium comprising an affinity chromatography ligand (e.g. camelid-derived single domain [VHH] antibody fragment, or protein A or an antibody fragment thereof, or protein G or an antibody fragment thereof) attached to a matrix such as, e.g., those described herein. Exemplary affinity chromatography medium used in a method
30 described herein include POROS® CaptureSelect® FcXP affinity resin (Thermo Fisher) and CaptureSelect® FcXP agarose affinity resin (Thermo Fisher). Further exemplary affinity chromatography medium include a medium having an amino acid sequence encoded by SEQ ID NO: 1 or variants thereof that specifically bind to the CH3 domain of human IgG. In one example, an exemplary affinity chromatography medium
35 comprises a VHH antigen-binding protein comprising a complementarity determining region (CDR) 1, CDR2 and/or CDR3 having an amino acid sequence of SEQ ID NO: 2,

3 or 4 respectively. Exemplary affinity chromatography resins are also described in US10259886. Exemplary protein A chromatography medium include MabSelect® Prisma protein A resins (Cytiva), Praesto® Jetted A50 protein A resins (Purolite Corp.), and Amsphere® A3 (JSR Corp.), MabSelect® SuRe® protein A resins (Cytiva), and
 5 MabSelect® SuRe® LX protein A resins (Cytiva). Exemplary protein G chromatography medium include Protein G Sepharose 4 Fast Flow resin (Cytiva), Protein G Resin (abbexa), Dynabeads® Protein G Magnetic Beads (ThermoFisher), Pierce® Protein G Agarose (ThermoFisher), Pierce® Protein G Plus Agarose (ThermoFisher), POROS® MabCapture® G Select (ThermoFisher), PROTEINDEX® rProtein G Agarose
 10 (Margvelgent Biosciences), and ProteinIso® Protein G Resin (TransGen Biotech Co., LTD).

The term “hydrophobic interaction chromatography medium” shall be taken to mean a chromatography medium comprising a hydrophobic ligand attached to a matrix such as, e.g., those described herein. Hydrophobic interaction chromatography (HIC)
 15 comprising a hydrophobic interaction chromatography medium separates molecules (e.g. proteins) based on the molecules hydrophobicity (i.e. aversion to water). Exemplary hydrophobic interaction chromatography medium include POROS® Ethyl Hydrophobic Interaction Chromatography (HIC) resin (ThermoFisher) and POROS® Benzyl Ultra Hydrophobic Interaction Chromatography (HIC) resin (ThermoFisher).

20 The term “mixed mode chromatography medium” shall be taken to mean a chromatography medium which allows for the separation of molecules (e.g. proteins) based on more than one form of interaction between the molecules and the medium. For example, mixed mode chromatography medium may comprise affinity chromatography ligands and ion exchange functional groups attached to a matrix. Exemplary mixed mode
 25 chromatography medium include CMM HyperCel (Sartorius), MEP HyperCel (Sartorius), HEA HyperCel (Sartorius), PPA HyperCel (Sartorius), and HA Ultrigel® (Sartorius).

The term “adsorption chromatography medium” shall be understood to mean a chromatography medium which allows the separation of a component in a mixture (e.g., proteins) by adsorption from a mobile phase into the stationary solid surface. Exemplary
 30 stationary phases included hydroxyapatite such as ceramic hydroxyapatite (CHT type I and type II, Bio-Rad Laboratories), HA Ultrigel hydroxyapatite (Pall Corp.), and ceramic fluoroapatite. (CFT Type I and Type II, Bio-Rad Laboratories).

The term “partition chromatography medium” shall be understood to mean a chromatography medium which allows for the separation of mixtures based on partition
 35 of a solute between two solvents one of which is immobilized by the substance in the separation unit (e.g., column).

The term “ligand” shall be taken to mean a molecule immobilised to a matrix of the chromatography medium which specifically binds to the protein (e.g., a constant domain of human IgG, such as a CH1, CH2, CH3 or CH4 domain of human IgG or combinations thereof). For example, the ligand is a camelid-derived single domain
5 [VHH] antibody fragment.

The term “camelid-derived single domain [VHH] antibody fragment” shall be taken to mean a VHH domain of a camelidae antibody. The camelidae antibody is an antibody produced from camels and llamas and has no CH1 domain normally present in human immunoglobulins and only one VHH domain. Exemplary affinity
10 chromatography resins comprising the camelid-derived [VHH] antibody fragment include CaptureSelect[®] antibody affinity chromatography resins (Thermo Fisher). For example, CaptureSelect[®] FcXL affinity resin, POROS[®] CaptureSelect[®] FcXP affinity resin, CaptureSelect IgG-CH1 affinity resin, and CaptureSelect FcXP agarose affinity resin. Further exemplary affinity chromatography resins include IgSelect[®] affinity resin
15 (Cytiva), HiTrap[®] IgSelect[®] affinity resin (Cytiva), Pierce[®] Protein G agarose affinity resin (Thermo Fisher), and Protein G sepharose 4 fast flow affinity resin (Cytiva).

The term “matrix” shall be taken to mean a support to which the ligand is immobilised. Exemplary matrices are cross-linked poly(styrene-divinylbenzene) matrix and agarose-based matrix.

20 The term “loading” as used herein, refers to the application of a sample (e.g., protein mixture) or solution (e.g., buffer) to the separation unit until the entire sample is applied to or contained within the unit.

As used herein, the term “washing” refers to the process of flowing a solution (e.g., a buffer) through the separation unit after loading so as to remove substances (e.g.,
25 proteins) not adsorbed or bound on the chromatography media, for example to remove substances or proteins from which the protein-of-interest is to be separated. Multiple washes are possible using different solutions.

The term “feeding” as used herein, refers to directing a solution (e.g., a flow through solution) from one separation unit to another separation unit.

30 The term “feed”, when used as a noun (e.g. “the feed”), refers to a supply of the mixture to be separated (e.g. the protein mixture containing the protein of interest).

As used herein, the term “in fluid communication” means that a flow of liquid occurs between two parts, i.e., between an outlet of one separation unit and an inlet of another separation unit. The connection between the two parts can be direct or it can be
35 interrupted by one or more valves or other parts. The term encompasses a connection

that is permanent as well as a connection that is not permanent, e.g., is interrupted by one or more valves such that the flow of liquid can be started and stopped when required.

The term “inlet” refers to any opening that enables or allows the introduction of a solution (e.g., protein mixture or buffer) onto the separation unit.

- 5 The term “outlet” refers to any opening that enables or allows the withdrawal or elution of solution (e.g., flow through or eluate) from the separation unit.

The term “immunoglobulin G (IgG)”, also known as “gamma globulin” or “immune globulin”, shall be taken to mean antibody of isotype G. There are several subclasses of IgG, for example, IgG1, IgG2, IgG3 and IgG4.

- 10 The term “plasma” shall refer to the straw-coloured/pale yellow component of blood obtained from one or more blood donor(s). Methods of obtaining plasma from a donor will be apparent to a skilled person and/or described herein. For example, plasma is obtained by removing red blood cells from donated blood. For example, plasma is obtained by plasmapheresis.

- 15 The term “plasma fraction” or “fraction thereof” shall refer to plasma which has been fractionated to isolate one or more desirable protein components from the plasma. For example, plasma may be fractionated to isolate cryo-precipitates (proteins that precipitate out of solution when a unit of fresh frozen plasma is slowly thawed in the cold) and cryosupernatant (also known as cryo-poor plasma). For example, plasma may
20 be fractionated by ethanol precipitation to produce IgG-containing Oncley fractions, Cohn fractions, ammonium sulphate precipitates, or Precipitates A (KN A), B (KN B), and the Precipitate of Supernatant B (KN B+1) from plasma as described in US patent 3,301,842. Plasma fractions include II+III precipitate produced according to Cohn methods such as Method 6, Cohn et. al. J. Am; Chem. Soc., 68 (3), 459-475 (1946),
25 Method 9, Oncley et al. J. Am; Chem. Soc., 71, 541-550 (1946), or the I+II+III precipitate, Method 10, Cohn et.al. J. Am; Chem. Soc., 72, 465-474 (1950); as well as the method of Deutsch et.al. J. Biol. Chem. 164, 109-118 (1946) or the Precipitate-A, B and the Precipitate of Supernatant B of Nitschmann and Kistler Vox Sang. 7, 414-424 (1962); Helv. Chim. Acta 37, 866-873 (1954). For example, the plasma may be
30 fractionated by octanoic acid fractionation as described in European application 893450. Typically, Cohn Fractions, and Kistler/Nitschmann Precipitate’s A (KN A), B (KN B) and the Precipitate of Supernatant B (KN B+1) exist as a suspended paste. Other purification techniques including chromatography may be used.

- 35 The term “cryo-precipitate” or “cryo-precipitates” refers to proteins in plasma that precipitate out of solution when a unit of fresh frozen plasma is slowly thawed in the

cold. Cryo-precipitates include factor VIII, fibrinogen, von Willebrand factor, factor XIII and platelet membrane microparticles.

The term “cryo-poor plasma” shall be taken to mean plasma removed of cryo-precipitates.

- 5 The term “cryo-rich plasma” shall be taken to mean plasma comprising components typically found in cryo-precipitates.

The term “dynamic binding capacity” or “DBC” of a chromatography medium shall be taken to refer to the maximum amount of protein that the medium will bind under operating conditions before significant breakthrough of unbound protein occurs.

- 10 The term “per mL of medium” shall be taken to refer to per mL of wet packed volume of medium.

- The term “bed height” shall be taken to mean the height at which the chromatography medium is packed into a separation unit (e.g., column). It will be apparent to the skilled person that reference to “total bed height” refers to the bed height of all separation units in the continuous chromatography set-up.
- 15

- The term “dissociation constant” shall refer to the pKa of a buffer. $pK_a = -\log_{10}(K_a)$, wherein K_a is the acid dissociation constant of the buffering agent of the buffer. For example, a wash buffer of 20 mM sodium dihydrogen phosphate, 40 mM sodium chloride at a pH of 7.4 comprises sodium dihydrogen phosphate as the buffering agent. Phosphoric acid has three dissociation constants (pKa1: 2.16, pKa2: 7.21, pKa3: 12.32).
- 20

- The term “industrial or commercial scale” or “large scale” or “manufacturing scale” shall refer to the amount of product that would be produced in a batch that was designed for clinical testing, formulation, sale and/or distribution to the public. For example, industrial scale refers to large scale purification of IgG from the plasma or fraction thereof to produce the plasma protein product.
- 25

- The term “plasma protein product” shall refer to a preparation, composition and/or protein product comprising a plasma protein (e.g. IgG) derived from the purification of the protein mixture (e.g., plasma or fraction thereof). Typically, the plasma protein is the predominant protein in the plasma protein product.
- 30

The term “pharmaceutical composition” shall be taken to mean a formulation of the protein product (e.g., the plasma protein product) with compounds generally accepted in the art for the delivery to mammals. Exemplary compounds include all pharmaceutically acceptable carriers, diluents or excipients thereof.

- 35 The term “treat” or “treatment” or “treating” shall be taken to mean administering a therapeutically effective amount of the composition or protein products of the

disclosure such that one or more symptoms or characteristics of the condition is reduced in the subject or that the subject is no longer clinically diagnosed with the condition.

The term “preventing”, “prevent” or “prevention” includes providing prophylaxis with respect to occurrence or recurrence of a specified condition in a subject. A subject
5 may be predisposed to or at risk of developing a condition but has not yet been diagnosed with the condition.

As used herein, the phrase “delaying progression of” includes reducing or slowing down the progression of a condition in a subject and/or at least one symptom of the condition.

10 The term “condition” shall be taken to mean a state of being or health status of a subject in need of treatment with IgG. Exemplary conditions include but are not limited to primary immunodeficiency disease (PI), chronic inflammatory demyelinating polyneuropathy (CIDP), and chronic immune thrombocytopenic purpura (ITP).

The term “subject” shall be taken to mean any animal including humans, for
15 example a mammal. Exemplary subjects include but are not limited to humans and non-human primates. For example, the subject is a human.

Continuous chromatography

The present disclosure provides a method of isolating a protein-of-interest
20 enriched solution and a plurality of protein-of-interest depleted fractions from a protein mixture using continuous chromatography.

The term “continuous chromatography” shall be taken to mean a chromatographic method comprising at least four separation units, wherein each unit comprises a chromatography medium capable of separating the protein-of-interest from the protein
25 mixture.

The term “separation unit” refers to an equipment onto which a chromatographic separation step can be performed and that is capable of comprising a chromatography medium. Separation units suitable for use in the present disclosure will be apparent to the skilled person and include, for example, a column, a cassette, a capsule and a filter
30 holder.

Continuous chromatography comprising at least four separation units involves the separation units being connected in an arrangement that allows the units to be operated in series and/or in parallel. In principle, a protein mixture maybe loaded onto a first and/or subsequent separation unit, whilst another separation unit is going through an
35 equilibration, wash, elution and/or regeneration phase simultaneously. For example, the first separation unit is being loaded, and at the same time the third separation unit is

undergoing a wash step. It will be apparent to the skilled person from the disclosure herein that such operation permits the separation units to operate independently and discordant.

It will be apparent to the skilled person from the disclosure that reference to a first, second, third, fourth, fifth and subsequent separation units is not to be taken as a reference to a specific separation unit and is for the purposes of comparison only. In particular, it will be understood that it is not reference to a defined physical set up or defined order of the separation units and is for the purposes of comparison only.

Continuous chromatography system

The chromatography system according to the present disclosure comprises at least first, second, third and fourth separation units, optionally a fifth and optionally further separation units, a switching valve arrangement and a valve control system.

In the illustrated examples, the separation units are shown as columns. In the Figures, each separation unit is designated a column number CX, where C may be 1, 2, 3, 4 or 5, depending on the number of columns. Each column C1-C5 has an inlet and an outlet. A fluid flow path extends through each column from the inlet to the outlet and is in contact with a chromatography medium within the column. An inlet flow path is provided in connection with each of the inlets of the columns C1-C5. A switching valve arrangement in connection with the inlet flow path is configured to selectively direct flow of one or more of a feed or wash buffer to the inlet of any one of the columns C1-C5.

As used herein, the term “switching valve arrangement” shall be taken to mean an arrangement of valves that define a network of flowpaths of the chromatography system. The network of flowpaths comprises solution flowpaths used in methods disclosed herein. The valves of the switching valve arrangement are operable to selectively direct solutions (e.g. protein mixture, wash buffer, elution buffer) along a flowpath in sequences in accordance with methods disclosed herein.

It will be apparent to the skilled person that flowpaths of the chromatography system may share one or more sections of flowpath in common. Similarly, valves may be used in the definition of more than one flowpath.

For example, the switching valve arrangement may include valves capable of defining more than one flowpath such as switching valves with multiple ports.

In accordance with the present disclosure, the valve control system acts to operate the valves of the switching valve arrangement to direct solutions in sequences in accordance with methods disclosed herein.

Reference to the separation units and/or flow paths by their respective functions or phase (e.g. feed, wash, feed loopback, wash loopback, elution) does not limit the

referenced separation unit and/or flow path to that function and is for the purposes of comparison only. It will be understood that each separation unit and/or flow path may be designated depending on the flow directed to that separation unit and/or flow path for that cycle segment and/or part thereof, and that the function of the separation units and/or flow paths may vary between cycle segments depending on the flow selectively directed thereto by the switching valve arrangement.

In one example, the chromatography system further comprises a protein sensor connected to each outlet of the separation units for detecting protein concentration in flow from the outlets.

10 In one example, the chromatography system further comprises a pressure sensor connected to each inlet for measuring pressure of flow to the inlets.

It will be appreciated that operation of the valves by the valve control system may be varied in response to data from the protein sensor and/or pressure sensor.

15 Feedback loops

As used herein, the term “feedback loop” refers to a process wherein a portion or all of an output solution (e.g., flow through from a load or wash) produced by a separation unit is returned as an input solution onto another separation unit.

