
1. A chromatography method of producing a target elution volume (238) comprising a first (P1) and a second (P2) target protein, the method comprising:
 - 5 - providing (100) a cation exchange chromatography column (230);
 - applying (102) a protein solution (202) on the column, the protein solution comprising at least the first target protein (P1) and the second target protein (P2) and optionally one or more further proteins (P3, P4, P5, P6);
 - inputting (106) an optimization criterion into a chromatography simulation
10 software (204), the optimization criterion being a desired property of the target elution volume in respect to the first and second target proteins comprised in the target elution volume;
 - computing (108), by the chromatography simulation software, an elution
15 buffer salt concentration (222) adapted to elute the first and the second target proteins from the chromatography column such that a target elution volume can be obtained that matches the optimization criterion best, the computation comprising computing a plurality of chromatography simulations as a function of multiple different elution buffer salt concentrations;
 - 20 - computing (110), by the chromatography simulation software, the pooling borders of the target elution volume as a function of at least the computed salt concentration and the input optimization criterion;
 - applying (112) an elution buffer (226) having the computed salt concentration on the chromatography column;
 - performing (114) the elution using the applied elution buffer; and
 - 25 - collecting (116) the computed target elution volume as a separate fraction using the computed pooling borders.

2. The chromatography method of claim 1, the optimization criterion being selected from a group comprising:
- a) a desired ratio or ratio range of the amounts of the first and of the second target proteins in the target elution volume;
 - 5 b) a desired amount or amount range of the first target protein in combination with a desired amount or amount range of the second target protein in the target elution volume;
 - c) a desired purity or purity range of the first target protein in combination with:
 - 10 ▪ a desired ratio of the amounts of the first and second protein in the target elution volume
 - a desired amount or amount range of the second target protein in the target elution volume;
 - a desired purity or purity range of the second target protein in
 - 15 the target elution volume;
 - d) a combination of two or more of the above-mentioned criteria.
3. The chromatography method of any one of the previous claims,
- 20 wherein the applied protein solution (202) comprises the one or more further proteins (P3,P4, P5, P6).
4. The chromatography method of any one of the previous claims,
- wherein the plurality of chromatography simulations are computed as a function of the multiple different elution buffer salt concentrations and as a function of multiple different elution buffer pH values, the method further comprising:
 - 25 - wherein the computing (108) comprises computing a combination of an elution buffer salt concentration (222) and an elution buffer pH value which in combination are adapted to elute the first and the second target proteins from the chromatography column such that a target elution volume can be ob-

- tained that matches the optimization criterion best, wherein the chromatography simulations are performed for identifying the combination of the combination of the elution buffer salt concentration (222) and the elution buffer pH;
- 5 - wherein the pooling borders of the target elution volume are computed as a function of at least the computed combination of the elution buffer salt concentration and the elution buffer pH value and the input optimization criterion; and
- wherein the elution buffer (226) that is applied on the column has the computed salt concentration and the computed pH value.
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5. The method of any one of the previous claims, the first and the second target proteins being proteins having a resolution factor of less than 0.75, and/or of less than 0.5.
- 15 6. The method of any one of the previous claims, the first and the second target protein being glycosylation variants of proteins having an identical amino acid sequence.
7. The method of any one of the previous claims, the first and the second target protein being antibody monomers having an identical amino acid sequence and comprising different numbers of glycosyl groups on the FAB fragment.
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8. The method of any one of the previous claims, wherein the applied protein solution comprises each of the target proteins (P1, P2) and each of the further proteins (P3-P6), if any, in a respective concentration of at least 0.5% by weight, in particular of at least 1% by weight, in particular at least 2% by weight.
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9. The method of any one of the previous claims, wherein the second target protein (P2) and one or more of the further proteins (P3-P6), if any, comprised in the applied protein solution have an affinity to the stationary phase of the column that is similar as the affinity of the first target protein to the stationary
- 30 phase leading to overlapping elution behaviors.

