

Claims

1. A polypeptide comprising the amino acid sequence of
TX₁LETX₂X₃X₄GX₅X₆X₇PX₈TIRVX₉Q (SEQ ID NO: 1)
5 wherein
X₁ is H, H-3Me or PyA-4;
X₂ is T, S, D or E;
X₃ is F, F-2-Br, F-2-Cl or F-3-F;
X₄ is W, Nal, or absent;
10 X₅ is D or *N*-Me-D;
X₆ is G, A or P;
X₇ is E or D;
X₈ is K or *N*-Me-K; and
X₉ is T or *N*-Me-T;
15 or a pharmaceutically acceptable salt thereof.
2. The polypeptide according to claim 1, wherein the polypeptide is covalently linked to a cyclization moiety.
- 20 3. The polypeptide according to any one of the preceding claims, wherein the cyclization moiety comprises the amino acid sequence of pGX₁₀, wherein X₁₀ is C, Q or E.
- 25 4. The polypeptide according to any of the preceding claims, wherein the polypeptide comprises or consists of the amino acid sequence
TX₁LETX₂X₃X₄GX₅X₆X₇PX₈TIRVX₉QpGX₁₀ (SEQ ID NO: 2)
wherein
X₁ is H, H-3Me or PyA-4;
X₂ is T, S, D or E;
30 X₃ is F, F-2-Br, F-2-Cl or F-3-F;
X₄ is W, Nal, or absent;
X₅ is D or *N*-Me-D;
X₆ is G, A or P;
X₇ is E or D;
35 X₈ is K or *N*-Me-K;

X₉ is T or *N*-Me-T; and

X₁₀ is C, Q or E;

or a pharmaceutically acceptable salt thereof.

- 5 5. The polypeptide according to any of claims 1 to 3, wherein the polypeptide comprises or consists of the amino acid sequence
TX₁LETX₂X₃GX₅X₆X₇PX₈TIRVX₉Q (SEQ ID NO: 3)
wherein
X₁ is H, H-3Me or PyA-4;
10 X₂ is T, S, D or E;
X₃ is F, F-2-Br, F-2-Cl or F-3-F;
X₅ is D or *N*-Me-D;
X₆ is G, A or P;
X₇ is E or D;
15 X₈ is K or *N*-Me-K; and
X₉ is T or *N*-Me-T;
or a pharmaceutically acceptable salt thereof.
- 20 6. The polypeptide according to claim 1, wherein the polypeptide comprises or consists of the amino acid sequence TX₁LETX₂X₃GX₅X₆X₇PX₈TIRVX₉QpGX₁₀
(SEQ ID NO: 4)
wherein
X₁ is H, H-3Me or PyA-4;
X₂ is T, S, D or E;
25 X₃ is F, F-2-Br, F-2-Cl or F-3-F;
X₅ is D or *N*-Me-D;
X₆ is G, A or P;
X₇ is E or D;
X₈ is K or *N*-Me-K;
30 X₉ is T or *N*-Me-T; and
X₁₀ is C, Q or E;
or a pharmaceutically acceptable salt thereof.

7. The polypeptide according to claim 1, wherein the polypeptide comprises or consists of the amino acid sequence TX₁LETTFX₄GX₅X₆X₇PX₈TIRVX₉QpGX₁₀ (SEQ ID NO: 5)
wherein
5 X₁ is H, H-3Me or PyA-4;
X₄ is W, Nal, or absent;
X₅ is D or *N*-Me-D;
X₆ is G, A or P;
X₇ is E or D;
10 X₈ is K or *N*-Me-K;
X₉ is T or *N*-Me-T; and
X₁₀ is C, Q or E;
or a pharmaceutically acceptable salt thereof.
- 15 8. The polypeptide according to claim 1, wherein the polypeptide comprises or consists of the amino acid sequence TX₁LETTFGX₅X₆X₇PX₈TIRVX₉QpGX₁₀ (SEQ ID NO: 6)
wherein
X₁ is H, H-3Me or PyA-4;
20 X₅ is D or *N*-Me-D;
X₆ is G, A or P;
X₇ is E or D;
X₈ is K or *N*-Me-K;
X₉ is T or *N*-Me-T; and
25 X₁₀ is C, Q or E;
or a pharmaceutically acceptable salt thereof.
9. The polypeptide according to claim 1, wherein the polypeptide comprises or consists of the amino acid sequence TX₁LETTFX₄GDGX₇PX₈TIRVX₉Q (SEQ ID
30 NO: 7), wherein
X₁ is H, or PyA-4;
X₄ is W or Nal;
X₇ is E or D;
X₈ is K or *N*-Me-K; and
35 X₉ is T or *N*-Me-T;