20 In the examples below, “loopback 1” represents a 1st loopback for the feed (post-load) FT and “loopback 2” represents a 2nd loopback for the wash (post-wash) FT.

Chromatography cycle

As used herein, the term “chromatography cycle” or “cycle” shall be taken to mean one round of performing each of the phases of loading, washing, eluting, 25 equilibrating first feedback loop, second feedback loop, and optionally stripping, sanitising, and/or regenerating performed on the chromatography media. A chromatography cycle may be performed across several separation units in parallel, with the phases staggered such that each separation unit is performing a different phase. Within one complete cycle, each of the separation units performs each of the cycle 30 phases.

The cycle may be divided into a number of cycle segments, which may be equal to the number of separation units. The cycle segments may be demarked by switching between cycle phases on the separation units. In the illustrated examples, the loading of the feed is the time-limiting step in the chromatography cycle. Loading is performed 35 successively across the respective columns and loading of each column demarks a respective cycle segment. The feed is applied substantially constantly, such that there is

constant load FT onto the respective columns. The other phases of the chromatography cycle are performed on the columns in subsequent cycle segments.

In the examples below, the loading of the feed is the time-limiting step in the chromatography cycle. Loading is performed successively across the respective columns
5 C1, C2, C3, C4 (and optionally C5) and loading of each column demarks the respective cycle segments S1, S2, S3, S4 (and optionally S5). The feed may be applied continuously, such that there is a constant flow of feed and also a constant flow of the feed (load) FT loopback onto the respective loopback column.

10 Single loopback continuous chromatography system

Figure 1 shows a portion of a simple two-column single feedback loop chromatography cycle. In this example column C1 is loaded with the feed comprising the protein mixture (feed), producing a feed flow through (FT). The feed FT is directed via a first loopback onto a column C2, producing a product (e.g. protein-of interest, POI)
15 depleted feed FT.

Figures 2 and 3 illustrate a cycle segment of a chromatography cycle on a four-column chromatography system, with a single feedback loop. In this example, the chromatography system comprises first, second, third and fourth separation units, in the form of columns C1, C2, C3 and C4 respectively.

20 As shown in Figure 2, in the illustrated cycle segment, the feed is directed to column C2, while the wash buffer (wash) is directed to column C1. The solid and dashed arrows indicate the flow paths of the wash buffer and feed, respectively.

Figure 3 illustrates the phases of the chromatography cycle. The columns C1, C2, C3, C4 may be understood as progressing clockwise through the illustrated phases. The
25 columns progress through the phases indicated in one quadrant in one cycle segment, before progressing to the next quadrant in the subsequent cycle segment. The outer ring shows the input of each phase, and the inner ring shows the output of each phase.

A schematic program for a chromatography cycle using the system of Figures 2 and 3 is set out in Table 1 below. The cycle segment illustrated in Figures 2 and 3
30 corresponds to the cycle segment S2 in Table 1 below.

Table 1: Chromatography cycle with single loopback

Cycle segment / Column	S1	S2	S3	S4
C1	Load (Feed)	Wash/ Elution/ Equilibration	Loopback	Loopback
C2	Loopback	Load (Feed)	Wash/ Elution/ Equilibration	Loopback
C3	Loopback	Loopback	Load (Feed)	Wash/ Elution/ Equilibration
C4	Wash/ Elution/ Equilibration	Loopback	Loopback	Load (Feed)

In this example, the feed FT and wash FT are pooled and directed onto columns C3 and C4 via a single feedback loop (dotted line in Figure 2, “Loopback” in Table 1 above). As a result, there is no separation of the feed FT and wash FT, or of product depleted feed FT and product depleted wash FT in this example.

Four column, two loopback continuous chromatography system

Embodiments of the present disclosure provide a chromatography system and associated method including two feedback loops.

Figures 4 and 5 illustrate a cycle segment of an example chromatography cycle on a four-column chromatography system, with two feedback loops. In this example, the chromatography system comprises first, second, third and fourth separation units, in the form of columns C1, C2, C3 and C4 respectively.

As shown in Figures 4 and 5, in the illustrated cycle segment, the feed (load) is directed to column C2, while the wash buffer (wash) is directed to column C1. The solid and dashed arrows indicate the flow paths of the wash buffer and feed, respectively, through the columns.

Figure 5 illustrates the phases of the chromatography cycle. The columns C1, C2, C3, C4 may be understood as progressing clockwise through the illustrated phases. The columns progress through the phases indicated in one quadrant in one cycle segment, before progressing to the next quadrant in the subsequent cycle segment. The outer ring shows the input of each phase, and the inner ring shows the output of each phase.

A schematic program for a chromatography cycle using the configuration of Figures 4 and 5 is set out in Table 2 below.

Table 2: Chromatography cycle with four columns and two loopbacks (fixed column connection)

Cycle time/ column	1/4	2/4	3/4	4/4
C1	Load (Feed)	Wash/ Elution/ Equilibration	Loopback 1	Loopback 2
C2	Loopback 2	Load (Feed)	Wash/ Elution/ Equilibration	Loopback 1
C3	Loopback 1	Loopback 2	Load (Feed)	Wash/ Elution/ Equilibration
C4	Wash/ Elution/ Equilibration	Loopback 1	Loopback 2	Load (Feed)

The other phases of the chromatography cycle are performed on the respective columns in subsequent cycle segments such that, in one complete cycle, each column C1-C4 performs each of the phases of load, wash/elution/equilibration, loopback 1 and loopback 2 in a predetermined order as indicated in Figure 5 or in Table 2 above. In a complete cycle, each column can be understood as progressing clockwise 360 degrees through the phases illustrated in Figure 5.

In this example, the feed (post-load) FT and wash (post-wash) FT are recycled by separate feedback loops. In the illustrated cycle segment, the feed FT is directed onto column C4 and the wash FT is directed onto column C3.

In each of the cycle segments S1-S4, the same pattern of column connections is maintained. When the feed (load) is directed to column CX, the feed FT (post-load FT / load loopback) is directed to column C(X+2). Similarly, when the wash buffer (wash) is directed to column CX, the wash FT (post-wash FT/wash loopback) is directed to column C(X+2). As such, in this example, C1 and C3 form a first interconnected pair of columns, separate from C2 and C4 which form a second interconnected pair of columns. The column connection remains the same regardless of which phase is being performed by the column. As such, in this example, the outlets may be considered defined by target column, rather than by phase.

The example of Figures 4 and 5 may be understood as a continuous chromatography process comprising at least a first chromatography cycle between a first separation unit and a third separation unit, and a second chromatography cycle between a second separation unit and a fourth separation unit.

In this example, each chromatography cycle occurs between two separation units connected in series. Accordingly, each additional chromatography cycle requires two additional separation units to be added to the method of the disclosure.

5 In this example, the first and second chromatography cycles operate independently and are discordant. In the context of the present application, the term “independent” shall be understood to mean that the first chromatography cycle is not influenced or controlled by another (e.g., the second) chromatography cycle.

10 In the context of the present disclosure, the term “discordant” shall be understood to mean that at least the first and second chromatography cycles operate out-of-sync. It will be apparent to the skilled person that this refers to the phase of the chromatography cycle. For example, a first chromatography cycle may be undergoing a loading phase on the first separation unit and a first loopback phase on the third separation unit, whilst the second chromatography cycle is undergoing a washing phase on the second separation unit and a second loopback phase on the fourth separation unit.

15 In this example, the first and second chromatography cycle, each comprise two “feedback” loops.

The present example provides a first feedback loop. For example, a first chromatography cycle first feedback loop and a second chromatography cycle first feedback loop.

20 The present example provides a first chromatography cycle first feedback loop comprising feeding a post-load flow through from an outlet of a first separation unit through an inlet onto a third separation unit.

The present example provides a second chromatography cycle first feedback loop comprising feeding a post-load flow through from an outlet of a second separation unit
25 through an inlet onto a fourth separation unit.

The present example provides a second feedback loop. For example, a first chromatography cycle second feedback loop and a second chromatography cycle second feedback loop.

30 The present example provides a first chromatography cycle second feedback loop comprising feeding a post-wash flow through from an outlet of a first separation unit through an inlet onto a third separation unit.

The present example provides a second chromatography cycle second feedback loop comprising feeding a post-wash flow through from an outlet of a second separation unit through an inlet onto a fourth separation unit.

35

Four column, two loopback continuous chromatography system (variable column connection)

Figures 6 to 9 illustrate another example of a four-column chromatography system, with two feedback loops, having an alternative configuration of column connection. A schematic program for a chromatography cycle using the configuration of Figures 6 to 9 is set out in Table 3 below.

In this example, the column connections are configured such that the order of the loopback phases is reversed compared to the system of Figures 4 and 5.

10 **Table 3: Chromatography cycle with four columns and two loopbacks (variable column connection)**

Cycle segment / column	S1	S2	S3	S4
C1	Load (Feed)	Wash/ Elution/ Equilibration	Loopback 2	Loopback 1
C2	Loopback 1	Load (Feed)	Wash/ Elution/ Equilibration	Loopback 2
C3	Loopback 2	Loopback 1	Load (Feed)	Wash/ Elution/ Equilibration
C4	Wash/ Elution/ Equilibration	Loopback 2	Loopback 1	Load (Feed)

Figures 6 and 7 illustrate one cycle segment of the chromatography cycle (segment S3 in Table 3 above), while Figures 8 and 9 illustrate the immediately subsequent cycle segment (segment S4 in Table 3 above).

Figures 6 and 8 illustrate the column connections in the respective cycle segments. The solid and dashed arrows indicate the flow paths of the wash buffer and feed, respectively, through the columns.

Figures 7 and 9 illustrate the phases of the chromatography cycle in the respective cycle segments. The columns C1, C2, C3, C4 may be understood as progressing clockwise through the illustrated phases. The columns progress through the phases indicated in one quadrant in one cycle segment, before progressing to the next quadrant in the subsequent cycle segment. The outer ring shows the input of each phase, and the inner ring shows the output of each phase. In a complete cycle, each column can be understood as progressing clockwise 360 degrees through the phases illustrated in Figures 7 and 9.

As shown in Figures 6 and 7, in the illustrated cycle segment, the feed (load) is directed to column C3, while the wash buffer (wash) is directed to column C2. The feed FT (feed loopback) is directed to column C4, while the wash FT (wash loopback) is directed to column C1.

5 In the subsequent cycle segment, as shown in Figures 8 and 9, the functions of each of the columns is varied, such that each column performs the next phase of the chromatography cycle according to the predetermined sequence set out in Table 3. The pattern of column connections remains the same between cycle segments. That is, in any given cycle segment, for feed (load) directed to column CX, the feed FT loopback
10 (loopback 1) is directed to column C(X+1), and for wash buffer (wash) directed to column CX, the wash FT loopback (loopback 2) is directed to column C(X+3).

In contrast to the system of Figures 4 and 5, in this example, the columns do not form pairs which are isolated from each other. Each column is connected to two other columns via two respective feedback loops. The valve switching arrangement selects the
15 appropriate feedback loop (or collection/waste flowpath) to direct the output of the column depending on the cycle phase being performed by the column in the given cycle segment. The column connection varied based on the phase is being performed by the column. As such, the outlets may be considered to be defined per phase rather than per column.

20

Five column, two loopback continuous chromatography system

Figure 10 illustrates a cycle segment of an example chromatography cycle using a five-column chromatography system with two feedback loops. In this example, the chromatography system comprises first, second, third, fourth and fifth separation units,
25 in the form of columns C1, C2, C3, C4 and C5 respectively. The columns C1, C2, C3, C4 and C5 may be understood as progressing clockwise through the illustrated phases. The columns progress through the phases indicated in one sector (one fifth of the circle in Figure 5) in one cycle segment, before progressing to the next quadrant in the subsequent cycle segment.

30 A schematic program for a full chromatography cycle using this configuration is set out in Table 4 below. Figure 10 illustrates the segment S4 shown in Table 4.

Table 4: Chromatography cycle with five columns and two loopbacks (fixed column connection)

Cycle segment / column	S1	S2	S3	S4	S5
C1	Load (Feed)	Wash	Elution/ Equilibration	Loopback 1	Loopback 2
C2	Loopback 2	Load (Feed)	Wash	Elution/ Equilibration	Loopback 1
C3	Loopback 1	Loopback 2	Load (Feed)	Wash	Elution/ Equilibration
C4	Elution/ Equilibration	Loopback 1	Loopback 2	Load (Feed)	Wash
C5	Wash	Elution/ Equilibration	Loopback 1	Loopback 2	Load (Feed)

In this example, a single feedback loop is provided in connection with the outlet of each column. The column connections are CX to C(X+2). As such, in any given cycle segment, when the feed is directed to column CX, the feed FT (Load loopback / loopback 1) is directed to column C(X+2). Similarly, when the wash is directed to column CX the wash FT (Wash LB / loopback 2) is directed to column C(X+2). The column connection remains the same regardless of which phase is being performed by the column. As such, in this example, the outlets may be considered defined by target column, rather than by phase.

This connection pattern may be understood as similar to that described in relation to Figures 4 and 5. However, the uneven number of columns breaks up the fixed connection of isolated 2-column pairs.

Figure 11 illustrates a cycle segment of another example chromatography cycle using a five-column chromatography system with two feedback loops, with an alternative connection configuration. In this example, the chromatography system comprises first, second, third, fourth and fifth separation units, in the form of columns C1, C2, C3, C4 and C5 respectively. The columns C1, C2, C3, C4 and C5 may be understood as progressing clockwise through the illustrated phases. The columns progress through the phases indicated in one sector (one fifth of the circle in Figure 5) in one cycle segment,

before progressing to the next quadrant in the subsequent cycle segment. A schematic program for a full chromatography cycle using this configuration is set out in Table 5 below. Figure 11 illustrates the segment S4 shown in Table 5.

5 **Table 5: Chromatography cycle with five columns and two loopbacks (variable column connection)**

Cycle segment / column	S1	S2	S3	S4	S5
C1	Load (Feed)	Wash	Elution/ Equilibration	Loopback 2	Loopback 1
C2	Loopback 1	Load (Feed)	Wash	Elution/ Equilibration	Loopback 2
C3	Loopback 2	Loopback 1	Load (Feed)	Wash	Elution/ Equilibration
C4	Elution/ Equilibration	Loopback 2	Loopback 1	Load (Feed)	Wash
C5	Wash	Elution/ Equilibration	Loopback 2	Loopback 1	Load (Feed)

The configuration of Figure 11 differs from that of Figure 10 in that the order of the loopback phases is reversed.

10 In this example, two feedback loops are provided in connection with the outlet of each column. The column connections are defined as feed: CX to C(X+1) and wash: CX to C(X+3). As such, in any given cycle segment, when the feed is directed to column CX the feed FT (Load loopback / loopback 1) is directed to column C(X+1). When the wash is directed to column CX, the wash FT (Wash LB / loopback 2) is directed to column C(X+2). The column connection varied based on the phase is being performed by the
15 column. In this example, the outlets may be considered defined by phase rather than by column.

 In Figures 10 and 11, the elution/equilibration phase is carried out by a separate column. The separation unit (column) selected to perform the elution/equilibration step in any given cycle segment may be designated an elution column for that cycle segment.
20 However, as shown in Figures 10 and 11, the elution phase may be partially applied to

the column receiving the wash buffer (the wash separation unit) at the end of that cycle segment. For example, Figure 10 shows elution applied to column C3 after washing. In the subsequent cycle segment (S5 as indicated in Table 5 above) column C3 performs the elution/equilibration phase. A 5-column setup may provide flexibility to adjust load
 5 phase to cover a varying titer of feed and/or a decreasing binding capacity of resin during the system lifetime.

