

WHAT IS CLAIMED IS:

1. A method for obtaining a composition comprising a high purity, propeptide depleted mature recombinant rVWF (mat-rVWF), said method comprising the steps of:
 - a) providing a solution comprising mat-rVWF/ rVWF-PP complex, mat-rVWF, and rVWF propeptide (rVWF-PP);
 - b) inducing dissociation of said mat-rVWF/ rVWF-PP complex in said solution in a) into mat-rVWF and rVWF-PP, wherein said dissociation occurs by disruption of the non-covalently associated mat-rVWF and rVWF-PP, wherein said dissociation is induced by:
 - i. addition of at least one chelating agent, or
 - ii. increasing the pH to a pH of at least 7; and
 - c) collecting said mat-rVWF to obtain a high purity, mat-rVWF composition, wherein said high purity, mat-rVWF composition comprises at least 95% mature rVWF and less than 5% rVWF-PP.
2. The method of claim 1, wherein said high purity, mat-rVWF composition comprises at least 96% mat-rVWF and less than 4% rVWF-PP, at least 97% mat-rVWF and less than 3% rVWF-PP, at least 98% mat-rVWF and less than 2% rVWF-PP, at least 99% mat-rVWF and less than 1% rVWF-PP, or at least 99.5% mat-rVWF and less than 0.5% rVWF-PP, or 99.9% mat-rVWF and less than 0.1% rVWF-PP.
3. The method according to any of the preceding claims, wherein said solution is selected from the group consisting of a cell culture medium, an antibody column flow-through solution, and a buffered solution.
4. The method according to any of the preceding claims, wherein said solution has been treated with furin prior to step a).
5. The method according to any of the preceding claims, wherein said solution is an antibody column flow-through solution.
6. The method according to any of the preceding claims, wherein said at least one chelating agent is a divalent cation chelating agent.

7. The method according to claim 6, wherein said divalent cation chelating agent is selected from the group consisting of EDTA, EGTA, CDTA, and citrate.
8. The method according to any of the preceding claims, wherein said pH is increased to at least 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, or 9.0.
9. The method according to any of the preceding claims, wherein said pH is increased to at least ~~about~~7.2 to ~~about~~7.8.
10. The method according to any of the preceding claims, wherein said pH is increased to at least ~~about~~7.6.
11. The method according to any of the preceding claims, wherein said pH is increased by the addition of basic amino acids, Tris, NaOH, Tricine, or ethanolamine.
12. The method according to any of the preceding claims, wherein said collecting in step b) of claim 1 comprises one or more protein separation methods.
13. The method according to claim 12, wherein said one or more protein separation methods is selected from the group consisting of ion exchange chromatography (IEC), size exclusion chromatography (SEC), physical size separation by membrane technology, and affinity chromatography.
14. The method according to claim 13, wherein said protein separation method is size exclusion chromatography (SEC).
15. The method according to claim 13, wherein said one or more protein separation method is ion exchange chromatography (IEC).
16. The method according to claim 15, wherein said ion exchange chromatography (IEC) is cation exchange chromatography.

17. The method according to claim 15, wherein said ion exchange chromatography (IEC) is a combination of anion exchange chromatography and cation exchange chromatography.

18. The method according to claim 12, wherein said one or more protein separation methods comprise a buffer system, wherein said buffer system comprises one or more buffers.

19. The method according to claim 18, wherein said one or more buffers includes wash buffers, wherein said one or more wash buffers include one, two, three, four, and/or five wash buffers, wherein when said one or more buffers includes five wash buffers, the first, second, third, and/or fifth wash buffers have a higher pH than the fourth wash buffer, and when said one or more buffers includes four wash buffers, the first, second, and/or fourth wash buffers have a higher pH than the third wash buffer.

20. The method according to claim 19, wherein the method further comprises a viral inactivation treatment step after the first wash buffer, and optionally the pH of the viral inactivation treatment step has a higher pH than said third and/or fourth wash buffer.

21. The method according to claim 18, wherein said one or more buffers comprise said one or more chelating agents.

22. The method according to claim 18, wherein said one or more buffers exhibit a pH of at least 7.

23. The method according to claim 12, wherein said one or more protein separation methods comprise a buffer system, wherein said buffer system comprises one or more loading buffers.

24. The method according to claim 23, wherein said one or more loading buffers comprise said one or more chelating agents.