or a pharmaceutically acceptable salt thereof.

10. The polypeptide according to any of the preceding claims, wherein said polypeptide is cyclic.

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11. The polypeptide according to any of the preceding claims, wherein said polypeptide is back-bone cyclized.

12. The polypeptide according to any of the preceding claims, wherein X_1 is H.

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13. The polypeptide according to any of the preceding claims, wherein X_1 is H-3Me.

14. The polypeptide according to any of the preceding claims, wherein X_1 is PyA-4.

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15. The polypeptide according to any of the preceding claims, wherein X_2 is T.

16. The polypeptide according to any of the preceding claims, wherein X_2 is S.

17. The polypeptide according to any of the preceding claims, wherein X_2 is D.

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18. The polypeptide according to any of the preceding claims, wherein X_2 is E.

19. The polypeptide according to any of the preceding claims, wherein X_3 is F.

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20. The polypeptide according to any of the preceding claims, wherein X_3 is F-2-Br.

21. The polypeptide according to any of the preceding claims, wherein X_3 is F-2-Cl.

22. The polypeptide according to any of the preceding claims, wherein X_3 is F-3-F.

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23. The polypeptide according to any of the preceding claims, wherein X_4 is W.

24. The polypeptide according to any of the preceding claims, wherein X_4 is NaI.

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25. The polypeptide according to any of the preceding claims, wherein X_5 is D.

26. The polypeptide according to any of the preceding claims, wherein X_5 is *N*-Me-D.

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27. The polypeptide according to any of the preceding claims, wherein X_6 is G.