Figures 12 and 13 show schematic chromatograms, illustrating the various phases of the chromatography cycle as applied to any one column CX. The outputs of the process may be divided into several fractions. For example, the output of the feed (load) phase
 10 may be separated into fractions as indicated below:

- a. initial fraction comprising pure buffer. This fraction may be directed to waste.
 - b. main fraction comprising undiluted product-depleted plasma. This fraction may be directed to a feedback loop.
- 15 Example fractions may be as indicated by numerals 1-6 below the diagram in Figure 12. The fractions 1-6 of Figure 12 are described in more detail below, along with example indications of the further direction of flow for each of the fractions.
1. Pure buffer of an equilibrated column when load has started → waste.
 2. Post-load plasma with no product → flow through fraction.
 - 20 3. Post-load plasma with product breakthrough → post-load loopback to another column.
 4. Post-wash with product and plasma → post-wash loopback to another column → undiluted product depleted plasma.
 5. Post-wash with product and little amount of plasma → post-wash loopback to
 25 another column → diluted product-depleted plasma.
 6. Post-wash with product and minimal amount of plasma → post-wash loopback to another column → waste.

As indicated by the black arrows in Figure 13, in the load/feed cycle segment, the post-load (feed) FT is directed from the outlet of column CX onto another column.
 30 Column CX receives feed (post-load) FT from another column in later cycle phase (loopback 1). Similarly, as indicated by the white arrows, in the wash cycle segment, the post-wash FT is directed from the outlet of column CX onto another column. Column CX receives post-wash FT from another column in a later cycle phase (loopback 2). In Figure 13, loopback 1 precedes loopback 2. However, in other examples, the order of
 35 loopback 1 and loopback 2 may be reversed.

It will be apparent to the skilled person from the disclosure herein that the presence of two feedback loops per chromatography cycle allows the post-load flow through and the post-wash flow through to be loaded separately onto chromatography media enabling efficient isolation of undiluted material for isolation of further proteins of interest. In any of the two-loopback configurations described above, the feed (post-load) FT and wash (post-wash) FT are maintained separated from each other. As a result, the product depleted feed FT and product depleted wash FT are also maintained separate from each other.

Examples of vessels which may be used to perform the continuous chromatography method using multiple feedback loops will be apparent to the skilled person and/or described herein. For example, the continuous chromatography method may be performed using, for example, Tricorn 5/100 (Cytiva) columns in a continuous system. In another example, the continuous chromatography method may be performed using, for example, YMC Eco plus columns in a continuous system. In one example, the continuous chromatography method may be performed using BioSMB PD System (Sartorius). In a further example, the continuous chromatography method may be performed using Äkta™ PCC system (GE Healthcare). In a further example, the continuous chromatography method may be performed using a BioSC system (Sartorius), for example, Sartorius Resolute® BioSC. Any of the vessels/systems mentioned above may be customised to perform the method according to the current disclosure.

In one example, the continuous chromatography is selected from the group consisting of simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC).

25

Simulated moving bed (SMB) chromatography

In one example, the continuous chromatography is simulated moving bed (SMB) chromatography. The term “simulated moving bed chromatography” or “SMB chromatography” refers to a chromatography method first described in US patent 2,985,589. Examples of SMB chromatography setup and/or apparatus will be apparent to the skilled person and/or described herein. The concept of simulated moving bed involves the use of multiple smaller columns (rather than one large column) containing a solid absorbent (e.g., an affinity resin) and performing one or more continuous chromatography steps (i.e., equilibration, binding, washing, eluting or stripping) simultaneously on different columns in a continuous loop.

35

One example of a SMB chromatography of the disclosure set up involves the use of four separation units, where each pair of separation units comprises the following exemplary chromatography cycle. A feed (containing adsorbable components (extract)) is loaded onto a first separation unit of the SMB chromatography setup, and at least some of the extract binds to the chromatography medium within the first separation unit. Meanwhile, less adsorbed components (raffinate) in the feed pass through the first separation unit and are loaded onto a third separation unit in a first feedback loop. A wash is loaded onto the first separation unit and the less adsorbed components (raffinate) in the wash flow through are loaded onto a third separation unit via a second feedback loop or removed from the SMB chromatography system as waste. An eluent is loaded onto the first column to collect the extract, followed by sanitisation and/or regeneration independently of the third separation unit. The third separation unit is then eluted, cleaned and/or re-equilibrated independently of the other separation units being loaded with a further sample. The process steps are continuously cycled between the four columns.

It will be appreciated that other examples of SMB chromatography according to the present disclosure may use an alternate configuration of columns and column connections, such as one or more of the examples described above.

20 Periodic counter-current chromatography (PCC)

In one example, the continuous chromatography is periodic counter-current chromatography (PCC). Examples of PCC setup and/or apparatus will be apparent to the skilled person and/or described herein. The concept of PCC involves the use of multiple columns containing a solid absorbent (e.g. affinity resin) and performing the chromatography steps in parallel in a quasi-continuous manner. The buffers used in binding, washing, and/or elution steps flow counter-current to the affinity resin.

One example of PCC setup involves the use of four columns. In a first step, a sample is loaded onto a first column above the DBC of the resin so that unbound product (e.g. IgG) breaks through the first column and is captured by the second column. In a second step, the first column is washed and the flow through is captured by a third column. Subsequently, the first column is eluted, cleaned and/or re-equilibrated independently of the second and third columns being loaded with a further sample. An additional sample is then loaded onto the second column above the DBC of the resin so that unbound product breaks through the second column and is captured by the third column. The second column is washed and the flow through is captured by a fourth column. The second column is then eluted, cleaned and/or re-equilibrated independently

of the other columns being loaded with a further sample. The process steps are continuously cycled between the four columns.

Another example of PCC setup involves the use of multiple columns. For example, a variation of the above PCC setup can involve use of multiple columns to
5 capture unbound product which simulates use of a large column.

Chromatography media

The present disclosure provides a isolating a protein-of-interest enriched solution and a plurality of protein-of-interest depleted fractions from a protein mixture using
10 continuous chromatography, wherein each separation unit comprises a chromatography medium capable of separating the protein-of-interest from the protein mixture.

It will be apparent to the skilled person from the disclosure herein that the chromatography media may be the same and/or different in each of the separation units. For example, the chromatography media in each of the separation units is different. In
15 another example, the chromatography media in each of the separation units is the same. In a further example, the chromatography media in some of the separation units is the same. For example, at least two of the separation units have the same chromatography media.

In one example, one or more of the chromatography media is selected from the
20 group consisting of a resin, a monolith and a membrane.

In one example, one or more of the chromatography media comprises a ligand capable of specifically binding to human IgG. The ligand may be capable of specifically binding to a constant domain of human IgG. For example, the ligand may be capable of specifically binding to a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, or
25 combinations thereof. For example, the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand capable of specifically binding the protein-of-interest.

Suitable chromatography resins will be apparent to the skilled person and/or described herein. In one example, the resin comprises a ligand of camelid-derived single
30 domain [VHH] antibody fragments. The skilled person will be aware that ligands based on camelid-derived single domain [VHH] antibody fragments are capable of specifically binding to all subclasses of IgG (IgG1, IgG2, IgG3, IgG4). Exemplary resins are the CaptureSelect® FcXP affinity chromatography resins (Thermo Fisher), CaptureSelect® FcXL affinity resin (Thermo Fisher), CaptureSelect® IgG-CH1 affinity resin (Thermo
35 Fisher), and CaptureSelect® FcXP agarose affinity resin (Thermo Fisher). Further exemplary chromatography resins include IgSelect® affinity resin (Cytiva), HiTrap®

IgSelect[®] affinity resin (Cytiva), Pierce[®] Protein G agarose affinity resin (Thermo Fisher), and Protein G sepharose 4 fast flow affinity resin (Cytiva).

In one example, one or more of the chromatography media comprise a camelid-derived single domain [VHH] antibody fragment and a cross-linked poly(styrene-divinylbenzene) matrix. For example, the chromatography resin is POROS[®] CaptureSelect[®] FcXP affinity resin (Thermo Fisher). The cross-linked poly(styrene-divinylbenzene) matrix allows the resin to withstand pressures of up to 100 bar.

In one example, one or more of the chromatography media comprises a camelid-derived single domain [VHH] antibody fragment and an agarose-based matrix. For example, the chromatography resin is CaptureSelect FcXP agarose affinity resin (Thermo Fisher).

Additional purification steps

Additional purification steps may be performed before or after the continuous chromatography step. In one example, additional purification steps may be performed before the continuous chromatography step. In one example, additional purification steps may be performed after the continuous chromatography step.

In one example, the method further comprises one or more steps selected from a group consisting of: precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation; ion exchange chromatography; viral inactivation; viral filtration and ultrafiltration/diafiltration. Additional purification steps will be apparent to the skilled person and/or described herein.

In one example, the method further comprises ethanol precipitation. For example, cold ethanol may be used to isolate and enrich IgG by removing albumin and α - and β -globulins from the plasma or fractions thereof. For example, as described in WO2011/149472.

In one example, the method further comprises immunoaffinity chromatography. For example, the method further comprises isoagglutinin affinity chromatography using Eshmuno anti-A and anti-B resin. For example, isoagglutinin affinity chromatography may be used to remove isoagglutinins A and B.

In one example, the method further comprises octanoic acid fractionation. Octanoic acid may be used to remove of plasma lipids and plasma proteins (other than IgG). For example, as described in WO2011/131787.

In one example, the method further comprises ion exchange chromatography. In one example, the ion exchange chromatography is anion exchange chromatography.

example, anion exchange chromatography may be used to remove IgA, remaining IgM and other plasma components (other than IgG).

The anion exchanger can be a resin-based anion exchanger, an anion exchange membrane adsorber, or any other format of anion exchanger with a positively charged
5 substrate for capturing negatively charged particles. In one example, the anion exchanger is an anion exchange membrane adsorber. In another example, the anion exchanger is a resin-based anion exchanger. In a further example, the anion exchanger is a monolithic anion exchanger.

In one example, the method further comprises anion exchange chromatography
10 using a resin-based anion exchanger. For example, the anion exchange chromatography resin is a strong anion exchanger. In one example, the strong anion exchange resin comprises a matrix consisting of a poly(styrene-divinylbenzene) matrix. In one example, the strong anion exchanger comprises a quaternized polyethyleneimine functional group. Suitable resin-based anion exchanges will be apparent to the skilled person and include,
15 for example, POROS™ HQ 50.

In one example, the anion exchange chromatography step is performed in flow through mode. In another example, the anion exchange chromatography step is performed in bind-and-elute mode.

In one example, the anion exchange chromatography step comprises a buffer
20 selected from the group consisting of sodium citrate, 2-(N-morpholino)ethanesulfonic acid (MES) buffer, sodium dihydrogen phosphate, Bis-Tris, phosphate, L-histidine and combinations thereof. In one example, the anion exchange chromatography step comprises a buffer comprising MES buffer. In another example, the anion exchange chromatography step comprises phosphate buffer.

25 In one example, the method further comprises viral inactivation. For example, viral inactivation may be effected by adjusting the solution to low pH. Low pH may be a pH of between 2 to 4. In one example, low pH viral inactivation is performed in the presence of caprylate. In another example, viral inactivation may be effected by contacting the plasma or fraction thereof, or an IgG-enriched preparation or IgG-
30 containing pharmaceutical composition with *n*-Octyl- β -D-Glucopyranoside (OG), thereby forming an OG-IgG mixture. In a further example, low pH viral inactivation is performed in the presence of *N,N*-Dimethylmyristylamine *N*-oxide (TDAO).

In a further example, viral inactivation may be effected by exposing the protein mixture, plasma or plasma fraction, a protein depleted preparation or composition (e.g.,
35 a plasma protein depleted preparation e.g., an IgG-depleted preparation) to a solvent-detergent inactivation step. Suitable solvent-detergent treatments would be apparent to

the skilled person and include, for example environmentally friendly detergents. Exemplary environmentally friendly detergents suitable for use in the present disclosure and in particular for use in inactivating lipid enveloped viruses include *N,N*-Dimethylmyristylamine *N*-oxide (TDAO), polysorbate 80 (PS80), polyoxyethylene (10) isooctylcyclohexyl ether (TRITON® X-100-reduced), and a non-ionic surfactant prepared from glucose and alcohol (e.g., Simulsol™ formulations). In one example, the detergent is *N,N*-Dimethylmyristylamine *N*-oxide (TDAO). In one example, the detergent is polysorbate 80. In another example, the detergent is polyoxyethylene (10) isooctylcyclohexyl ether (TRITON® X-100-reduced). In a further example, the detergent is a non-ionic surfactant prepared from glucose and alcohol.

In one example, the method further comprises viral filtration. For example, viral filtration membranes of pore sizes from 15-20 nm may be used to remove microbes and viruses from a solution or eluate or pharmaceutical composition. Exemplary nanofilters include Planova S20N (Asahi), Virosart HC (Sartorius) and Planova 20N (Asahi).

In one example, the method further comprises ultrafiltration/diafiltration. An exemplary ultrafiltration/diafiltration membrane is Pellicon 2 Cassettes (Millipore) or Polyethersulfone or Hydrosart cassettes (Sartorius).

Assaying Activity

In one example, the activity of the purified protein (e.g., a plasma protein product purified or produced by the present disclosure) is assessed.

Analysis of IgG composition

Methods of determining yield, purity and IgG subclass distribution will be apparent to the skilled person and/or described herein.

In one example, purity is determined by SDS-PAGE and MALDI-TOF-MS peptide fingerprint analysis. Briefly, purified plasma protein products or pharmaceutical composition described herein is loaded onto a suitable SDS-PAGE gel (e.g. 8-16% TRIS-glycine), along with a protein size marker and a positive control for the protein of interest (e.g., IgG such as Privigen) under reduced and non-reduced conditions. Proteins are separated based on size and protein bands of interest are isolated, processed and analysed by MALDI-TOF-MS.

In another example, impurities in the preparation or pharmaceutical composition described herein are measured in an Enzyme-Linked Immunosorbent Assay (ELISA) using impurity (e.g. IgA) specific antibodies. For example, the ELISA is performed using commercially available methods.

In one example, purity, yield and/or subclass distribution of IgG is determined by nephelometry.

Stability of plasma and plasma fraction

5 The stability of the plasma or plasma fraction for loading onto a chromatography medium described herein can be determined by assessing the pro-coagulant activity, proteolytic activity and particle size of the plasma or fraction thereof. Methods for assessing pro-coagulant activity, proteolytic activity and particle size will be apparent to a skilled person and/or described herein. Briefly, the plasma or plasma fraction is
10 freeze/thawed in one or more cycles, stored at between 2°C and 32°C (e.g. 2°C, 10°C, 18°C, 21°C, 28°C or 32°C) for 24 or up to 48 hrs and analysed using one or more of the methods described below. In one example, the plasma or plasma fraction is thawed in one or more cycles at a temperature of 32°C, stored for 24 or up to 48 hours and analysed using one or more of the methods described below. In another example, the plasma or
15 plasma fraction is thawed in one or more cycles at a temperature of 32°C, stored for 24 or up to 48 hours and analysed using one or more of the methods described below and then cooled and stored at a temperature of 21°C. In one example, the plasma or plasma fraction is thawed at a temperature of 32°C and at a temperature of 21°C before the continuous chromatography.

20 In one example, the pro-coagulant activity in the plasma or plasma fraction can be determined using an *in vitro* coagulation assay, e.g., activated partial thromboplastin time (NaPTT) assay. The NaPTT assay measures the rate at which one or more coagulation factors (e.g., fibrinogen, prothrombin, proaccelerin, anti-hemophilic factor, Stuart-Prower factor, plasma thromboplastin antecedent and Hegeman factor) are
25 activated or form in plasma, or a fraction thereof, when coagulation activators (e.g. silica, kaolin, ellagic acid) are added to the assay.

In one example, proteolytic activity in the plasma or plasma fraction can be assessed by measuring the activity of thrombin, general serine proteases, kallikrein, plasmin and FXa e.g. using commercially available kits, such as thrombin activity assay
30 kit (S-2238), general serine protease assay kit (S-2288), kallikrein activity assay kit (S-2302), plasmin activity assay kit (S-2251) and FXa activity kit (S-2765).

In one example, the size of any particles in the plasma or plasma fraction is assessed by microflow imaging (MFI) and polydispersity index is calculated. Calculation of the polydispersity index will be apparent to the skilled person.