25. The method according to claims 23 or 24, wherein said one or more loading buffers exhibit a pH of at least 7.

26. The method according to claim 12, wherein said one or more protein separation methods comprise a buffer system, wherein said buffer system comprises one or more load, wash, and/or elution buffers.
27. The method according to claim 26, wherein said one or more load, wash, and/or elution buffers comprise said one or more chelating agents.
28. The method according to claims 26 or 27, wherein said one or more load, wash, and/or elution buffers exhibit a pH of at least 7.
29. The method according to any of claims 26 through 28, wherein said one or more load, wash, and/or elution buffers comprise said one or more chelating agents and exhibit a pH of at least 7.
30. The method according to any of claims 18 through 29, wherein said one or more buffers of said buffering system is selected from the group consisting of glycine, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), Tris_HCl (Tris(hydroxymethyl)-aminomethane), histidine, imidazole, acetate, citrate, and MES, ~~and~~ (2-(N-morpholino)ethanesulfonic acid).
31. The method according to any of claims 18 through 30, wherein said buffer further comprises one or more monovalent cations.
32. The method according to claim 31, wherein said one or more monovalent cations are selected from the group consisting of Na⁺, K⁺, Li⁺, and Cs⁺.
33. The method according to claim 31, wherein said monovalent cation is Na⁺.
34. The method according to any of claims 18 through 33, wherein said buffer further comprises one or more monovalent, divalent and/or trivalent anions.

35. The method according to claim 34, wherein said one or more monovalent, divalent and/or trivalent anions are selected from the group consisting of Cl^- , acetate $^-$, SO_4^{2-} , Br^- , and citrate $^{3-}$.
36. The method according to any of claims 18 through 35, wherein said buffer system comprises at least one buffer exhibiting a conductivity of ≥ 0.5 mS/cm at 25°C.
37. The method according to any of claims 18 through 36, wherein said buffer system comprises at least one buffer exhibiting a conductivity of 15.0 ± 0.2 mS/cm at 25°C.
38. The method according to any of claims 18 through 37, wherein said buffer further comprises one or more nonionic detergents.
39. The method according to claim 38, wherein said nonionic detergent is selected from the group consisting of Triton X 100, Tween 80, and Tween 20.
40. The method according to any of claims 18 through 39, wherein said buffer further comprises one or more additional substances selected from the group consisting of non-reducing sugars, sugar alcohols, and polyols.
41. The method according to any of the preceding claims, wherein said high purity mat-rVWF composition comprises a host cell (HC) impurity level of ≤ 2.0 %.
42. The method according to any of the preceding claims, wherein said high purity, mat-rVWF composition comprises a host cell (HC) impurity level of ≤ 0.6 %.
43. The method according to any of the preceding claims, wherein said solution comprising mat-rVWF/ rVWF-PP complex, mat-rVWF, and rVWF-PP is derived from a capture step for rVWF.
44. The method according to any of the preceding claims, wherein said solution comprising mat-rVWF/ rVWF-PP complex, mat-rVWF, and rVWF-PP is derived from a method comprising a FVIII immunoaffinity step and anion exchange chromatography step.

45. A method for obtaining a composition comprising a high purity, propeptide depleted mature recombinant rVWF (high purity mat-rVWF), said method comprising the steps of:

- a) loading a solution comprising pro-rVWF, mat-rVWF/ rVWF-PP complex, mat-rVWF, and/or rVWF propeptide (rVWF-PP) onto an anion exchange column, wherein said pro-rVWF, mat-rVWF/ rVWF-PP complex, and mat-rVWF are bound to said anion exchange column;
- b) washing said anion exchange column in a) containing said bound pro-rVWF, mat-rVWF/ rVWF-PP complex, and mat-rVWF with one or more wash buffers;
- c) treating said column in b) comprising the bound pro-rVWF, mat-rVWF/ rVWF-PP complex, and mat-rVWF with furin, wherein said furin cleaves said pro-rVWF into mat-rVWF and rVWF-PP;
- d) eluting said bound pro-rVWF, mat-rVWF/ rVWF-PP complex, and mat-rVWF from the column in c) with an elution buffer, wherein said elution buffer induces dissociation of said rVWF-PP from mat-rVWF non-covalently associated with said rVWF-PP, and wherein said dissociation is induced by:
 - i. addition of at least one chelating agent into said elution buffer, or
 - ii. increasing the pH of said elution buffer to a pH of at least 7; and
- e) collecting said mat-rVWF separately from said rVWF-PP to obtain a high purity mat-rVWF composition, wherein said high purity mat-rVWF composition comprises at least 95% mature rVWF and less than 5% rVWF-PP.

46. The method according to claim 45, wherein said one or more buffers includes wash buffers, wherein said one or more wash buffers include one, two, three, four, and/or five wash buffers, wherein when said one or more buffers includes five wash buffers, the first, second, third, and/or fifth wash buffers have a higher pH than the fourth wash buffer, and when said one or more buffers includes four wash buffers, the first, second, and/or fourth wash buffers have a higher pH than the third wash buffer.

47. The method according to claim 46, wherein the method further comprises a viral inactivation treatment step after the first wash buffer, and optionally the pH of the viral inactivation treatment step has a higher pH than said third and/or fourth wash buffer.