10. The method of any one of the previous claims, wherein the total amount of protein in the protein solution applied to the column is identical to or smaller than the maximum protein load capacity of the column, and is in particular in the range of 50% to 100%, e.g. 50% to 90%, of the maximum protein load capacity.
11. The method of any one of the previous claims, wherein the computed pooling borders of the target elution volume are specified in the form of a collection start time offset and a collection stop time offset, the method comprising:
- continuously monitoring, by an automated chromatography system comprising the chromatography column, the time lapsed since the starting of the elution;
 - automatically starting the collecting of the eluted elution buffer by the chromatography system when the lapsed time equals the collection start time offset; and
 - stopping the collecting of the eluted elution buffer by the chromatography system when the lapsed time equals the collection stop time offset.
12. The chromatography method of any one of the previous claims,
- wherein each of the plurality of chromatography simulations is a simulation of a chromatography process using two or more elution steps, whereby in each elution step, an elution buffer with a different elution salt concentration is used,
 - wherein the computed elution buffer salt concentration is a series of different, elution-step specific elution buffer salt concentrations, and
 - wherein the applying of the elution buffer having the computed salt concentration on the chromatography column comprises step-wise ap-

plying a series of elution buffers having the different salt concentrations in accordance with the computed series of elution-step specific salt concentrations.

- 5 13. The chromatography method of any one of the previous claims, the method comprising:
- 10 – inputting (104) the amount of each of the first and second target proteins (P1-P2) comprised in the applied protein solution and optionally also the amount of each of one or more further proteins (P3-P6) comprised in the applied protein solution, if any, into the chromatography simulation software (204);
 - the simulations being computed as a function of a set of parameter values comprising at least:
 - 15 • the dimension of the provided cation exchange chromatography column; and
 - the amounts of the first and second target proteins and optionally also the amounts of the one or more further proteins applied on the column.
- 20 14. The chromatography method of claim 12, the set of parameters further comprising:
- a predefined pH value of the elution buffer,
 - a flow rate of the elution buffer through the column;
 - 25 – chemical properties of the proteins (P1, P2, P3-P6) of the applied protein solution.

15. The method of any one of the previous claims, wherein the chromatography simulation software is configured to use a combination of mathematical models for computing the simulations *r* and/or for computing the pooling borders of the target elution volume, the models comprising:
- 5 – a column model (206) being configured to interrelate the concentration of each of the proteins, the salt concentration and the pH-value in the elution buffer in the interstitial volume of the column (230); and
 - a pore model (208) being configured to interrelate the concentration of each of the proteins, the salt concentration and the pH value in the
10 elution buffer in the pore volume of the stationary phase of the column (230); and
 - a reaction model (210) being configured to interrelate the concentration of each of the proteins in the stationary phase (228), the elution
15 buffer salt concentration and at least some of the chemical properties of each of the proteins in the protein solution.
16. A chromatography control system comprising a simulation software, the simulation software being configured for performing a method of obtaining pooling
20 borders of a target elution volume (238) comprising a first (P1) and a second (P2) target protein, the chromatography control system being configured for:
- receiving (106) an optimization criterion and inputting the optimization
25 criterion into the chromatography simulation software (204), the optimization criterion being a desired property of the target elution volume in respect to the first and second target proteins comprised in the target elution volume;
 - computing (108), using the chromatography simulation software, an elution buffer salt concentration (222) adapted to elute the first and the second target proteins from the chromatography column such that a target elution volume can be obtained that matches the optimization

criterion best, the computing comprising computing a plurality of chromatography simulations as a function of multiple different elution buffer salt concentrations ;

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- computing (110) the pooling borders of the target elution volume as a function of at least the computed salt concentration and the input optimization criterion;
 - outputting the computed salt concentration and pooling borders.