35

Pharmaceutical Compositions

The present disclosure provides a pharmaceutical composition comprising a protein-of-interest purified or produced by a method of the disclosure. The present disclosure also provides a pharmaceutical composition comprising IgG purified or
5 produced as a protein-of-interest by a method of the disclosure. The present disclosure further provides a pharmaceutical composition comprising one or more plasma protein products from the protein-of-interest depleted preparation purified or produced by a method of the disclosure.

Proteins purified by the disclosure (*syn.* active ingredients) are useful for
10 formulating into a pharmaceutical composition for parenteral, such as intravenous administration or subcutaneous administration, for therapeutic and prophylactic treatment.

The compositions for administration will commonly comprise a solution of the protein dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A
15 variety of aqueous carriers can be used, e.g., buffered saline and the like. The compositions may contain pharmaceutically acceptable carriers as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

The concentration of the protein in these formulations can vary widely, and will
20 be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. The vehicles may contain minor amounts of additives that enhance isotonicity and chemical stability, e.g., buffers and preservatives. For example, the pharmaceutical
25 composition comprises proline as a stabilising agent.

Suitable pharmaceutical compositions in accordance with the disclosure will generally include an amount of the protein admixed with an acceptable pharmaceutical carrier, such as a sterile aqueous solution, to give a range of final concentrations, depending on the intended use. The techniques of preparation are generally known in the
30 art as exemplified by Remington's Pharmaceutical Sciences, 16th Ed. Mack Publishing Company, 1980.

In one example, the plasma protein product is selected from a group consisting of an immunoglobulin G (IgG), apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen,
35 a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-

thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof.

In one example, the pharmaceutical composition comprises IgG purified or produced by a method of the present disclosure. In one example, the composition comprises IgG at a concentration of 1 to 5% w/v, 5 to 15% w/v, or 8 to 12% w/v. For example, the concentration of the IgG in the pharmaceutical composition is 1%, 2%, 3%, 4%, 5%, or 6%, or 7%, or 8%, or 9%, or 10%, or 11%, or 12%, or 13%, or 14%, or 15% w/v. For intravenous use, 1% w/v (i.e. 10g IgG/L) may be used. For intravenous use, 10% w/v (i.e. 100g IgG/L) may be used.

For subcutaneous administration, a higher concentration may be used. For example, 15 to 35% w/v, or 20 to 30% w/v. In one example, the concentration of the IgG in the pharmaceutical composition is 16%, or 17%, or 18%, or 19%, or 20%, or 21%, or 22%, or 23%, or 24%, or 25%, or 26% w/v.

Method of use

As discussed herein, the present disclosure provides a method of treating, preventing and/or delaying progression of a condition in a subject, comprising administering IgG or a pharmaceutical formulation to the subject.

In one example, the condition is selected from a group consisting of primary immunodeficiency disease (PI), chronic inflammatory demyelinating polyneuropathy (CIDP), and chronic immune thrombocytopenic purpura (ITP).

Table of Sequences

SEQ ID NO: 1	QVQLQESGGGLVQAGDSLRISCRASGLTVNDLYMGWFR QAPGKEREFVGRVTPGDNTDYTTYVDSVKGRFTISRDSA KNTVYLQMNSLKLEDTAVYLCAGRRFGSSEWDYWGQG TQVTVSS
SEQ ID NO: 2	GLTVNDLYMG
SEQ ID NO: 3	RVTPGDNTDYTTYVDSVK
SEQ ID NO: 4	RRFGSSEWDY

The present disclosure includes the following non-limiting Examples.

EXAMPLES

Example 1: Continuous capture of IgG with efficient isolation of undiluted product-depleted plasma material for further use

An affinity chromatography resin POROS® CaptureSelect® FcXP affinity resin (Thermo Fisher) was used to investigate the use of multiple feedback loops in a continuous chromatography setup. In this example, the affinity chromatography resin contained a ligand capable of binding to a CH3 domain of human IgG, specifically camelid-derived single domain [VHH] antibody fragment, and a matrix of cross-linked poly(styrene-divinylbenzene). The affinity chromatography resin was packed in YMC Eco plus columns (diameter 1cm) and chromatography performed on a BioSMB PD System (Sartorius). The columns had the following bed heights: A (6.1cm); B (6.0cm); C (5.9cm) and D (6.2cm).

Cryo-rich plasma (CRP) (prepared by CSL Behring) was thawed at 37 °C and filtered through a 1.2 µm and 0.45+0.2 µm capsule filters at pressure between 0.5-1.0 bar. Filtered CRP at room temperature was used as the feed material.

Table 6 describes the chromatography running conditions and buffers used.

Table 6: Chromatography buffers

Chromatography phase	Buffer
Equilibration	20 mM NaH ₂ PO ₄ , 500 mM NaCl, pH 7.4
Column Wash	20 mM NaH ₂ PO ₄ , 500 mM NaCl, pH 7.4
Elution	20 mM acetic acid buffer, pH 4.0
Regeneration	20% EtOH and 20 mM NaOH

The continuous chromatography capture program was set up with one or two loopbacks, as shown in **Figure 1** (A and B single loopback; C-G two loopbacks) and **Tables 7** and **8**. Using two loopbacks parallel the load-flow through and wash-flow through streams were independently recycled and product-depleted feed separated from the diluted wash.

Table 7: Continuous capture program setup with 4 columns and 1 loopback

Cycle time/ Column	1/4	2/4	3/4	4/4
C1	Load	Wash/ Elution/ Equilibration	Loopback	Loopback
C2	Loopback	Load	Wash/ Elution/ Equilibration	Loopback
C3	Loopback	Loopback	Load	Wash/ Elution/ Equilibration
C4	Wash/ Elution/Equilibration	Loopback	Loopback	Load

Table 8: Continuous capture program setup with 4 columns and 2 loopbacks

Cycle time/ Column	1/4	2/4	3/4	4/4
C1	Load	Wash/ Elution/ Equilibration	Loopback 1	Loopback 2
C2	Loopback 2	Load	Wash/ Elution/ Equilibration	Loopback 1
C3	Loopback 1	Loopback 2	Load	Wash/ Elution/ Equilibration
C4	Wash/ Elution/Equilibration	Loopback 1	Loopback 2	Load

- 5 The use of two loopbacks yielded a recovery rate of 93 % with a dilution factor of 9 %.

Example 2: Continuous capture of IgG with double wash step

- 10 In a further example an affinity chromatography resin POROS® CaptureSelect® FcXP affinity resin (Thermo Fisher) was used to investigate the use of multiple feedback loops in a continuous chromatography setup. The continuous chromatography setup was as described for Example 1 above, with the addition of a second wash step in the column wash phase, as indicated below.

Chromatography phase	Buffer
Equilibration	20 mM NaH ₂ PO ₄ , 500 mM NaCl, pH 7.4
Column Wash	First wash

	10 mM NaH ₂ PO ₄ , 1 M NaCl, pH 7.4
	<u>Second wash</u>
	10 mM NaH ₂ PO ₄ , pH 6.8
Elution	20 mM acetic acid buffer, pH 4.0
Regeneration	20% EtOH and 20 mM NaOH

The second wash step was performed with a different wash buffer to the first wash step, having no salt and a lower conductivity. The use of a second wash step was found to aid in removal of salt from the column prior to the elution phase.

5

Clauses

The following statements describe certain examples according to the present disclosure.

- 10
- 15
- 20
- 25
- 30
- 1A.
- A method of isolating a protein-of-interest enriched solution and a plurality of protein-of-interest depleted fractions from a protein mixture comprising a protein-of-interest using a continuous chromatography system comprising at least four separation units, each separation unit comprising a respective chromatography medium capable of separating and retaining a portion of a protein-of-interest from a protein mixture, wherein the method comprises:
- performing at least one chromatography cycle comprising a series of cycle segments, wherein a cycle segment comprises:
- a. loading a feed comprising the protein mixture comprising the protein-of-interest onto a feed separation unit, wherein a portion of a protein-of-interest content of the feed is retained in the feed separation unit, thereby producing a feed flow through;
- b. directing the feed flow through from the feed separation unit onto a feed loopback separation unit via a feed flow through feedback loop, wherein at least a portion of a protein-of-interest content of the feed flow through is retained in the feed loopback separation unit, thereby producing a protein-of-interest depleted feed flow through fraction;
- c. loading a wash buffer onto a wash separation unit containing retained protein-of-interest from a previous cycle segment, thereby producing a wash flow through;
- d. directing the wash flow through from the wash separation unit onto a wash loopback separation unit via a wash flow through feedback loop, wherein a portion of a protein-of-interest content of the wash flow through is retained

- in the wash loopback separation unit, thereby producing a protein-of-interest depleted wash flow through fraction; and
- e. loading an elution buffer onto the wash separation unit and/or onto an elution separation unit to elute retained protein-of-interest from the respective chromatography medium, thereby producing a protein-of-interest enriched solution,
- wherein the feed flow through and the wash flow through are maintained separate from each other.
- 10 2A. The method of example 1A, wherein a function of one or more of the at least four separation units varies between successive cycle segments.
- 3A. The method of example 2A, wherein, within one chromatography cycle, the functions of the at least four separation units vary such that each separation unit performs phases of the chromatography cycle, the phases including: loading, washing, eluting, equilibrating, feed loopback, wash loopback, and/or combinations thereof.
- 15 4A. The method of example 2A or example 3A, wherein performing the chromatography cycle comprises loading the feed comprising the protein mixture onto each of the at least four separation units successively, wherein the loading of the feed onto each successive separation unit demarks a respective cycle segment.
- 20 5A. The method of example 4A, wherein loading the feed is performed substantially continuously across successive cycle segments such that a flow rate of the feed during the chromatography cycle is substantially constant.
- 25 6A. The method of any one of the preceding examples, wherein the number of cycle segments in one chromatography cycle is equal to the number of separation units.
- 30 7A. The method of any one of the preceding examples, wherein a load time taken to perform step a) is equal to a cycle time of one chromatography cycle divided by the number of separation units.

- 8A. The method of example 3A, wherein the functions of each separation unit vary such that each separation unit performs each of the phases in a predetermined sequence.
- 5 9A. The method of example 8A, wherein the function of each of the separation units varies based on the function performed by the respective separation unit in one or more preceding cycle segments.
- 10A. The method of any one of examples 1A to 9A, wherein each separation unit
10 comprises:
an inlet;
an outlet; and
a fluid flow path extending through the separation unit from the inlet to the outlet and in contact with the chromatography medium.
- 15 11A. The method of example 10A, wherein each inlet and/or each outlet is connected to a switching valve arrangement.
- 12A. The method of any one of examples 1A to 11A, comprising determining a protein
20 concentration of one or more of:
the feed flow-through;
the wash flow through;
the protein-of-interest depleted feed flow through fraction;
the protein-of-interest depleted wash flow through fraction; and/or
25 the protein-of-interest enriched solution.
- 13A. The method of example 12A, wherein determining the protein concentration comprises determining the protein concentration comprises performing a spectroscopic analysis, optionally wherein determining the protein concentration comprises one or
30 more of:
(a) performing absorbance spectroscopy;
(b) performing reflection spectroscopy;
(c) detecting an optical density (OD);
(d) performing Raman spectroscopy; and/or
35 (e) performing Near-infrared (NIR) spectroscopy.

- 14A. The method of example 13A, wherein detecting the optical density (OD) comprises detecting a UV absorption.
- 5 15A. The method of example 14A, comprising measuring UV absorption at one or more of 300 nm, 280 nm, 245 nm, 224 nm, and 214 nm.
- 10 16A. The method of any one of examples 1A to 15A, wherein the protein-of-interest depleted wash flow through fraction is further fractionated into a low protein concentration fraction and a high protein concentration fraction.
- 15 17A. The method of example 16A, wherein the further fractionation is based on a determined protein concentration of the protein-of-interest depleted wash flow through fraction and/or a volume of wash buffer passed over the chromatography media.
- 20 18A. The method of example 16A or example 17A, wherein the method further comprises pooling the protein-of-interest depleted feed flow through fraction and the high protein concentration fraction from the further fractionated protein-of-interest depleted wash flow through fraction to produce a protein-of-interest depleted preparation.
- 25 19A. The method of any one of examples 1A to 18A, wherein the continuous chromatography system is selected from the group consisting of simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC).
- 30 20A. The method of any one of examples 1A to 19A, wherein one or more of the chromatography media is selected from the group consisting of an ion exchange chromatography medium, an affinity chromatography medium, a hydrophobic interaction chromatography medium, a mixed mode chromatography medium, an adsorption chromatography medium and a partition chromatography medium.
- 35 21A. The method of example 20A, wherein the ion exchange chromatography medium is an anion exchange chromatography medium or a cation exchange chromatography medium.

- 22A. The method of any one of examples 1A to 21A, wherein one or more of the chromatography media is selected from the group consisting of a membrane, a monolith and a resin.
- 23A. The method of any one of examples 1A to 22A, wherein one or more of the chromatography media comprise a ligand capable of specifically binding the protein-of-interest.
- 24A. The method of any one of examples 1A to 23A, wherein one or more of the chromatography media comprise a ligand capable of specifically binding human IgG, for example to a constant domain of human IgG, for example CH1, CH2, CH3, CH4 or combinations thereof.
- 25A. The method of example 24A, wherein the ligand comprises a camelid-derived single domain [VHH] antibody fragment.
- 26A. The method of example 25A, wherein the ligand comprises:
 an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;
 a framework region comprising an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;
 an amino acid sequence that comprises framework regions (FR)1, 2, 3 and 4, and complementarity determining regions (CDRs)1, 2 and 3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:
- a. the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
 - b. the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
 - c. the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1, and
 - d. wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1, optionally wherein the antigen binding protein specifically binds to the Fc domain of a

human IgG molecule and does not bind to an IgG molecule of murine origin or bovine origin.

- 27A. The method of any one of examples 1A to 26A, wherein one or more of the chromatography media comprise a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix.
- 28A. The method of any one of examples 23A to 27A, wherein one or more of the chromatography media comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix.
- 29A. The method of any one of examples 21A to 28A, wherein one or more of the separation units comprises an affinity chromatography medium and one or more of the separation units comprises an ion exchange chromatography medium.
- 30A. The method of example 29A, wherein the affinity chromatography medium comprises a ligand capable of specifically binding to a human IgG, for example to a constant domain of human IgG, for example CH1, CH2, CH3, CH4 or combinations thereof.
- 31A. The method of any one of examples 1A to 30A, wherein one or more of the separation units is loaded with the feed comprising the protein-of-interest at a concentration above a dynamic binding capacity (DBC) of the chromatography medium of the respective separation unit.
- 32A. The method of example 31A, wherein the DBC of at least one chromatography medium is at least 5 mg protein-of-interest per mL of the chromatography medium.
- 33A. The method of example 31A or 32A, wherein one or more of the separation units is loaded with the feed comprising the protein-of-interest at a concentration up to the DBC of the chromatography medium.
- 34A. The method of example 33A, wherein the DBC of one or more of the separation units is loaded with the protein-of-interest at a concentration of up to 40 mg protein-of-interest per mL of the chromatography medium.

- 35A. The method of any one of examples 1A to 34A, wherein the chromatography media have a total bed height of between 2 cm and 30 cm.
- 36A. The method of any one of examples 1A to 35A, wherein one or more of the separation units are selected from the group consisting of a column, a cassette, a capsule and a filter holder.
- 37A. The method of example 36A, wherein each separation unit is a column.
- 38A. The method of example 37A, wherein each column has a diameter of between 5 cm and 200 cm.
- 39A. The method of any one of examples 1A to 38A, wherein the wash buffer has a pH of between 5 and 10 and a dissociation constant (pKa) between 6.8 and 8.5 at 25°C.
- 40A. The method of any one of examples 1A to 39A, wherein the wash buffer comprises a buffering agent selected from a group consisting of sodium dihydrogen phosphate, imidazole, Tris, glycylglycine, 3-morpholinopropane-1-sulfonic acid (MOPS), piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES), bis[(2-hydroxyethyl)amino]acetic acid (Bicine), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sulfurous acid, 4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS), N-(Hydroxyethyl)piperazine-N'-2-hydroxypropanesulfonic acid (HEPPSO), 4-(N-Morpholino)butanesulfonic acid (MOBS), Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) (POPSO), N-[Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid (TAPSO), Tricine, triethanolamine (TEA) and combinations thereof.
- 41A. The method of example 40A, wherein the buffering agent is at a concentration of between 5 mM to 200 mM.
- 42A. The method of any one of examples 1A to 41A, wherein the wash buffer further comprises sodium chloride and/or a divalent salt at a concentration of up to 1000 mM.