48. The method according to any of claims 45 through 47, wherein a) and b) occur simultaneously in a single step.

49. The method according to any of claims 45 through 48, wherein said solution in a) comprises the flow through from a monoclonal antibody column, wherein said monoclonal antibody is a FVIII monoclonal antibody.
50. The method according to any of claims 45 through 49, wherein said solution in a) is selected from the group consisting of a cell culture medium, an antibody column flow-through solution, and a buffered solution.
51. The method according to any of claims 45 through 50, wherein said at least one chelating agent is a divalent cation chelating agent.
52. The method according to claim 51, wherein said divalent cation chelating agent is selected from the group consisting of EDTA, EGTA, CDTA, and citrate.
53. The method according to any of claims 45 through 52, wherein said pH is increased to at least 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0.
54. The method according to any of claims 45 through 53, wherein said pH is increased to at least ~~about~~7.2 to ~~about~~7.8.
55. The method according to any of claims 45 through 54, wherein said pH is increased to at least ~~about~~7.6.
56. The method according to any of claims 45 through 55, wherein said pH is increased by the addition of basic amino acids.
57. The method according to any of claims 45 through 55, wherein said one or more wash buffers in b) comprise said one or more chelating agents.
58. The method according to any of claims 45 through 55, wherein said one or more wash buffers in b) exhibit a pH of at least 7.

59. The method according to any of claims 45 through 55, wherein said one or more wash buffers in b) comprise said one or more chelating agents and exhibit a pH of at least 7.
60. The method according to any of claims 45 through 55, wherein said method further comprises a step of viral inactivation, wherein said viral inactivation occurs before, after, or concurrently with the washing step and/or the elution step, but before the collecting step.
61. The method according to claim 60, wherein said viral inactivation treatment inactivates lipid enveloped viruses.
62. The method according to claims 60 or 61, wherein said viral inactivation treatment is an S/D treatment.
63. The method according to any of claims 45 through 62, wherein said one or more buffers comprise a buffer selected from the group consisting of glycine HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), TrisHCl (Tris(hydroxymethyl)-aminomethane), histidine, imidazole, acetate citrate, MES, and 2-(N-morpholino)ethanesulfonic acid.
64. The method according to any of claims 45 through 63, wherein said one or more buffers further comprise one or more monovalent cations.
65. The method according to claim 64, wherein said one or more monovalent cations are selected from the group consisting of Na⁺, K⁺, Li⁺, and Cs⁺.
66. The method according to claim 65, wherein said monovalent cation is Na⁺.
67. The method according to any of claims 45 through 66, wherein said one or more buffers further comprise one or more monovalent, divalent, and/or trivalent anions.
68. The method according to claim 67, wherein said one or more monovalent, divalent and/or trivalent anions are selected from the group consisting of Cl⁻, acetate⁻, SO₄²⁻, Br⁻, and citrate³⁻.

69. The method according to any of claims 45 through 68, wherein said one or more buffers comprise at least one buffer exhibiting a conductivity of ≥ 0.5 mS/cm at 25°C.
70. The method according to any of claims 45 through 69, wherein said one or more buffers comprise at least one buffer exhibiting a conductivity of 15.0 ± 0.2 mS/cm at 25°C.
71. The method according to any of claims 45 through 70 wherein said one or more buffers further comprise one or more nonionic detergents.
72. The method according to claim 71, wherein said nonionic detergent is selected from the group consisting of Triton X 100, Tween 80, and Tween 20.
73. The method according to any of claims 45 through 72, wherein said one or more buffers further comprise one or more additional substances selected from the group consisting of non-reducing sugars, sugar alcohols, and polyols.
74. The method according to any of claims 45 through 73, wherein said high purity mat-rVWF composition comprises a host cell (HC) impurity level of ≤ 2.0 %.
75. The method according to any of claims 45 through 74, wherein said high purity mat-rVWF composition comprises a host cell (HC) impurity level of ≤ 0.6 %.
76. The method according to any of the preceding claims, wherein said high purity mat-rVWF composition is used for the production of a pharmaceutical composition.
77. A pharmaceutical composition comprising a high purity mat-rVWF generated by the method according to any of the preceding claims and a pharmaceutically acceptable buffer.
- ~~78. The pharmaceutical composition according to claim 77, wherein the composition comprises 50 mM Glycine, 10 mM Taurine, 5% (w/w) Sucrose, 5% (w/w) D-Mannitol, 0.1%~~

~~Polysorbate 80, 2 mM CaCl₂, 150 mM NaCl, wherein said composition has a pH of about pH 7.4.~~

78. The method according to any of the preceding claims, wherein said high purity mat-rVWF composition is lyophilized after purification.