28. The polypeptide according to any of the preceding claims, wherein X_6 is A.
29. The polypeptide according to any of the preceding claims, wherein X_6 is P.
- 5 30. The polypeptide according to any of the preceding claims, wherein X_7 is E.
31. The polypeptide according to any of the preceding claims, wherein X_7 is D.
- 10 32. The polypeptide according to any of the preceding claims, wherein X_8 is K.
33. The polypeptide according to any of the preceding claims, wherein X_8 is *N*-Me-K.
- 15 34. The polypeptide according to any of the preceding claims, wherein X_9 is T.
35. The polypeptide according to any of the preceding claims, wherein X_9 is *N*-Me-T.
- 20 36. The polypeptide according to any of the preceding claims, wherein X_{10} is C.
37. The polypeptide according to any of the preceding claims, wherein X_{10} is Q.
38. The polypeptide according to any of the preceding claims, wherein X_{10} is E.
- 25 39. The polypeptide according to any of the preceding claims, wherein X_1 is H, X_2 is T, X_3 is F, X_4 is W, X_5 is D, and X_6 is G.
40. The polypeptide according to any of the preceding claims, wherein X_1 is H, X_2 is T, X_3 is F, X_4 is W, X_5 is D, X_6 is G, and X_7 is E.
- 30 41. The polypeptide according to any of the preceding claims, wherein X_1 is H, X_2 is T, X_3 is F, X_4 is W, X_5 is D, X_6 is G, and X_7 is D.
- 35 42. The polypeptide according to any of the preceding claims, wherein X_1 is H, X_2 is T, X_3 is F, X_4 is Nal, X_5 is D, X_6 is G, and X_7 is E.
43. The polypeptide according to any of the preceding claims, wherein X_1 is H, X_2 is PyA-4, X_3 is F, X_4 is W, X_5 is D, X_6 is G, and X_7 is E.
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44. The polypeptide according to any of the preceding claims, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of:
THLETTFWGDGE (SEQ ID NO: 8),
5 THLETTFWGDGD (SEQ ID NO: 9),
THLETTF(Nal)GDGE (SEQ ID NO: 10), and
T(PyA-4)LETTFWGDGE (SEQ ID NO: 11).
45. The polypeptide according to any of the preceding claims, wherein the
10 polypeptide comprises the amino acid sequence THLETTFWGDGE (SEQ ID NO: 8).
46. The polypeptide according to any of the preceding claims, wherein the
15 polypeptide comprises the amino acid sequence THLETTFWGDGD (SEQ ID NO: 9).
47. The polypeptide according to any of the preceding claims, wherein the
polypeptide comprises the amino acid sequence THLETTF(Nal)GDGE (SEQ ID
20 NO: 10).
48. The polypeptide according to any of the preceding claims, wherein the
polypeptide comprises the amino acid sequence T(PyA-4)LETTFWGDGE
(SEQ ID NO: 11).
- 25 49. The polypeptide according to any one of the preceding claims, wherein the polypeptide is a cyclic polypeptide comprising or consisting of the amino acid sequence TX₁LETTFX₄GDGEPKTIRVTQpGX₁₀ (SEQ ID NO: 13)
wherein
X₁ is H or H-3Me;
30 X₄ is W or absent;
X₁₀ is C, Q or E;
or a pharmaceutically acceptable salt thereof.
50. The polypeptide according to any one of the preceding claims, wherein the
35 polypeptide is a cyclic polypeptide comprising or consisting of the amino acid sequence THLETTFWGDGEPKTIRVTQ (SEQ ID NO: 419).

51. The polypeptide according to any one of the preceding claims, wherein the polypeptide is a cyclic polypeptide comprising or consisting of the amino acid sequence THLETTFGDGEPKTIRVTQ (SEQ ID NO: 420).
52. The polypeptide according to any one of the preceding claims, wherein the polypeptide is a cyclic polypeptide comprising or consisting of the amino acid sequence TH(3-Me)LETTFWGDGEPKTIRVTQ (SEQ ID NO: 421).
53. The polypeptide according to any one of the preceding claims, wherein the polypeptide is a cyclic polypeptide comprising or consisting of the amino acid sequence TH(3-Me)LETTFGDGEPKTIRVTQ (SEQ ID NO: 422).
54. The polypeptide according to any of the preceding claims, wherein the polypeptide comprises at least 20 amino acid residues, such as at least 21 amino acid residues, such as at least 22 amino acid residues, such as at least 23 amino acid residues, such as at least 24 amino acid residues, such as at least 25 amino acid residues, such as at least 26 amino acid residues, such as at least 27 amino acid residues, such as at least 28 amino acid residues, such as at least 29 amino acid residues, such as at least 30 amino acid residues, such as at least 31 amino acid residues, such as at least 32 amino acid residues, such as at least 33 amino acid residues, such as at least 34 amino acid residues, such as at least 35 amino acid residues, such as at least 36 amino acid residues, such as at least 37 amino acid residues.
55. The polypeptide according to any of the preceding claims, wherein the polypeptide comprises no more than 50 amino acid residues, such as no more than 45 amino acid residues, such as no more than 40 amino acid residues, such as no more than 35 amino acid residues, such as no more than 30 amino acid residues, such as no more than 29 amino acid residues, such as no more than 28 amino acid residues, such as no more than 27 amino acid residues, such as no more than 26 amino acid residues, such as no more than 25 amino acid residues, such as no more than 24 amino acid residues, such as no more than 23 amino acid residues, such as no more than 22 amino acid residues,

such as no more than 21 amino acid residues, such as no more than 20 amino acid residues.