- 43A. The method of any one of examples 1A to 42A, wherein the wash buffer comprises 20 mM sodium dihydrogen phosphate, 500 mM sodium chloride and is at a pH of 7.4.
- 5 44A. The method of any one of examples 1A to 43A, wherein the elution buffer has a pH of between 3 and 5.
- 45A. The method of any one of examples 1A to 44A, wherein the elution buffer is or comprises a phosphate buffer, an acetate buffer or an acetic acid buffer.
- 10 46A. The method of any one of examples 1A to 45A, wherein the elution buffer comprises 20mM phosphate buffer, acetate buffer or acetic acid buffer having a pH of between 3 and 5.
- 15 47A. The method of any one of examples 1A to 46A, wherein the elution buffer contacts the chromatography medium for up to 5 minutes.
- 48A. The method of any one of examples 1A to 47A, wherein the method further comprises equilibrating at least one chromatography medium with an
20 equilibration buffer.
- 49A. The method of example 48A, comprising loading the equilibration buffer onto the wash separation unit or onto the elution separation unit after loading the elution
25 buffer.
- 50A. The method of example 49A, wherein the equilibration buffer has a pH of between 7 and 8.
- 51A. The method of example 49A or 50A, wherein the equilibration buffer is at a pH
30 of 7.4.
- 52A. The method of any one of examples 49A to 51A, wherein the equilibration buffer comprises 20 mM sodium dihydrogen phosphate, 1000 mM sodium chloride and is at a pH of 7.4.
- 35

- 53A. The method of any one of examples 1A to 52A, wherein the method further comprises regenerating one or more of the chromatography media.
- 54A. The method of any one of examples 1A to 50A, wherein the protein-of-interest is
5 a plasma protein.
- 55A. The method of example 54A, wherein the protein-of-interest is a plasma protein selected from the group consisting of immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1-
10 antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, a coagulation factor, a serine protease inhibitor
15 and combinations thereof.
- 56A. The method of any one of examples 1A to 55A, wherein the protein-of-interest is immunoglobulin G (IgG).
- 20 57A. The method of any one of examples 1A to 56A, wherein the protein-of-interest is an albumin.
- 58A. The method of any one of examples 1A to 57A, wherein the protein mixture is plasma or a plasma fraction.
25
- 59A. The method of example 58A, wherein the plasma fraction is selected from a group consisting of cryo-rich plasma, cryo-poor plasma, Supernatant I (SN I), Cohn Fraction II (Fr II), Cohn Fraction II+III (Fr II+III), Cohn Fraction I+II+III (FrI+II+III), Kistler/Nitschmann Precipitate A (KN A), Kistler/Nitschmann
30 Precipitate B (KN B), Kistler/Nitschmann Precipitate of Supernatant B (KN B+1), and combinations thereof.
- 60A. The method of example 58A or 59A, wherein the plasma or plasma fraction is thawed at a temperature of at least 32°C.
35

- 61A. The method of any one of examples 58A to 60A, wherein the plasma or plasma fraction is at a temperature in the range of from 2°C to 28°C before loading.
- 5 62A. The method of example 61A, wherein the plasma or plasma fraction is at a temperature of 21°C before loading.
- 63A. The method of any one of examples 1A to 62A, wherein at least 75% of the protein-of-interest is recovered from the protein mixture.
- 10 64A. The method of any one of examples 55A to 63A, wherein at least 75% of IgG is recovered from the plasma or fraction thereof.
- 65A. The method of any one of examples 56A to 64A, wherein the protein-of-interest enriched solution comprises a purity of at least 95% IgG.
- 15 66A. The method of any one of examples 56A to 65, wherein the protein-of-interest enriched solution comprises polyvalent IgG.
- 67A. The method of any one of examples 56A to 66A, wherein the IgG is further purified from the protein-of-interest enriched solution.
- 20 68A. The method of example 67A, wherein the IgG is further purified from the protein-of-interest enriched solution by one or more purification steps selected from the group consisting of: precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation; chromatography; viral inactivation; viral filtration; ultrafiltration/diafiltration and combinations thereof.
- 25 69A. The method of any one of examples 1A to 68A, wherein the method further comprises formulating the protein-of-interest enriched solution into a pharmaceutical composition.
- 30 70A. The method of any one of examples 18A to 62A, wherein the protein-of-interest depleted preparation comprises one or more plasma protein products.

- 71A. The method of example 70A, wherein the method further comprises purifying one or more of the plasma protein products from the protein-of-interest depleted preparation.
- 5 72A. The method of example 70A or 71A, wherein the one or more plasma protein products is further purified from the protein-of-interest depleted preparation by one or more purification steps selected from the group consisting of precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation; chromatography; viral inactivation; viral filtration; ultrafiltration/diafiltration and combinations thereof.
- 10
- 73A. The method of example 72A, wherein the one or more plasma protein products is further purified from the protein-of-interest depleted preparation using a continuous chromatography system.
- 15
- 74A. The method of any one of examples 70A to 73A, wherein the one or more plasma protein products purified from the protein-of-interest depleted preparation is selected from a group consisting of immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1-antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof.
- 20
- 25
- 75A. The method of example 74A, wherein the one or more plasma protein products purified from the protein-of-interest depleted preparation is an albumin.
- 30 76A. The method of any one of examples 74A to 75A, wherein the method further comprises formulating the purified plasma protein products from the protein-of-interest depleted preparation into a pharmaceutical composition.
- 77A. A pharmaceutical composition comprising a protein-of-interest purified or produced by a method of any one of examples 1A to 69A.
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- 78A. A pharmaceutical composition comprising IgG purified or produced as a protein-of-interest by a method of any one of examples 1A to 69A.
- 5 79A. The pharmaceutical composition of example 77A or 78A, wherein the pharmaceutical composition comprises:
10% (w/v) IgG; or
20% (w/v) IgG.
- 10 80A. The pharmaceutical composition of example 78A or 79A, wherein the IgG is polyvalent IgG.
- 81A. The pharmaceutical composition of any one of examples 77A to 80A, wherein the pharmaceutical composition comprises a purity of at least 98% IgG.
- 15 82A. The pharmaceutical composition of any one of examples 77A to 81A, wherein the pharmaceutical composition comprises a nominal osmolality of 320 mOsm/kg.
- 83A. The pharmaceutical composition of any one of examples 77A to 82A, wherein the pharmaceutical composition comprises a pH of between 4.6 and 5.0.
- 20 84A. The pharmaceutical composition of any one of examples 77A to 83A, wherein the pharmaceutical composition comprises a pH of 4.8.
- 85A. The pharmaceutical composition of any one of examples 77A to 84A, wherein the pharmaceutical composition further comprises 250 mmol/L of L-proline.
- 25 86A. The pharmaceutical composition of any one of examples 77A to 85A, wherein the pharmaceutical composition comprises a sodium content of ≤ 1 mmol/L.
- 30 87A. The pharmaceutical composition of any one of examples 77A to 86A, wherein the pharmaceutical composition comprises an IgA content of ≤ 0.05 mg/mL.
- 88A. The pharmaceutical composition of any one of examples 77A to 87A, wherein the pharmaceutical composition comprises a Prekallikrein activator (PKA) level of $\leq$
- 35 35 IU/mL.

- 89A. The pharmaceutical composition of any one of examples 77A to 88A, wherein the pharmaceutical composition comprises 10% (w/v) polyvalent IgG, 250 mM proline and a pH of 4.8.
- 5 90A. The pharmaceutical composition of any one of examples 77A to 89A, wherein the pharmaceutical composition comprises 20% (w/v) polyvalent IgG, 250 mM proline, 20 µg/mL PS80 and a pH of 4.8.
- 10 91A. A pharmaceutical composition comprising one or more plasma protein products from the protein-of-interest depleted preparation purified or produced by a method of any one of examples 18A to 76A.
- 15 92A. The pharmaceutical composition of example 91A, wherein the plasma protein product is selected from a group consisting of an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor 20 VIII and Factor IX and combinations thereof.
- 25 93A. Use of IgG purified or produced as a protein-of-interest by a method of any one of examples 1A to 76A or the one or more plasma protein products from the protein-of-interest depleted preparation purified or produced by a method of any one of examples 18A to 76A in the manufacture of a medicament for treating, preventing and/or delaying progression of a condition in a subject.
- 30 94A. The pharmaceutical composition of any one of examples 77A to 91A, for use in treating, preventing and/or delaying progression of a condition in a subject.
- 95A. A method of treating, preventing and/or delaying progression of a condition in a subject, the method comprising administering the pharmaceutical composition of any one of examples 77A to 92A to the subject.
- 35 96A. The use of example 93A, or the pharmaceutical composition of example 94A, or the method of example 95A, wherein the condition is selected from a group

consisting of immune conditions, particular autoimmune diseases and certain neurological diseases. These conditions include Rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), Antiphospholipid syndrome, immune thrombocytopenia (ITP), Kawasaki disease, Guillain Barre syndrome (GBS),
 5 multiple sclerosis (MS), chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), myasthenia gravis (MG), skin blistering diseases, scleroderma, Dermatomyositis, Polymyositis, Alzheimer's Disease, Parkinson's Disease, Alzheimer's Disease related to Downs Syndrome, cerebral amyloid angiopathy, Dementia with Lewy bodies, Fronto- temporal lobar
 10 degeneration, vascular dementia, cell and organ transplant and combinations thereof.

97A. A continuous chromatography system for separating a protein-of-interest from a protein mixture, wherein the system comprises:
 15 at least four separation units, each separation unit comprising:
 an inlet;
 an outlet;
 a fluid flow path extending through the separation unit from the inlet to the outlet; and
 20 a chromatography medium in contact with the fluid flow path and capable of separating at least a portion of the protein-of-interest from the protein mixture;
 a switching valve arrangement configured to:
 selectively direct flow of one or more of a feed comprising the protein mixture, a wash buffer and an eluting agent to the inlet of any one of the separation units;
 25 selectively direct flow from the outlet of each separation unit to:
 at least one feedback loop in fluid communication with the inlet of another one of the separation units;
 at least one collection flow path; and
 a waste flow path; and
 30 a valve control system operable to control the switching valve arrangement, wherein the valve control system is configured to operate the switching valve arrangement such that:
 the feed is directed to the inlet of a selected first separation unit;
 the wash buffer is directed to the inlet of a selected second separation unit;
 35 a feed flow through from the outlet of the first separation unit and a wash flow through from the outlet of the second separation unit are directed to respective

feedback loops, wherein the feed flow through and the wash flow through are maintained separate from each other.

5 98A. The system of example 97A, wherein the switching valve arrangement is configured to direct flow from the outlet of each separation unit to a selected one of two feedback loops in fluid communication respectively with the inlets of two other separation units.

10 99A. The system of example 97A or example 98A, wherein the at least one collection flow path comprises one or more of:

- a protein-of-interest enriched solution collection flow path;
- a protein-of-interest depleted feed flow through collection flow path; and
- a protein-of-interest depleted wash flow through collection flow path.

15 100A. The system of any one of examples 97A to 99A, wherein the valve control system is configured to operate the switching valve arrangement such that:

- the feed flow through from the outlet of the first separation unit is directed to the inlet of a third separation unit via a feed flow through feedback loop; and
- 20 the wash flow through from the outlet of the second separation unit is directed to the inlet of a fourth separation unit via a wash flow through feedback loop.

25 101A. The system of example 100A wherein the valve control system is configured to operate the switching valve arrangement such that a protein-of-interest depleted feed from the outlet of the third separation unit is directed to the at least one collection flow path.

30 102A. The system of example 100A or example 101A wherein the valve control system is configured to operate the switching valve arrangement such that protein-of-interest depleted wash flow through is directed from the outlet of the fourth separation unit to the at least one collection flow path and/or to the waste flow path.

35 103A. The system of any one of examples 97A to example 102A, wherein the valve control system is configured to operate the switching valve arrangement to:
direct flow of an elution buffer to the inlet of at least one selected separation unit; and

direct flow of a protein-of-interest enriched solution from the outlet of the at least one separation unit selected to receive the elution buffer to the at least one collection flow path collection flow path.

- 5 104A. The system of any one of examples 97A to 103A, further comprising a protein sensor connected to each outlet of the separation units.
- 105A. The system of example 104A, wherein the protein sensor is configured to determine a protein concentration in a flow from the outlet.
- 10 106A. The system of example 105A, wherein the protein sensor comprises one or more of: an optical absorbance detector; a Raman spectrometer; and a near-infrared spectrometer.
- 15 107A. The system of example 106A, wherein the optical absorbance detector is configured to detect an optical density (OD) of the flow.
- 108A. The system of example 106A or 107A, wherein the optical absorbance detector is configured to detect a UV absorption of the flow.
- 20 109A. The system of example 108A, wherein the optical absorbance detector is configured to measure UV absorption at one or more of 300 nm, 280 nm, 245 nm, 224 nm, and 214 nm.
- 25 110A. The system of any one of examples 97A to 109A, further comprising a pressure sensor connected to each inlet of the separation units.
- 111A. The system of any one of examples 97A to 110A, wherein the chromatography system is selected from the group consisting of: simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC).
- 30 112A. The system of any one of examples 97A to 111A, wherein one or more of the chromatography media is selected from the group consisting of an ion exchange chromatography medium, an affinity chromatography medium, a hydrophobic interaction chromatography medium, a mixed mode chromatography medium, an adsorption chromatography medium, and a partition chromatography medium.
- 35

- 113A. The system of examples 112A, wherein the ion exchange chromatography medium is an anion exchange chromatography medium or a cation exchange chromatography medium.
- 5
- 114A. The system of any one of examples 97A to 113A, wherein one or more of the chromatography media is selected from the group consisting of a membrane, a monolith and a resin.
- 10 115A. The system of any one of examples 97A to 114A, wherein one or more of the chromatography media comprise a ligand capable of specifically binding the protein-of-interest.
- 15 116A. The system of any one of examples 97A to 115A, wherein one or more of the chromatography media comprise a ligand capable of specifically binding to human IgG, for example to a constant domain of human IgG, for example .
- 20 117A. The system of example 116A, wherein the ligand comprises a camelid-derived single domain [VHH] antibody fragment.
- 118A. The system of example 115A, wherein the ligand comprises:
an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;
a framework region comprising an amino acid sequence set forth in SEQ ID
25 NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;
an amino acid sequence that comprises framework regions (FR)1, 2, 3 and 4, and complementarity determining regions (CDRs)1, 2 and 3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:
30 the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
35 the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the

- framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1, and
- wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1, optionally wherein
- 5 the antigen binding protein specifically binds to the Fc domain of a human IgG molecule and does not bind to an IgG molecule of murine origin or bovine origin.
- 119A. The system of any one of examples 97A to 118A, wherein one or more of the chromatography media comprise a matrix selected from the group consisting of a
- 10 cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix.
- 120A. The system of any one of examples 116A to 119A, wherein one or more of the chromatography media comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix.
- 15 121A. The system of any one of examples 112A to 120A, wherein the first chromatography medium comprises an affinity chromatography medium and the second chromatography medium comprises an ion exchange chromatography medium.
- 20 122A. The system of example 121A, wherein the first affinity chromatography medium comprises a ligand is capable of specifically binding to a human IgG, for example to a constant domain of human IgG, for example CH1, CH2, CH3, CH4 or combinations thereof.
- 25 123A. The system of any one of examples 97A to 122A, wherein the chromatography media have a total bed height of between 2 cm and 30 cm.
- 124A. The system of any one of examples 97A to 123A, wherein one or more of the
- 30 separation units are selected from the group consisting of a column, a cassette, a capsule and a filter holder.
- 125A. The system of example 124A, wherein each separation unit is a column.
- 35 126A. The system of example 125A, wherein each column has a diameter of between 5 cm and 200 cm.

