74. The composition comprising a purified protein or subunit of a protein according to any one of items 71 to 73, wherein the concentration of the purified protein or subunit of a protein is at least 0.1 mg/mL.

75. The composition comprising a purified protein or subunit of a protein according to any one of items 71 to 73, wherein the concentration of the purified protein or subunit of a protein is at least 0.3 mg/mL.

76. The composition according to any one of items 71 to 75 for use as a medicament.

77. A method of subjecting Factor VIII (FVIII) to furin protease treatment.

78. The method according to item 77, wherein FVIII is recombinant FVIII (rFVIII).

79. The method according to item 77 or 78, wherein the FVIII comprises single chain FVIII.

80. The method according to any one of items 77 to 79, wherein the furin protease treatment is performed using furin at a final concentration of more than 100 IU/mL.

81. The method according to any one of items 77 to 80, wherein the method additionally comprises a step of separating the furin protease from the FVIII.

82. The method according to any one of items 77 to 81, wherein the method is for increasing the activity of FVIII.

83. A composition comprising FVIII, wherein the FVIII is obtainable according to any one of items 77 to 82.

84. The composition comprising FVIII according to item 83 for use as a medicament.

85. The composition comprising FVIII according to item 83 or 84 for use in the treatment of a bleeding disorder.

86. The composition comprising FVIII according to any one of items 83 to 85 for use in the treatment of hemophilia A.

In a first aspect, the present disclosure provides a method for purifying a Factor VIII (FVIII) subspecies from a composition comprising several FVIII subspecies, wherein ~~the~~ a FVIII subspecies is defined as one~~a~~ FVIII heavy chain that is associated with ~~a~~ one FVIII light chain, said method comprising the steps of:

(1) subjecting the composition comprising several FVIII subspecies to anion exchange chromatography, and collecting the fraction of the eluate comprising said FVIII subspecies to be purified, wherein elution is performed by linear gradient elution, and wherein the gradient of the linear gradient elution has a length of at least 16 column volumes;

(2) subjecting the fraction of the eluate of step (1) comprising said FVIII subspecies to be purified to size exclusion chromatography, and collecting the fraction of the eluate comprising said FVIII subspecies to be purified; and

(3) concentrating the fraction of the eluate of step (2) comprising said FVIII subspecies to be purified; ~~wherein~~ whereby the weight ratio of the FVIII subspecies to be purified to all other FVIII subspecies comprised in the composition is increased, wherein said other FVIII subspecies comprise a different FVIII heavy chain and/or a different FVIII light chain than said FVIII subspecies to be purified.

~~In a second aspect, the present disclosure provides a composition comprising a purified Factor VIII (FVIII) subspecies, wherein the FVIII subspecies is a FVIII heavy chain that is associated with a FVIII light chain, and wherein the weight ratio of the purified FVIII subspecies in the composition to all other FVIII subspecies in the composition is at least 8.~~

~~In a third aspect, the present disclosure provides the composition according to the second aspect for use in the treatment of a bleeding disorder.~~

~~In a fourth aspect, the present disclosure provides the composition according to the second aspect for use in the treatment of hemophilia A.~~

~~In a fifth aspect, the present disclosure provides use of the composition according to the second aspect in the manufacture of a medicament for treating a bleeding disorder.~~

~~In a sixth aspect, the present disclosure provides use of the composition according to the second aspect in the manufacture of a medicament for treating hemophilia A.~~

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Blood Coagulation Cascade including the tissue factor dependent or extrinsic pathway on the right-hand side and the contact activation or intrinsic pathway on the left-hand side with black arrows indicating activation of the respective blood coagulation factor and dashed lines labelled *"feed forw."* and *"inact."* showing feed forward inactivating effect, respectively. Illustration based on Schaller et al. (2008).

Figure 2: (A) Schematic presentation of factor VIII single chain molecule on top and the thereof derived heavy chain fragments on the left-hand side and light chain fragments on the right-hand side. Illustration based on (Schaller et al.,2008). The particular protein domains A1, A2, B, A3 and C1 plus C2 are shown in different grey shades. The B-domain is distributed to different extend to heavy- and light chain fragments. White spaces between the coloured domain areas represent highly acid sequences with different functions. (B) FVIII heterogeneity and molecular species. Domain structure of FVIII. Brackets indicate major HC/B domain species with terminating amino acids present in FL-FVIII.

Figure 3: Schematic presentation of the three most common types of elution modes, which are (1) Isocratic elution shown as dash-dot line, (2) Step elution shown as dashed line and (3) Gradient elution shown as solid line.

Figure 4: Chromatogram of the elution phase of FVIII molecular subspecies on AIEX Mono 10Q resin separated with standard buffers QA1 and QB1 at pH 6.7. Gradient: 135.0 - 750.0 mM sodium chloride in eight column volumes. Column dimensions: 0.5 cm inner diameter x 5.0 cm bed height, 0.98 mL column volume.