56. The polypeptide according to any of the preceding claims, wherein the polypeptide comprises in the range of 19 to 50 amino acid residues, such as in the range of 19 to 45 amino acid residues, such as in the range of 19 to 40 amino acid residues, such as in the range of 19 to 35 amino acid residues, such as in the range of 19 to 30 amino acid residues, such as in the range of 19 to 25 amino acid residues, such as in the range of 19 to 23 amino acid residues, such as in the range of 20 to 23 amino acid residues, such as in the range of 20 to 22 amino acid residues.
57. The polypeptide according to any one of the preceding claims, wherein the polypeptide is capable of binding to PSD-95.
58. The polypeptide according to any one of the preceding claims, wherein the polypeptide is capable of inhibiting binding of nNOS to the PDZ2 domain of PSD-95.
59. The polypeptide according to any one of the preceding claims, wherein the polypeptide binds to PSD-95-PDZ2 with a K_d of less than 100 μM , such as less than 75 μM , such as less than 50 μM , such as less than 25 μM , such as less than 20 μM , such as less than 15 μM , such as less than 10 μM , such as less than 5 μM , such as less than 4 μM , such as less than 3 μM , such as less than 2 μM , such as less than 1 μM .
60. The polypeptide according to any one of the preceding claims, wherein the compound has a K_i value for inhibiting binding of nNOS to PDZ2 domain of PSD-95 of less than 100 μM , such as less than 75 μM , such as less than 50 μM , such as less than 10 μM , such as less than 5 μM , such as less than 2.5 μM , such as less than 1 μM .
61. The polypeptide according to any one of the preceding claims, wherein the polypeptide is further conjugated to a moiety.

62. The polypeptide according to claim 61, wherein the moiety is selected from the group consisting of PEG, monosaccharides, fluorophores, chromophores, radioactive compounds, and cell-penetrating peptides.
- 5 63. The polypeptide according to claim 61, wherein the moiety is a detectable moiety.
64. The polypeptide according to any one of the preceding claims, wherein the polypeptide is further modified by glycosylation, PEGylation, amidation,
10 esterification, acylation, acetylation and/or alkylation.
65. The polypeptide according to any one of the preceding claims, wherein one or more of the amino acid residues are alkylated, such as methylated.
- 15 66. The polypeptide according to any one of claims 1, 10, 11, or 54 to 65, wherein the polypeptide comprises or consist of an amino acid sequence selected from the group consisting of SEQ ID NO: 14 to 136 and SEQ ID NO: 139 to 433.
- 20 67. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(THLETTFWGDGE**PKTIRVTQpG**(E α)) (SEQ ID NO: 423).
68. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(THLETTFGDGE**PKTIRVTQpG**(E α)) (SEQ ID NO: 424).
- 25 69. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(**TH(3-Me)**LETTFWGDGE**PKTIRVTQpG**(E α)) (SEQ ID NO: 425).
- 30 70. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(**TH(3-Me)**LETTFGDGE**PKTIRVTQpG**(E α)) (SEQ ID NO: 426).
71. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(THLETTFWGDGE**PKTIRVTQpG**(Q α)) (SEQ ID NO: 14).
- 35 72. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(THLETTFGDGE**PKTIRVTQpG**(Q α)) (SEQ ID NO: 15).

73. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(TH(3-Me)LETTFWGDGEPKTIRVTQpG(Qα)) (SEQ ID NO: 16).
74. The polypeptide according to any of the preceding claims, wherein the polypeptide is cyclo-(TH(3-Me)LETTFGDGEPKTIRVTQpG(Qα)) (SEQ ID NO: 17).
75. A composition comprising the polypeptide according to any of the preceding claims.
76. The composition according to claim 75, wherein the composition is a pharmaceutical composition.
77. A polynucleotide encoding the polypeptide as defined in any one of claims 1 to 74.
78. A vector comprising a polynucleotide as defined in claim 77.
79. A host cell comprising the polynucleotide according to claim 77 or the vector according to claim 78.
80. The host cell according to claim 79, wherein the host cell is a bacterial cell.
81. The host cell according to claim 79, wherein the host cell is a mammalian cell.
82. The host cell according to claim 79, wherein the host cell is a human cell.
83. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use as a medicament.
84. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use in prevention and/or treatment of an excitotoxic-related disease in a subject.