The following statements describe further examples according to the present disclosure.

1B. A method of isolating a protein-of-interest enriched solution and a plurality of protein-of-interest depleted fractions from a protein mixture using continuous chromatography comprising at least four separation units, wherein the method
5 comprises:

(i) a first chromatography cycle between a first separation unit and a third separation unit comprising:

- 10 a. loading a protein mixture comprising a protein-of-interest through an inlet onto the first separation unit comprising a first chromatography medium capable of separating the protein-of-interest from the protein mixture, wherein at least some of the protein-of-interest from the protein mixture is retained by the first chromatography medium, thereby producing a first chromatography cycle post-load flow through from the first separation unit through an outlet;
- 15 b. feeding the first chromatography cycle post-load flow through from an outlet of the first separation unit through an inlet onto a third separation unit via a first chromatography cycle first feedback loop in fluid communication between the outlet of the first separation unit and the inlet of the third separation unit, wherein the third separation unit comprises a third
20 chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the third chromatography medium, thereby producing a first chromatography cycle first protein-of-interest depleted flow through fraction from the third separation unit through
25 an outlet;
- c. loading a wash buffer through the inlet onto the first separation unit, wherein loading the wash buffer onto the first separation unit produces a first chromatography cycle post-wash flow through from the first separation unit through the outlet;
- 30 d. feeding the first chromatography cycle post-wash flow through from the outlet of the first separation unit through the inlet onto the third separation unit via a first chromatography cycle second feedback loop; wherein at least some of the protein-of-interest from the post-wash flow through is retained by the third chromatography medium, thereby producing a first
35 chromatography cycle second protein-of-interest depleted flow through fraction from the third separation unit through the outlet;

- e. loading an elution buffer through the inlet onto the first separation unit, eluting the retained protein-of-interest from the first chromatography medium thereby producing a first chromatography cycle first protein-of-interest enriched solution from the first separation unit through the outlet;
5 and
- f. loading an elution buffer through the inlet onto the third separation unit, eluting the retained protein-of-interest from the third chromatography medium thereby producing a first chromatography cycle second protein-of-interest enriched solution from the third separation unit through the outlet;
10 and
- (ii) a second chromatography cycle between a second separation unit and a fourth separation unit comprising:
 - a. loading the protein mixture comprising the protein-of-interest through an inlet onto the second separation unit comprising a second chromatography medium capable of separating the protein-of-interest from the protein mixture, wherein at least some of the protein-of-interest from the protein mixture is retained by the second chromatography medium, thereby producing a second chromatography cycle post-load flow through from the second separation unit through an outlet;
15
 - b. feeding the second chromatography cycle post-load flow through from an outlet of the second separation unit through an inlet onto a fourth separation unit via a second chromatography cycle first feedback loop in fluid communication between the outlet of the second separation unit and the inlet of the fourth separation unit, wherein the fourth separation unit comprises a fourth chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the fourth chromatography medium, thereby producing a second chromatography cycle first protein-of-interest depleted flow through fraction from the fourth separation unit through an outlet;
20
25
30
 - c. loading a wash buffer through the inlet onto the second separation unit, wherein loading the wash buffer onto the second separation unit produces a second chromatography cycle post-wash flow through from the second separation unit through the outlet;
 - d. feeding the second chromatography cycle post-wash flow through from the outlet of the second separation unit through the inlet onto the fourth
35

- 5 separation unit via a second chromatography cycle second feedback loop;
wherein at least some of the protein-of-interest from the post-wash flow
through is retained by the fourth chromatography medium, thereby
producing a second chromatography cycle second protein-of-interest
depleted flow through fraction from the fourth separation unit through the
outlet;
- 10 e. loading an elution buffer through the inlet onto the second separation unit,
eluting the retained protein-of-interest from the second chromatography
medium thereby producing a second chromatography cycle first protein-of-
interest enriched solution from the second separation unit through the outlet;
and
- 15 f. loading an elution buffer through the inlet onto the fourth separation unit,
eluting the retained protein-of-interest from the fourth chromatography
medium thereby producing a second chromatography cycle second protein-
of-interest enriched solution from the fourth separation unit through the
outlet;
- wherein the method comprises repeating steps (i) and (ii) one or more times; and wherein
the first and second chromatography cycles operate independently and are discordant
such that the first chromatography cycle first feedback loop and the second
20 chromatography cycle second feedback loop operate in parallel.

- 2B. The method of example 1B, wherein the method further comprises
- (i) a third chromatography cycle between the third separation unit and the first
separation unit comprising:
- 25 a. loading a protein mixture comprising a protein-of-interest through an inlet
onto the third separation unit comprising the third chromatography medium
capable of separating the protein-of-interest from the protein mixture,
wherein at least some of the protein-of-interest from the protein mixture is
retained by the third chromatography medium, thereby producing a third
30 chromatography cycle post-load flow through from the third separation unit
through an outlet;
- b. feeding the third chromatography cycle post-load flow through from an
outlet of the third separation unit through an inlet onto the first separation
unit via a third chromatography cycle first feedback loop in fluid
35 communication between the outlet of the third separation unit and the inlet
of the first separation unit, wherein the first separation unit comprises a first

- chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the first chromatography medium, thereby producing a third chromatography cycle first protein-of-interest depleted flow through fraction from the first separation unit through an outlet;
- 5 c. loading a wash buffer through the inlet onto the third separation unit, wherein loading the wash buffer onto the third separation unit produces a third chromatography cycle post-wash flow through from the third separation unit through the outlet;
- 10 d. feeding the third chromatography cycle post-wash flow through from the outlet of the third separation unit through the inlet onto the first separation unit via a third chromatography cycle second feedback loop; wherein at least some of the protein-of-interest from the post-wash flow through is retained by the first chromatography medium, thereby producing a third chromatography cycle second protein-of-interest depleted flow through fraction from the first separation unit through the outlet;
- 15 e. loading an elution buffer through the inlet onto the third separation unit, eluting the retained protein-of-interest from the third chromatography medium thereby producing a third chromatography cycle first protein-of-interest enriched solution from the third separation unit through the outlet; and
- 20 f. loading an elution buffer through the inlet onto the first separation unit, eluting the retained protein-of-interest from the first chromatography medium thereby producing a third chromatography cycle second protein-of-interest enriched solution from the first separation unit through the outlet; and
- 25 (ii) a fourth chromatography cycle between a fourth separation unit and a second separation unit comprising:
- 30 a. loading the protein mixture comprising the protein-of-interest through an inlet onto the fourth separation unit comprising a fourth chromatography medium capable of separating the protein-of-interest from the protein mixture, wherein at least some of the protein-of-interest from the protein mixture is retained by the fourth chromatography medium, thereby producing a fourth chromatography cycle post-load flow through from the fourth separation unit through an outlet;
- 35

- 5 b. feeding the fourth chromatography cycle post-load flow through from an outlet of the fourth separation unit through an inlet onto a second separation unit via a fourth chromatography cycle first feedback loop in fluid communication between the outlet of the fourth separation unit and the inlet of the second separation unit, wherein the second separation unit comprises a second chromatography medium capable of separating the protein-of-interest from the post-load flow through; wherein at least some of the protein-of-interest from the post-load flow through is retained by the second chromatography medium, thereby producing a fourth chromatography cycle first protein-of-interest depleted flow through fraction from the second separation unit through an outlet;
- 10 c. loading a wash buffer through the inlet onto the fourth separation unit, wherein loading the wash buffer onto the fourth separation unit produces a fourth chromatography cycle post-wash flow through from the fourth separation unit through the outlet;
- 15 d. feeding the fourth chromatography cycle post-wash flow through from the outlet of the fourth separation unit through the inlet onto the second separation unit via a fourth chromatography cycle second feedback loop; wherein at least some of the protein-of-interest from the post-wash flow through is retained by the second chromatography medium, thereby producing a fourth chromatography cycle second protein-of-interest depleted flow through fraction from the second separation unit through the outlet;
- 20 e. loading an elution buffer through the inlet onto the fourth separation unit, eluting the retained protein-of-interest from the fourth chromatography medium thereby producing a fourth chromatography cycle first protein-of-interest enriched solution from the fourth separation unit through the outlet; and
- 25 f. loading an elution buffer through the inlet onto the second separation unit, eluting the retained protein-of-interest from the second chromatography medium thereby producing a fourth chromatography cycle second protein-of-interest enriched solution from the second separation unit through the outlet;
- 30 wherein the method comprises repeating steps (i) and (ii) one or more times; and wherein the third and fourth chromatography cycles operate independently and are discordant such that the third chromatography cycle first feedback loop and the fourth chromatography cycle second feedback loop operate in parallel.

- 3B. The method of example 1B or 2B, wherein the method comprises:
- (a) sequentially cycling through the first chromatography cycle between the first separation unit and the third separation unit; and the third chromatography cycle between the third separation unit and the first separation unit; and
 - 5 (b) sequentially cycling through the second chromatography cycle between the second separation unit and the fourth separation unit; and the fourth chromatography cycle between the fourth separation unit and the second separation unit;
- wherein the method comprises repeating steps (a) and (b) one or more times.
- 10 4B. The method of any one of examples 1B to 3B, wherein each inlet and/or each outlet is connected to a switching valve arrangement.
- 5B. The method of example 4B, wherein the switching valve arrangement permits
- 15 switching between different phases on the separation unit.
- 6B. The method of example 5B, wherein the different phases are selected from the group consisting of loading, washing, eluting, equilibration, first loopback, second loopback and combinations thereof.
- 20 7B. The method of any one of examples 1B to 6B, wherein a protein sensor is connected to each outlet of the separation units.
- 8B. The method of example 9B, wherein the protein sensor detects the protein
- 25 concentration in the post-load flow-through, the post-wash flow through, the first protein-of-interest depleted flow through fraction, the second protein-of-interest depleted flow through fraction and/or the protein-of-interest enriched solution.
- 9B. The method of example 7B or 8B, wherein the protein sensor is an optical
- 30 absorbance detector.
- 10B. The method of example 8B or 9B, wherein detecting the protein concentration comprises determining the optical density (OD).
- 35 11B. The method of example 9B or 10B, wherein the optical absorbance detector detects UV absorption.

12B. The method of example 11B, wherein UV absorption is measured at one or more of 300 nm, 280 nm, 245 nm, 224 nm, and 214 nm.

13B. The method of any one of examples 1B to 12B, wherein the first and/or second
5 and/or third and/or fourth chromatography cycle second protein-of-interest depleted flow through fraction is further fractionated into a low protein concentration fraction and a high protein concentration fraction.

14B. The method of example 13B, wherein the second protein depleted flow through
10 fraction is fractionated into the low protein concentration fraction and the high protein concentration fraction based on the protein concentration and/or the number of column volumes passed over the chromatography media.

15B. The method of examples 13B or 14B, wherein the method further comprises
15 pooling the first protein-of-interest depleted flow through fraction(s) and the high protein concentration fraction(s) from the second protein-of-interest depleted flow through fraction(s) to produce a protein-of-interest depleted preparation.

16B. The method of any one of examples 1B to 15B, wherein the method further
20 comprises pooling the protein-of-interest enriched solution(s) to produce a protein-of-interest enriched preparation.

17B. The method of any one of examples 1B to 16B, wherein the continuous
25 chromatography system is selected from the group consisting of simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC).

18B. The method of any one of examples 1B to 17B, wherein one or more of the
30 chromatography media is selected from the group consisting of an ion exchange chromatography medium, an affinity chromatography medium, a hydrophobic interaction chromatography medium, a mixed mode chromatography medium, an adsorption chromatography medium and a partition chromatography medium.

19B. The method of example 18B, wherein the ion exchange chromatography medium
35 is an anion exchange chromatography medium or a cation exchange chromatography medium.

- 20B. The method of any one of examples 1B to 19B, wherein one or more of the chromatography media is selected from the group consisting of a membrane, a monolith and a resin.
- 5 21B. The method of any one of examples 1B to 20B, wherein one or more of the chromatography media comprise a ligand capable of specifically binding the protein-of-interest.
- 10 22B. The method of example 21B, wherein the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand capable of specifically binding the protein-of-interest.
- 23B. The method of any one of examples 1B to 22B, wherein one or more of the chromatography media comprise a ligand capable of specifically binding to a CH3
15 domain of human IgG.
- 24B. The method of example 23B, wherein the ligand comprises a camelid-derived single domain [VHH] antibody fragment.
- 20 25B. The method of example 24B, wherein the ligand comprises:
- (a) an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;
 - (b) a framework region comprising an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set
25 forth in SEQ ID NO: 1;
 - (c) an amino acid sequence that comprises framework regions (FR)1, 2, 3 and 4, and complementarity determining regions (CDRs)1, 2 and 3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:
 - i. the CDR1 has an amino acid sequence selected from the group consisting
30 of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
 - ii. the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
 - iii. the CDR3 has an amino acid sequence having at least 80% sequence identity
35 with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the

- framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1, and
- iv. wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1, optionally wherein the antigen binding protein specifically binds to the Fc domain of a human IgG molecule and does not bind to an IgG molecule of murine origin or bovine origin.
- 26B. The method of any one of examples 1B to 25B, wherein one or more of the chromatography media comprise a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix.
- 27B. The method of any one of examples 23B to 26B, wherein one or more of the chromatography media comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix.
- 28B. The method of any one of examples 18B to 27B, wherein the first separation unit comprises an affinity chromatography medium and the third separation unit comprises an ion exchange chromatography medium.
- 29B. The method of example 28B, wherein the first separation unit affinity chromatography medium comprises a ligand capable of specifically binding to a CH3 domain of human IgG.
- 30B. The method of any one of examples 1B to 29B wherein the first and/or second separation unit is loaded with the protein mixture at a concentration above a dynamic binding capacity (DBC) of the first chromatography medium.
- 31B. The method of example 30B, wherein the DBC of the first chromatography medium is at least 5 mg protein-of-interest per mL of the chromatography medium.
- 32B. The method of example 30B or 31B, wherein the first separation unit and one or more additional separation units are loaded with the protein-of-interest at a concentration up to the DBC of the chromatography medium.