Figure 5: SDS page gel electrophoresis of the separation of FVIII molecular subspecies on Mono 10Q resin separated with standard buffers QA1 and QB1 at pH 6.7. Gradient: 135.0 - 750.0 mM sodium chloride in eight column volumes. M) Molecular weight marker, AS) Standard of the commercially available FVIII drug substance, showing all relevant heavy- and light chain species, SB) Sample buffer, Fractions B7-C8, NE) Posteluate. From top to bottom: Full length single chain (300 kDa), 180 kDa heavy chain, 150 kDa truncated heavy chain, 120 kDa extended light chain, 110 kDa truncated heavy chain, 90 kDa heavy chain without B-domain, 80 kDa light chain.

Figure 6: Overlay of FVIII molecular subspecies separation on AIEX Mono 10Q resin separated with standard buffers QA1 and QB1 at pH 6.7. (1) Gradient: 135.0 - 750.0 mM sodium chloride in eight column volumes, Broken line: Conductivity, Solid line: Absorbance at 280 nm. (2) Gradient: 135.0 - 750.0 mM sodium chloride in 16 column volumes, Dashed line: Conductivity, Dotted line: Absorbance at 280 nm. Column dimensions: 0.5 cm inner diameter x 5.0 cm bed height, 0.98 mL column volume.

CLAIMS

1. A method for purifying a Factor VIII (FVIII) subspecies from a composition comprising several FVIII subspecies, wherein ~~the~~ a FVIII subspecies is defined as one~~a~~ FVIII heavy chain that is associated with one~~a~~ FVIII light chain, said method comprising the steps of:

   (1) subjecting the composition comprising several FVIII subspecies to anion exchange chromatography, and collecting the fraction of the eluate comprising said FVIII subspecies to be purified, wherein elution is performed by linear gradient elution, and wherein the gradient of the linear gradient elution has a length of at least 16 column volumes;

   (2) subjecting the fraction of the eluate of step (1) comprising said FVIII subspecies to be purified to size exclusion chromatography, and collecting the fraction of the eluate comprising said FVIII subspecies to be purified; and

   (3) concentrating the fraction of the eluate of step (2) comprising said FVIII subspecies to be purified;

   ~~wherein~~ whereby the weight ratio of the FVIII subspecies to be purified to all other FVIII subspecies comprised in the composition is increased, wherein said other FVIII subspecies comprise a different FVIII heavy chain and/or a different FVIII light chain than said FVIII subspecies to be purified.

2. The method according to claim 1, wherein the method additionally comprises the following step (0) prior to step (1):

   (0) subjecting the several FVIII subspecies comprised in the composition to furin protease treatment.

3. The method according to claim 2, wherein the FVIII light chain is the FVIII 80 kDa light chain.

4. The method according to any one of claims 1 to 3, wherein the concentration step (3) is a step of subjecting the fraction of the eluate of step (2) comprising said FVIII subspecies to be purified to anion exchange chromatography, and collecting the fraction of the eluate comprising said FVIII subspecies to be purified.

5. The method according to claim 4, wherein in step (3) a SourceQ resin is used for anion exchange chromatography.

6. The method according to any one of claims 1 to 5, wherein ~~FVIII is recombinant FVIII (rFVIII) and~~ the FVIII subspecies are ~~is a~~ recombinant FVIII (rFVIII) subspecies.

7. The method according to any one of claims 1 to 6, wherein the FVIII subspecies to be purified is the FVIII 180 kDa heavy chain that is associated with a FVIII light chain, or the FVIII 150 kDa heavy chain that is associated with a FVIII light chain, or the FVIII 110 kDa heavy chain that is associated with a FVIII light chain, or the FVIII 90 kDa heavy chain that is associated with a FVIII light chain.

8. The method according to any one of claims 1 to 7, wherein in step (1) a high resolution Q-resin with a bead size of less than 20 µm is used for anion exchange chromatography.

9. The method according to any one of claims 1 to 8, wherein in step (2) a size exclusion chromatography resin with a resolution range of 10000 Da to 60000 Da is used for size exclusion chromatography.

10. The method according to any one of claims 1 to 9, wherein ~~elution~~ in step (1) ~~is performed by linear gradient elution, and wherein~~ the gradient of the linear gradient elution has a length of ~~at least 16 column volumes,~~ at least 24 column volumes, or at least 32 column volumes.

~~11. A composition comprising a purified Factor VIII (FVIII) subspecies, wherein the FVIII subspecies is a FVIII heavy chain that is associated with a FVIII light chain, and wherein the weight ratio of the purified FVIII subspecies in the composition to all other FVIII subspecies in the composition is at least 8.~~

~~12. The composition according to claim 11, wherein the weight ratio of the purified FVIII subspecies in the composition to all other FVIII subspecies in the composition is at least 9.~~

~~13. The composition according to claim 11 or 12, wherein the concentration of the purified FVIII subspecies is at least 0.1 mg/mL, or at least 0.3 mg/mL.~~

~~14. The composition according to any one of claims 11 to 13 for use in the treatment of a bleeding disorder.~~

~~15. The composition according to any one of claims 11 to 14 for use in the treatment of hemophilia A.~~

~~16. Use of the composition according to any one of claims 11 to 13 in the manufacture of a medicament for treating a bleeding disorder.~~

~~17. Use of the composition according to any one of claims 11 to 13 in the manufacture of a medicament for treating hemophilia A.~~