- 5 85. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use according to claim 84, wherein the excitotoxic-related disease is stroke, such as ischemic stroke.
- 10 86. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use according to claim 85, wherein the polypeptide is administered in combination with reperfusion therapy, such as in combination with administration of a thrombolytic agent.
- 15 87. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use according to claim 84, wherein the excitotoxic-related disease is ischemic or traumatic injury of the CNS, such as spinal cord injury and traumatic brain injury.
- 20 88. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use according to claim 84, wherein the excitotoxic-related disease is epilepsy.
- 25 89. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use according to claim 84, wherein the excitotoxic-related disease is a neurodegenerative
- 30 disease of the CNS.
- 35 90. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use according to claim 84, wherein the neurodegenerative disease of the CNS is selected from the group consisting of Alzheimer's disease, Huntington's disease and Parkinson's disease.

- 5 91. The polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79 for use in prevention and/or treatment of neuropathic pain in a subject.
- 10 92. A method of preventing and/or treating an excitotoxicity-related disease and/or neuropathic pain, said method comprising administering a therapeutically effective amount of the polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79, to a subject in need thereof.
- 15 93. The method according to claim 92, wherein the polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79, is administered in combination with reperfusion therapy, such as in combination with administration of a thrombolytic agent.
- 20 94. Use of the polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the vector according to claim 78, or the host cell according to claim 79, for the manufacture of a medicament for the treatment and/or prevention of an excitotoxicity-related disease and/or neuropathic pain in a subject.
- 25 95. A kit of parts comprising at least two separate unit dosage forms (A) and (B), wherein
(A) comprises the polypeptide according to any one of claims 1 to 74, the composition according to claim 75, the polynucleotide according to claim 77, the
30 vector according to claim 78, or the host cell according to claim 79,; and
(B) comprises a thrombolytic agent.
- 35 96. The kit of parts according to claim 95 for use in in treating, preventing, reducing and/or delaying development of an excitotoxic-related disease and/or pain, wherein (A) and (B) are administered simultaneously, sequentially or separately to the subject.

- 5 97. A method for manufacturing the polypeptide according to any of claims 1 to 74, said method comprising the steps of:
- a) preparing a peptide using Fmoc/tBu-based solid-phase peptide synthesis (SPPS), and
 - b) cyclization of said peptide via native chemical ligation (NCL).
- 10 98. The method according to claim 97, wherein step b) involves oxidizing a C-terminal hydrazine group to an azide and reacting said azide with a thiol group of the N-terminal Cys, followed by transthioesterification to form an amide bond linkage.
- 15 99. The method according to claim 97, further comprising a step following step b), wherein a fluorophore is conjugated to the polypeptide.
- 20 100. A method for manufacturing the polypeptide according to any of claims 1 to 74, said method comprising the steps of:
- a) Providing a cellulose membrane;
 - b) Coupling of PEG spacer and adding a mixture of Fmoc/Boc-Gly to the cellulose membrane provided in step a);
 - c) Capping the membrane prepared in step b) with acetic anhydride;
 - d) Adding quasi-orthogonal protected AA to the product of step c);
 - 25 e) Preparing the remaining polypeptide using Fmoc/tBu-based solid-phase peptide synthesis (SPPS) on the AA of step d);
 - f) Removing the quasi-orthogonal protecting group from the polypeptide generated in step e) and cyclizing the polypeptide;
 - g) Cleaving side-chain protecting groups from the polypeptide generated in step f); and
 - 30 h) Cleaving the polypeptide from the cellulose membrane.