- 33B. The method of example 32B, wherein the DBC of the first separation unit and one or more additional separation units are loaded with the protein-of-interest at a concentration of up to 40 mg protein-of-interest per mL of the chromatography medium.
- 5 34B. The method of any one of examples 1B to 33B, wherein the chromatography media have a total bed height of between 2 cm and 30 cm.
- 35B. The method of any one of examples 1B to 34, wherein one or more of the separation units are selected from the group consisting of a column, a cassette, a capsule and a filter holder.
- 10 36B. The method of example 35B, wherein each separation unit is a column.
- 37B. The method of example 36B, wherein the column has a diameter of between 5 cm and 200 cm.
- 15 38B. The method of any one of examples 1B to 37B, wherein the wash buffer has a pH of between 5 and 10 and a dissociation constant (pKa) between 6.8 and 8.5 at 25°C.
- 20 39B. The method of any one of examples 1B to 35B, wherein the wash buffer comprises a buffering agent selected from a group consisting of sodium dihydrogen phosphate, imidazole, Tris, glycylglycine, 3-morpholinopropane-1-sulfonic acid (MOPS), piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES), bis[(2-hydroxyethyl)amino]acetic acid (Bicine), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sulfurous acid, 4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS), N-(Hydroxyethyl)piperazine-N'-2-hydroxypropanesulfonic acid (HEPPSO), 4-(N-Morpholino)butanesulfonic acid (MOBS), Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) (POPSO), N-
- 25 [Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid (TAPSO), Tricine, triethanolamine (TEA) and combinations thereof.
- 30 40B. The method of example 39B, wherein the buffering agent is at a concentration of between 5 mM to 200 mM.
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- 41B. The method of any one of examples 1B to 40B, wherein the wash buffer further comprises sodium chloride and/or a divalent salt at a concentration of up to 1000 mM.
- 42B. The method of any one of examples 1B to 41B, wherein the wash buffer comprises
5 20 mM sodium dihydrogen phosphate, 500 mM sodium chloride and is at a pH of 7.4.
- 43B. The method of any one of examples 1B to 42B, wherein the elution buffer has a pH of between 3 and 5.
- 10 44B. The method of any one of examples 1B to 43B, wherein the elution buffer is or comprises a phosphate buffer, an acetate buffer or an acetic acid buffer.
- 45B. The method of any one of examples 1B to 44B, wherein the elution buffer comprises 20mM phosphate buffer, acetate buffer or acetic acid buffer having a pH of
15 between 3 and 5.
- 46B. The method of any one of examples 1B to 45B, wherein the elution buffer contacts the chromatography medium for up to 5 minutes.
- 20 47B. The method of any one of examples 1B to 46B, wherein the method further comprises equilibrating the first chromatography medium before loading the protein mixture with an equilibration buffer.
- 48B. The method of example 47B, wherein the equilibration buffer has a pH of between
25 7 and 8.
- 49B. The method of example 47B or 48B, wherein the equilibration buffer is at a pH of 7.4.
- 30 50B. The method of any one of examples 47B to 49B, wherein the equilibration buffer comprises 20 mM sodium dihydrogen phosphate, 1000 mM sodium chloride and is at a pH of 7.4.
- 51B. The method of any one of examples 1B to 50B, wherein the method further
35 comprises regenerating one or more of the chromatography media.

- 52B. The method of any one of examples 1B to 48B, wherein the protein-of-interest is a plasma protein.
- 53B. The method of example 52B, wherein the protein-of-interest is a plasma protein
5 selected from the group consisting of immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein
10 product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, a coagulation factor, a serine protease inhibitor and combinations thereof.
- 54B. The method of any one of examples 1B to 53B, wherein the protein-of-interest is immunoglobulin G (IgG).
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- 55B. The method of any one of examples 1B to 53B, wherein the protein-of-interest is an albumin.
- 56B. The method of any one of examples 1B to 55B, wherein the protein mixture is
20 plasma or a plasma fraction.
- 57B. The method of example 56B, wherein the plasma fraction is selected from a group consisting of cryo-rich plasma, cryo-poor plasma, Supernatant I (SN I), Cohn Fraction II (Fr II), Cohn Fraction II+III (Fr II+III), Cohn Fraction I+II+III (FrI+II+III),
25 Kistler/Nitschmann Precipitate A (KN A), Kistler/Nitschmann Precipitate B (KN B), Kistler/Nitschmann Precipitate of Supernatant B (KN B+1), and combinations thereof.
- 58B. The method of example 56B or 57B, wherein the plasma or plasma fraction is thawed at a temperature of at least 32°C.
30
- 59B. The method of any one of examples 56B to 58B, wherein the plasma or plasma fraction is at a temperature in the range of from 2°C to 28°C before loading onto the first chromatography medium and/or second chromatography medium.

- 60B. The method of example 59B, wherein the plasma or plasma fraction is at a temperature of 21°C before loading onto the first chromatography medium and/or second chromatography medium.
- 5 61B. The method of any one of examples 1B to 60B, wherein at least 75% of the protein-of-interest is recovered from the protein mixture.
- 62B. The method of any one of examples 53B to 61B, wherein at least 75% of IgG is recovered from the plasma or fraction thereof.
- 10 63B. The method of any one of examples 54B to 62B, wherein the protein-of-interest enriched solution comprises a purity of at least 95% IgG.
- 64B. The method of any one of examples 54B to 63B, wherein the protein-of-interest
15 enriched solution comprises polyvalent IgG.
- 65B. The method of any one of examples 54B to 64B, wherein the IgG is further purified from the protein-of-interest enriched solution.
- 20 66B. The method of example 65B, wherein the IgG is further purified from the protein-of-interest enriched solution by one or more purification steps selected from the group consisting of ethanol precipitation, octanoic acid fractionation, chromatography, viral inactivation, viral filtration, ultrafiltration/diafiltration and combinations thereof.
- 25 67B. The method of any one of examples 1B to 66B, wherein the method further comprises formulating the protein-of-interest enriched solution into a pharmaceutical composition.
- 68B. The method of any one of examples 15B to 60B, wherein the protein-of-interest
30 depleted preparation comprises one or more plasma protein products.
- 69B. The method of example 68B, wherein the method further comprises purifying one or more of the plasma protein products from the protein-of-interest depleted preparation.
- 35 70B. The method of example 68B or 69B, wherein the one or more plasma protein products is further purified from the protein-of-interest depleted preparation by one or

more purification steps selected from the group consisting of ethanol precipitation, octanoic acid fractionation, chromatography, viral inactivation, viral filtration, ultrafiltration/diafiltration and combinations thereof.

- 5 71B. The method of example 70B, wherein the one or more plasma protein products is further purified from the protein-of-interest depleted preparation using a continuous chromatography system.

- 72B. The method of any one of examples 68B to 71B, wherein the one or more plasma
10 protein products purified from the protein-of-interest depleted preparation is selected from a group consisting of immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1
15 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof.

- 20 73B. The method of example 72B, wherein the one or more plasma protein products purified from the protein-of-interest depleted preparation is an albumin.

- 74B. The method of any one of examples 69B to 73B, wherein the method further comprises formulating the purified plasma protein products from the protein-of-interest
25 depleted preparation into a pharmaceutical composition.

75B. A pharmaceutical composition comprising a protein-of-interest purified or produced by a method of any one of examples 1B to 67B.

- 30 76B. A pharmaceutical composition comprising IgG purified or produced as a protein-of-interest by a method of any one of examples 1B to 67B.

77B. The pharmaceutical composition of example 75B or 76B, wherein the pharmaceutical composition comprises:

- 35 (a) 10% (w/v) IgG; or
(b) 20% (w/v) IgG.

- 78B. The pharmaceutical composition of example 76B or 77B, wherein the IgG is polyvalent IgG.
- 79B. The pharmaceutical composition of any one of examples 75B to 78B, wherein the
5 pharmaceutical composition comprises a purity of at least 98% IgG.
- 80B. The pharmaceutical composition of any one of examples 75B to 79B, wherein the pharmaceutical composition comprises a nominal osmolality of 320 mOsm/kg.
- 10 81B. The pharmaceutical composition of any one of examples 75B to 80B, wherein the pharmaceutical composition comprises a pH of between 4.6 and 5.0.
- 82B. The pharmaceutical composition of any one of examples 75B to 81B, wherein the pharmaceutical composition comprises a pH of 4.8.
- 15 83B. The pharmaceutical composition of any one of examples 75B to 82B, wherein the pharmaceutical composition further comprises 250 mmol/L of L-proline.
- 84B. The pharmaceutical composition of any one of examples 75B to 83B, wherein the
20 pharmaceutical composition comprises a sodium content of ≤ 1 mmol/L.
- 85B. The pharmaceutical composition of any one of examples 75B to 84B, wherein the pharmaceutical composition comprises an IgA content of ≤ 0.05 mg/mL.
- 25 86B. The pharmaceutical composition of any one of examples 75B to 85B, wherein the pharmaceutical composition comprises a Prekallikrein activator (PKA) level of ≤ 35 IU/mL.
- 87B. The pharmaceutical composition of any one of examples 75B to 86B, wherein the
30 pharmaceutical composition comprises 10% (w/v) polyvalent IgG, 250 mM proline and a pH of 4.8.
- 88B. The pharmaceutical composition of any one of examples 75B to 87B, wherein the pharmaceutical composition comprises 20% (w/v) polyvalent IgG, 250 mM proline, 20
35 $\mu\text{g/mL}$ PS80 and a pH of 4.8.

- 89B. A pharmaceutical composition comprising one or more plasma protein products from the protein-of-interest depleted preparation purified or produced by a method of any one of examples 15B or 74B.
- 5 90B. The pharmaceutical composition of example 89B, wherein the plasma protein product is selected from a group consisting of an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof.
- 10 91B. Use of IgG purified or produced as a protein-of-interest by a method of any one of examples 1B to 74B or the one or more plasma protein products from the protein-of-interest depleted preparation purified or produced by a method of any one of examples 15 to 74 in the manufacture of a medicament for treating, preventing and/or delaying progression of a condition in a subject.
- 15 92B. The pharmaceutical composition of any one of examples 75B to 90B, for use in treating, preventing and/or delaying progression of a condition in a subject.
- 20 93B. A method of treating, preventing and/or delaying progression of a condition in a subject, the method comprising administering the pharmaceutical composition of any one of examples 75B to 90B to the subject.
- 25 94B. The use of example 91B, or the pharmaceutical composition of example 92B, or the method of example 93B, wherein the condition is selected from a group consisting of immune conditions, particular autoimmune diseases and certain neurological diseases.
- 30 These conditions include Rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), Antiphospholipid syndrome, immune thrombocytopenia (ITP), Kawasaki disease, Guillain Barre syndrome (GBS), multiple sclerosis (MS), chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), myasthenia gravis (MG), skin blistering diseases, scleroderma, Dermatomyositis, Polymyositis, Alzheimer's Disease, Parkinson's Disease, Alzheimer's Disease related to
- 35 Down's Syndrome, cerebral amyloid angiopathy, Dementia with Lewy bodies, Fronto-

temporal lobar degeneration, vascular dementia, cell and organ transplant and combinations thereof.

95B. A continuous chromatography system for separating a protein-of-interest from a protein mixture, wherein the system comprises:

- 5 a first separation unit comprising: a first chromatography medium capable of separating the protein-of-interest from the protein mixture; and a first separation unit inlet and a first separation unit outlet defining a flowpath through the first separation unit and in contact with at least a portion of the first chromatography medium;
- 10 a second separation unit comprising: a second chromatography medium capable of separating the protein-of-interest from the protein mixture; and a second separation unit inlet and a second separation unit outlet defining a flowpath through the second separation unit and in contact with at least a portion of the second chromatography medium;
- 15 a third separation unit comprising: a third chromatography medium capable of separating the protein-of-interest from the protein mixture; and a third separation unit inlet and a third separation unit outlet defining a flowpath through the third separation unit and in contact with at least a portion of the third chromatography medium;
- a fourth separation unit comprising: a fourth chromatography medium capable of separating the protein-of-interest from the protein mixture; and a fourth separation unit inlet and a fourth separation unit outlet defining a flowpath through the fourth separation unit and in contact with at least a portion of the fourth chromatography;
- 20 a switching valve arrangement operable to:
 - selectively direct flow of a feed of the protein mixture to be separated to the inlet
 - 25 of the first, second, third or fourth separation unit;
 - selectively direct flow of a wash buffer to the inlet of the first, second, third or fourth separation unit;
 - selectively direct flow from the first separation unit outlet to: the third separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath;
 - 30 selectively direct flow from the second separation unit outlet to: the fourth separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath;
 - selectively direct flow from the third separation unit outlet to: the first separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath;

selectively direct flow from the fourth separation unit outlet to: the second separation unit inlet, a wash buffer output flowpath, or protein-of-interest depleted feed flowpath; and

5 a valve control system configured to operate the switching valve arrangement to sequentially cycle through:

a first chromatography cycle in which:

a first chromatography cycle first feedback loop is defined in which the feed flows through the first and third separation units in series and to the protein-of-interest depleted feed flowpath, and

10 a first chromatography cycle second feedback loop is defined in which the wash buffer flows through the fourth and second separation units in series and to the wash buffer output flowpath; and

a second chromatography cycle in which:

15 a second chromatography cycle first feedback loop is defined in which the feed flows through the second and fourth separation units in series and to the protein-of-interest depleted feed flowpath, and

a second chromatography cycle second feedback loop is defined in which the wash buffer flows through the first and third separation units in series and to the wash buffer output flowpath.

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96B. A separation system according to example 95B, wherein the switching valve arrangement is further operable to:

selectively direct flow of an elution buffer to the inlet of the first, second, third or fourth separation units,

25 selectively direct flow from the first, second, third and fourth separation unit outlets to a protein-of-interest enriched solution flowpath,

wherein:

30 during the first chromatography cycle, the valve control system is further configured to operate the switching valve arrangement to direct flow of the elution buffer to the fourth separation unit inlet such that flow of the wash buffer can be discontinued to be replaced with flow of the elution buffer through the fourth and second separation units in series and to the protein-of-interest enriched solution flowpath;

35 during the second chromatography cycle, the valve control system is further configured to operate the switching valve arrangement to direct flow of the elution buffer to the first separation unit inlet such that flow of the wash buffer

can be discontinued to be replaced with flow of the elution buffer through the first and third separation units in series and to the protein-of-interest enriched solution flowpath.

- 5 97B. The system of example 95B or example 96B, wherein the valve control system is further configured to operate the switching valve arrangement to sequentially cycle through:

a third chromatography cycle in which:

- 10 a third chromatography cycle first feedback loop is defined in which the feed flows through the third and first separation units in series and to the protein-of-interest depleted feed flowpath, and

a third chromatography cycle second feedback loop is defined in which the wash buffer flows through the second and fourth separation units in series and to the wash buffer output flowpath; and

- 15 a fourth chromatography cycle in which:

a fourth chromatography cycle first feedback loop is defined in which the feed flows through the fourth and second separation units in series and to the protein-of-interest depleted feed flowpath, and

- 20 a fourth chromatography cycle second feedback loop is defined in which the wash buffer flows through the third and first separation units in series and to the wash buffer output flowpath.

- 98B. The system of example 97B, wherein:

- 25 during the third chromatography cycle, the valve control system is further configured to operate the switching valve arrangement to direct flow of the elution buffer to the second separation unit inlet such that flow of the wash buffer can be discontinued to be replaced with flow of the elution buffer through the second and fourth separation units in series and to the protein-of-interest enriched solution flowpath; and

- 30 during the fourth chromatography cycle, the valve control system is further configured to operate the switching valve arrangement to direct flow of the elution buffer to the third separation unit inlet such that flow of the wash buffer can be discontinued to be replaced with flow of the elution buffer through the third and first separation units in series and to the protein-of-interest enriched solution flowpath.
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99B. The system of any one of examples 95B to 98B, further comprising a protein sensor connected to each outlet of the separation units.

100B. The system of example 99B, wherein the protein sensor detects the protein
5 concentration in flow from the outlet.

101B. The system of example 99B or 100B, wherein the protein sensor is an optical absorbance detector.

10 102B. The system of example 101B, wherein detecting the protein concentration comprises determining the optical density (OD).

103B. The method of example 101B or 102B, wherein the optical absorbance detector detects UV absorption.

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104B. The method of example 103B, wherein UV absorption is measured at one or more of 300 nm, 280 nm, 245 nm, 224 nm, and 214 nm.

105B. The system of any one of examples 95B to 104B, further comprising a pressure
20 sensor connected to each inlet of the separation units.

106B. The system of any one of examples 95B to 105B, wherein the system is selected from the group consisting of simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC).

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107B. The system of any one of examples 95B to 106B, wherein one or more of the chromatography media is selected from the group consisting of an ion exchange chromatography medium, an affinity chromatography medium, a hydrophobic interaction chromatography medium, a mixed mode chromatography medium, an
30 adsorption chromatography medium, and a partition chromatography medium.

108B. The system of examples 107B, wherein the ion exchange chromatography medium is an anion exchange chromatography medium or a cation exchange chromatography medium.