10 17. The chromatography control system of claim 16, the system further being configured for:

- receiving dimensions of a cation exchange chromatography column (230);
 - receiving (105) the amounts of the first and second target proteins and optionally also the amounts of the one or more further proteins applied on the column;
 - wherein the simulations and/or the pooling borders are computed as a function of a set of parameter values comprising at least:
 - the dimension of the provided cation exchange chromatography column; and
 - the amounts of the first and second target proteins and optionally also the amounts of the one or more further proteins applied on the column.
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18. The chromatography control system of any one of claims 16-17,

- wherein the plurality of chromatography simulations are computed as a function of the multiple different elution buffer salt concentrations and as a function of multiple different elution buffer pH values, the method further comprising:
 - 5 - wherein the computing (108) comprises computing a combination of an elution buffer salt concentration (222) and an elution buffer pH value which in combination are adapted to elute the first and the second target proteins from the chromatography column such that a target elution volume can be obtained that matches the optimization criterion best;
 - 10 - wherein the computing (110) of the pooling borders of the target elution volume is computed as a function of at least the computed combination of the elution buffer salt concentration and the elution buffer pH value and the input optimization criterion; and
 - 15 - wherein the computed pH value is output in addition to the computed elution buffer (226).

19. The chromatography control system of any one of claims 16-18, wherein
- 20 the chromatography control system is configured to control a buffer mixing unit as to automatically generate an elution buffer having the output elution salt concentration; and/or
 - 25 the chromatography control system is configured to control a buffer mixing unit as to automatically generate an elution buffer having both the salt concentration and the pH value computed in combination and output according to claim 18; and/or
 - 30 the chromatography control system is configured to control an elution buffer selection unit adapted to automatically select one out of a plurality of available elution buffers having different salt concentrations, the selected elution buffer having the output salt concentration; and/or
 - 30 the chromatography control system is configured to control an elution buffer selection unit adapted to automatically select one out of a plurality of available elution buffers having different salt concentrations and

different pH values, the selected elution buffer having both the salt concentration and pH value computed in combination and output according to claim 18; and/or

5 the chromatography control system is configured to control a buffer application unit configured to automatically apply an automatically generated or selected elution buffer on the chromatography column, the applied elution buffer having the output salt concentration or having both the salt concentration and pH value computed in combination and output according to claim 18;

10 the chromatography control system is configured to control an elution volume collection unit of a chromatography system such that the computed target elution volume is automatically collected as a separate fraction in accordance with the computed pooling borders.

15 20. A chromatography system comprising the chromatography control system of claim 19, and further comprising the buffer mixing unit and/or the elution buffer selection unit and/or the buffer application unit and/or the automated elution volume collection unit.

20 21. A computer program for managing a chromatography process such that a target elution volume (238) comprising a first (P1) and a second (P2) target protein is obtained, the computer program comprising a chromatography simulation software and being configured for:

25 – receiving (106) an optimization criterion being a desired property of a target elution volume in respect to a first and second target proteins comprised in the target elution volume;

30 – computing (108), using the chromatography simulation software, an elution buffer salt concentration (222) adapted to elute the first and the second target proteins from the chromatography column such that a target elution volume can be obtained that matches the optimization

criterion best, the computing comprising computing a plurality of chromatography simulations as a function of multiple different elution buffer salt concentrations;

- 5 – computing (110) the pooling borders of the target elution volume as a function of at least the computed salt concentration and the input optimization criterion; and
- 10 – outputting the computed salt concentration and/or the pooling borders for enabling a user to control a chromatography system such that a target elution volume comprising the first and the second target proteins in accordance with the optimization criterion is obtained and/or using the computed salt concentration and/or the pooling borders for automatically or semi-automatically controlling a chromatography system such that a target elution volume comprising the first and the second target proteins in accordance with the optimization criterion is obtained.
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22. The computer program of claim 21, further configured for generating control commands for automatically or semi-automatically controlling one or more units of a chromatography system.