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- 109B. The system of any one of examples 95B to 108B, wherein one or more of the chromatography media is selected from the group consisting of a membrane, a monolith and a resin.
- 5 110B. The system of any one of examples 95B to 109B, wherein one or more of the chromatography media comprise a ligand capable of specifically binding the protein-of-interest.
- 10 111B. The system of example 110B, wherein the first chromatography medium and the second chromatography medium, and optionally one or more additional chromatography media, comprise a ligand capable of specifically binding the protein-of-interest.
- 112B. The system of any one of examples 95B to 111B, wherein one or more of the chromatography media comprise a ligand capable of specifically binding to a CH3
- 15 domain of human IgG.
- 113B. The system of example 112B, wherein the ligand comprises a camelid-derived single domain [VHH] antibody fragment.
- 20 114B. The system of example 110B, wherein the ligand comprises:
- (a) an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;
 - (b) a framework region comprising an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;
 - 25 (c) an amino acid sequence that comprises framework regions (FR)1, 2, 3 and 4, and complementarity determining regions (CDRs)1, 2 and 3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:
 - i. the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
 - 30 ii. the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
 - iii. the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the
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framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1, and

iv. wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1, optionally wherein the antigen binding protein specifically binds to the Fc domain of a human IgG molecule and does not bind to an IgG molecule of murine origin or bovine origin.

115B. The system of any one of examples 95B to 114B, wherein one or more of the chromatography media comprise a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix.

116B. The system of any one of examples 112B to 115B, wherein one or more of the chromatography media comprise a ligand comprising a VHH antibody fragment conjugated to a cross-linked poly(styrene-divinylbenzene) matrix.

117B. The system of any one of examples 107B to 116B, wherein the first chromatography medium comprises an affinity chromatography medium and the second chromatography medium comprises an ion exchange chromatography medium.

118B. The system of example 117B, wherein the first affinity chromatography medium comprises a ligand is capable of specifically binding to a CH3 domain of human IgG.

119B. The system of any one of examples 95B to 118B, wherein the chromatography media have a total bed height of between 2 cm and 30 cm.

120B. The system of any one of examples 95B to 119B, wherein one or more of the separation units are selected from the group consisting of a column, a cassette, a capsule and a filter holder.

121B. The system of example 120B, wherein each separation unit is a column.

122B. The system of example 121B, wherein the column has a diameter of between 5 cm and 200 cm.

CLAIMS

1. A method of isolating a protein-of-interest enriched solution and a plurality of protein-of-interest depleted fractions from a protein mixture comprising a protein-of-interest using a continuous chromatography system comprising at least four separation units, each separation unit comprising a respective chromatography medium capable of separating and retaining a portion of a protein-of-interest from a protein mixture, wherein the method comprises:
performing at least one chromatography cycle comprising a series of cycle segments, wherein a cycle segment comprises:
 - a. loading a feed comprising the protein mixture comprising the protein-of-interest onto a feed separation unit, wherein a portion of a protein-of-interest content of the feed is retained in the feed separation unit, thereby producing a feed flow through;
 - b. directing the feed flow through from the feed separation unit onto a feed loopback separation unit via a feed flow through feedback loop, wherein at least a portion of a protein-of-interest content of the feed flow through is retained in the feed loopback separation unit, thereby producing a protein-of-interest depleted feed flow through fraction;
 - c. loading a wash buffer onto a wash separation unit containing retained protein-of-interest from a previous cycle segment, thereby producing a wash flow through;
 - d. directing the wash flow through from the wash separation unit onto a wash loopback separation unit via a wash flow through feedback loop, wherein a portion of a protein-of-interest content of the wash flow through is retained in the wash loopback separation unit, thereby producing a protein-of-interest depleted wash flow through fraction; and
 - e. loading an elution buffer onto the wash separation unit and/or onto an elution separation unit to elute retained protein-of-interest from the respective chromatography medium, thereby producing a protein-of-interest enriched solution,wherein the feed flow through and the wash flow through are maintained separate from each other.
2. The method of claim 1, wherein a function of one or more of the at least four separation units varies between successive cycle segments such that each separation unit performs phases of the chromatography cycle, the phases including: loading, washing, eluting, equilibrating, feed loopback, wash loopback, and/or combinations thereof, optionally wherein the functions of each separation unit vary such that each separation unit performs each of the phases in a predetermined sequence.

3. The method of claim 1 or claim 2, wherein performing the chromatography cycle comprises loading the feed comprising the protein mixture onto each of the at least four separation units successively, wherein the loading of the feed onto each successive separation unit demarks a respective cycle segment, optionally wherein loading the feed is performed substantially continuously across successive cycle segments such that a flow rate of the feed during the chromatography cycle is substantially constant.

4. The method of any one of claims 1 to 3, comprising determining a protein concentration of one or more of the feed flow-through, the wash flow through, the protein-of-interest depleted feed flow through fraction, the protein-of-interest depleted wash flow through fraction and the protein-of-interest enriched solution, optionally wherein determining the protein concentration comprises performing a spectroscopic analysis, optionally wherein determining the protein concentration comprises one or more of:

- (a) performing absorbance spectroscopy;
- (b) performing reflection spectroscopy;
- (c) detecting an optical density (OD), optionally wherein determining the protein concentration comprises detecting a UV absorption, preferably comprising measuring UV absorption at one or more of 300 nm, 280 nm, 245 nm, 224 nm, and 214 nm;
- (d) performing Raman spectroscopy; and/or
- (e) performing near-infrared (NIR) spectroscopy.

5. The method of any one of claims 1 to 4, wherein the protein-of-interest depleted wash flow through fraction is further fractionated into a low protein concentration fraction and a high protein concentration fraction, optionally wherein the further fractionation is based on a determined protein concentration of the protein-of-interest depleted wash flow through fraction and/or a volume of wash buffer passed over the chromatography media.

6. The method of claim 5, wherein the method further comprises pooling the protein-of-interest depleted feed flow through fraction and the high protein concentration fraction from the further fractionated protein-of-interest depleted wash flow through fraction to produce a protein-of-interest depleted preparation.

7. The method of any one of claim 6, wherein the protein-of-interest depleted preparation comprises one or more plasma protein products, for example immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1-antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated

clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof, optionally wherein the method further comprises purifying one or more of the plasma protein products from the protein-of-interest depleted preparation, for example, wherein the one or more plasma protein products is further purified from the protein-of-interest depleted preparation by one or more purification steps selected from the group consisting of precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation; chromatography; viral inactivation; viral filtration; ultrafiltration/diafiltration and combinations thereof, for example, wherein the one or more plasma protein products is further purified from the protein-of-interest depleted preparation using a continuous chromatography system.

8. The method of any one of claims 1 to 7, wherein the continuous chromatography system is selected from the group consisting of simulated moving bed (SMB) chromatography and periodic counter-current chromatography (PCC) and/or wherein one or more of the chromatography media is selected from the group consisting of an ion exchange chromatography medium, an affinity chromatography medium, a hydrophobic interaction chromatography medium, a mixed mode chromatography medium, an adsorption chromatography medium and a partition chromatography medium, wherein, for example:

- (a) the ion exchange chromatography medium is an anion exchange chromatography medium or a cation exchange chromatography medium;
- (b) one or more of the chromatography media is selected from the group consisting of a membrane, a monolith and a resin;
- (c) one or more of the chromatography media comprise a ligand capable of specifically binding the protein-of-interest.

9. The method of any one of claims 1 to 8, wherein one or more of the chromatography media comprise:

(A) a ligand capable of specifically binding to human IgG, optionally wherein the ligand is capable of specifically binding to a constant domain of human IgG, optionally wherein the ligand is capable of specifically binding to a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, or combinations thereof; for example, a camelid-derived single domain [VHH] antibody fragment, optionally wherein the ligand comprises:

- (a) an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1;

- (b) a framework region comprising an amino acid sequence set forth in SEQ ID NO: 1 or a sequence comprising at least 50% amino acid identity to a sequence set forth in SEQ ID NO: 1; or
- (c) an amino acid sequence that comprises framework regions (FR)1, 2, 3 and 4, and complementarity determining regions (CDRs)1, 2 and 3, that are operably linked in the order FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein:
 - i. the CDR1 has an amino acid sequence selected from the group consisting of SEQ ID No: 2 or an amino acid sequence that differs from SEQ ID NO: 2 in one or two of the amino acid residues;
 - ii. the CDR2 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 3; and,
 - iii. the CDR3 has an amino acid sequence having at least 80% sequence identity with an amino acid sequence of SEQ ID NO: 4; and, wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of any one of SEQ ID NO: 1, and
 - iv. wherein each of the framework regions has at least 50% amino acid identity with the framework amino acid sequence of SEQ ID NO: 1, optionally wherein the antigen binding protein specifically binds to the Fc domain of a human IgG molecule and does not bind to an IgG molecule of murine origin or bovine origin;

and/or

(B) a matrix selected from the group consisting of a cross-linked poly(styrene-divinylbenzene) matrix and an agarose-based matrix.

10. The method of claim 8 or claim 9, wherein one or more of the separation units comprises an affinity chromatography medium, for example a ligand capable of specifically binding to human IgG, optionally wherein the ligand is capable of specifically binding to a constant domain of human IgG, optionally wherein the ligand is capable of specifically binding to a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, or combinations thereof, and one or more of the separation units comprises an ion exchange chromatography medium.

11. The method of any one of claims 1 to 10, wherein:

- (a) one or more of the separation units is loaded with the protein mixture at a concentration above a dynamic binding capacity (DBC) of the chromatography medium;
- (b) one or more of the separation units are loaded with the protein-of-interest at a concentration up to the DBC of the chromatography medium;
- (c) the chromatography media have a total bed height of between 2 cm and 30 cm;

- (d) one or more of the separation units are selected from the group consisting of a column, a cassette, a capsule and a filter holder; for example, a column, such as a column with a diameter of between 5 cm and 200 cm;
 - (e) the wash buffer has a pH of between 5 and 10 and a dissociation constant (pKa) between 6.8 and 8.5 at 25°C, preferably wherein the wash buffer comprises a buffering agent selected from a group consisting of sodium dihydrogen phosphate, imidazole, Tris, glycylglycine, 3-morpholinopropane-1-sulfonic acid (MOPS), piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES), bis[(2-hydroxyethyl)amino]acetic acid (Bicine), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sulfurous acid, 4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS), N-(Hydroxyethyl)piperazine-N'-2-hydroxypropanesulfonic acid (HEPPSO), 4-(N-Morpholino)butanesulfonic acid (MOBS), Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) (POPSO), N-[Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid (TAPSO), Tricine, triethanolamine (TEA) and combinations thereof; and optionally wherein the buffering agent is at a concentration of between 5 mM to 200 mM;
 - (f) the wash buffer further comprises sodium chloride and/or a divalent salt at a concentration of up to 1000 mM, for example, 20 mM sodium dihydrogen phosphate, 500 mM sodium chloride and is at a pH of 7.4;
 - (g) the elution buffer has a pH of between 3 and 5, optionally wherein the elution buffer is or comprises a phosphate buffer, an acetate buffer or an acetic acid buffer;
 - (h) the elution buffer contacts the chromatography medium for up to 5 minutes;
 - (i) the method further comprises equilibrating at least one chromatography medium with an equilibration buffer before loading the protein mixture, optionally wherein the equilibration buffer has a pH of between 7 and 8, such as pH of 7.4, optionally wherein the equilibration buffer comprises 20 mM sodium dihydrogen phosphate, 1000 mM sodium chloride and is at a pH of 7.4; and/or
 - (j) the method further comprises regenerating one or more of the chromatography media.
12. The method of any one of claims 1 to 11, wherein:
- (a) the protein-of-interest is a plasma protein, for example a plasma protein selected from the group consisting of immunoglobulin G (IgG), an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product,

- alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H, a coagulation factor, a serine protease inhibitor and combinations thereof;
- (b) the protein mixture is plasma or a plasma fraction, for example, the plasma fraction is selected from a group consisting cryo-rich plasma, cryo-poor plasma, Supernatant I (SN I), Cohn Fraction II (Fr II), Cohn Fraction II+III (Fr II+III), Cohn Fraction I+II+III (Fr I+II+III), Kistler/Nitschmann Precipitate A (KN A), Kistler/Nitschmann Precipitate B (KN B), Kistler/Nitschmann Precipitate of Supernatant B (KN B+1), and combinations thereof, optionally wherein the plasma or plasma fraction is thawed at a temperature of at least 32°C, for example, the plasma or plasma fraction is at a temperature in the range of from 2°C to 28°C before loading, such as a temperature of 21°C;
- (c) at least 75% of the protein-of-interest is recovered from the protein mixture;
- (d) the protein-of-interest is IgG, and at least 75% of IgG is recovered from the plasma or fraction thereof;
- (e) the protein-of-interest is IgG, and the protein-of-interest enriched solution comprises a purity of at least 95% IgG;
- (f) the protein-of-interest is IgG, and the protein-of-interest enriched solution comprises polyvalent IgG; and/or
- (g) the protein-of-interest is IgG, and the IgG is further purified from the protein-of-interest enriched solution, for example, the IgG is further purified from the protein-of-interest enriched solution by one or more purification steps selected from the group consisting of precipitation, such as ethanol, ammonium sulphate and octanoic acid fractionation; chromatography; viral inactivation; viral filtration; ultrafiltration/diafiltration and combinations thereof.
13. A pharmaceutical composition comprising:
- (a) a protein-of-interest purified or produced by a method of any one of claims 1 to 12;
- (b) IgG purified or produced as a protein of interest by a method of any one of claims 1 to 12; or
- (c) one or more plasma protein products from the protein-of-interest depleted preparation purified or produced by a method of claims 6 or 7, for example wherein the plasma protein product is selected from a group consisting of an apolipoprotein A1, an albumin, a serine protease, a plasmin, plasminogen, a FXa, an alpha-1- antitrypsin, an IgA, an IgM, a factor VIII, a fibrinogen, a von Willebrand factor, an activated clotting factor, factor XIII, a contact system factor, a PKA, a factor IX, a prothrombin complex, a C1 esterase inhibitor, a protein C, an anti-thrombin III, a RhD immunoglobulin protein product, alpha acid glycoprotein, haptoglobin, hemopexin, transferrin, Factor H,

coagulation factors such as Factor VII, Factor VIII and Factor IX and combinations thereof.

14. The pharmaceutical composition of claim 13, for use in treating, preventing and/or delaying progression of a condition in a subject, such as a condition selected from a group consisting of immune conditions, particular autoimmune diseases and certain neurological diseases. These conditions include Rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), Antiphospholipid syndrome, immune thrombocytopenia (ITP), Kawasaki disease, Guillain Barre syndrome (GBS), multiple sclerosis (MS), chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), myasthenia gravis (MG), skin blistering diseases, scleroderma, Dermatomyositis, Polymyositis, Alzheimer's Disease, Parkinson's Disease, Alzheimer's Disease related to Downs Syndrome, cerebral amyloid angiopathy, Dementia with Lewy bodies, Fronto- temporal lobar degeneration, vascular dementia, cell and organ transplant and combinations thereof.

15. A continuous chromatography system for separating a protein-of-interest from a protein mixture, wherein the system comprises:

at least four separation units, each separation unit comprising:

an inlet;

an outlet;

a fluid flow path extending through the separation unit from the inlet to the outlet;

and

a chromatography medium in contact with the fluid flow path and capable of separating at least a portion of the protein-of-interest from the protein mixture;

a switching valve arrangement configured to:

selectively direct flow of one or more of a feed comprising the protein mixture, a wash buffer and an eluting agent to the inlet of any one of the separation units;

selectively direct flow from the outlet of each separation unit to:

at least one feedback loop in fluid communication with the inlet of another one of the separation units;

at least one collection flow path; and

a waste flow path; and

a valve control system operable to control the switching valve arrangement, wherein the valve control system is configured to operate the switching valve arrangement such that:

the feed is directed to the inlet of a selected first separation unit;

the wash buffer is directed to the inlet of a selected second separation unit;

a feed flow through from the outlet of the first separation unit and a wash flow

through from the outlet of the second separation unit are directed to respective feedback loops, wherein the feed flow through and the wash flow through are maintained separate from each other.