CLAIMS

1. A single chain polypeptide comprising, from N-terminal to C-terminal:

(a) an N-terminal fragment of a reporter protein;

(b) a linker comprising a neurotoxin cleavage site, in which the neurotoxin cleavage site is a *C. botulinum* neurotoxin (BoNT) cleavage site, and/or a Tetanus neurotoxin cleavage site; and

(c) a C-terminal fragment of the reporter protein;

in which the N-terminal and the C-terminal fragments collectively comprise a functional reporter protein sequence; and

in which the linker functionally joins the N-terminal fragment and the C-terminal fragment to generate a functional reporter fusion protein.

2. A single chain polypeptide according to Claim 1, in which the reporter protein is a luciferase protein.

3. A single chain polypeptide according to Claim 1, in which the N-terminal fragment has a sequence of amino acids 1-159 of luciferase of SEQ ID NO: 1 (N-nano$_{1-159}$), and the C-terminal fragment has a sequence of amino acids 160-170 of luciferase of SEQ ID NO: 1 (C-nano$_{160-170}$).

4. A single chain polypeptide according to Claim 3, in which the N-terminal fragment comprises a sequence with at least 90% sequence identity to amino acids 1-159 of luciferase of SEQ ID NO: 1 (N-nano$_{1-159}$), and the C-terminal fragment comprises a sequence with at least 90% sequence identity amino acids 160-170 of luciferase of SEQ ID NO: 1 (C-nano$_{160-170}$).

5. A single chain polypeptide according to Claim 3, in which the N-terminal fragment comprises a sequence having less than 10 amino acid residues substitutions, deletions, or additions relative to amino acids 1-159 of luciferase of SEQ ID NO: 1 (N-nano$_{1-159}$), and the C-terminal fragment comprises a sequence having less than 3 amino acid residues substitutions, deletions, or additions relative to amino acids 160-170 of luciferase of SEQ ID NO: 1 (C-nano$_{160-170}$).

6. A single chain polypeptide according to Claim 1, in which the BoNT cleavage site is recognized by BoNT of serotype A, E, C, B, D, F, or G.

7. A single chain polypeptide according to Claim 1, in which the BoNT cleavage site is from a SNARE protein.

8. A single chain polypeptide according to Claim 7, in which the SNARE protein is SNAP-25, synaptobrevin (VAMP), or syntaxin.

9. A single chain polypeptide according to Claim 7, in which the SNARE protein is amino acids 141-206 of human SNAP-25b.

10. A single chain polypeptide according to Claim 7, in which the SNARE protein is amino acids 35-96 of human VAMP1.

11. A single chain polypeptide according to Claim 7, in which the SNARE protein is amino acids 1-206 of human SNAP25b.

12. A single chain polypeptide according to Claim 1, in which the linker further comprises a neurotoxin binding fragment.

13. A single chain polypeptide according to Claim 12, in which the neurotoxin binding fragment is located between the N-terminal fragment and the neurotoxin cleavage site.

14. A single chain polypeptide according to Claim 12, in which the neurotoxin binding fragment is a human, mouse, rat, or primate neurotoxin receptor.

15. A single chain polypeptide according to Claim 14, in which the neurotoxin receptor is SV2C.

16. A single chain polypeptide according to Claim 14, in which the neurotoxin receptor comprises amino acids 529-566 of human SV2C.

17. A single chain polypeptide according to Claim 14, in which the neurotoxin receptor is SYTI.

18. A single chain polypeptide according to Claim 14, in which the neurotoxin receptor comprises amino acids 32-52 of SYTI.

19. A single chain polypeptide according to Claim 14, in which the neurotoxin receptor is SYT2.

20. A single chain polypeptide according to Claim 14, in which the neurotoxin receptor comprises amino acids 40-60 of SYT2.

21. A single chain polypeptide according to Claim 2, in which the linker further comprises an intact second reporter polypeptide located between the N-terminal fragment and the neurotoxin cleavage site.

22. A single chain polypeptide according to Claim 21, in which the second reporter polypeptide is firefly luciferase (e.g., *Photinus pyralis*), bacterial luciferase (e.g., *Vibrio fischeri*, *Vibrio harveyi*), sea pansy luciferase (*Renilla reniformis*), dinoflagellate luciferase, *Gaussia* luciferase, or copepod luciferase.

23. A single chain polypeptide according to Claim 2, further comprising a polyhistidine affinity tag.

24. A single chain polypeptide according to Claim 2, in which the linker further comprises one or more spacers located between the neurotoxin cleavage site and the N-terminal fragment, and one or more spacers located between the neurotoxin cleavage site and the C-terminal fragment, or a combination thereof.

25. A single chain polypeptide according to Claim 13, in which the linker further comprises one or more spacers located between the neurotoxin binding fragment and the N-terminal fragment.

26. A single chain polypeptide according to Claim 13, in which the linker further comprises one or more spacers located between the neurotoxin binding fragment and the neurotoxin cleavage site.

27. A single chain polypeptide according to Claim 21, in which the linker further comprises one or more spacers located between the second reporter polypeptide and the N-terminal fragment.

28. A single chain polypeptide according to Claim 21, in which the linker further comprises one or more spacers located between the second reporter polypeptide and the neurotoxin cleavage site.

29. A nucleic acid comprising a nucleotide sequence that encodes the single chain polypeptide according to Claim 2.

30. A nucleic acid vector comprising a nucleic acid according to Claim 29.

31. A cell comprising a nucleic acid according to Claim 29.

32. A method for determining potency of *C. botulinum neurotoxin* (BoNT), comprising:

(a) contacting the BoNT to a single chain polypeptide under conditions appropriate for BoNT activity, in which the single chain polypeptide comprises:

(i) an N-terminal fragment of a reporter protein;

(ii) a linker comprising a BoNT cleavage site; and

(iii) a C-terminal fragment of the reporter protein; and

(b) determining reporter activity of the polypeptide, as compared to a reference, thereby determining potency, in which the BoNT is determined to be more potent than the reference if it decreases reporter activity compared to the reference.

33. A method for detecting *C. botulinum neurotoxin* (BoNT) activity in a sample, comprising:

(a) contacting the sample to a single chain polypeptide under conditions appropriate for BoNT activity, in which the single chain polypeptide comprises:

(i) an N-terminal fragment of a reporter protein;

(ii) a linker comprising a BoNT cleavage site; and

(iii) a C-terminal fragment of the reporter protein; and

(b) determining reporter activity of the polypeptide, as compared to reporter activity of the polypeptide in the absence of the sample, in which a decrease of reporter activity indicates neurotoxin activity in the sample.

34. A method according to Claim 33, in which the single chain polypeptide is expressed by a neuronal cell and the method further comprises after the contacting step:

incubating the neuronal cells for a period from about 12 hours to about 60 hours, and harvesting lysate from the neuronal cells.

35. A kit comprising:

(a) one or more single chain polypeptides described in Claim 2, with each or a combination of the single chain polypeptides packaged into a separate container;

(b) one or more nucleic acids or nucleic acid vectors comprising a nucleotide sequence that encodes the one or more single chain polypeptide of (a), with each or a combination of the nucleic acids or nucleic acid vectors packaged into a separate container; and/or

(c) one or more cells that express the one or more single chain polypeptide of (a), with each or a combination of the cells packaged into a separate container.

CLAIMS

1. A single chain polypeptide comprising, from N-terminal to C-terminal:

(a) an N-terminal fragment of a reporter protein;

(b) a linker comprising a neurotoxin binding fragment and a neurotoxin cleavage site, in which the neurotoxin cleavage site is a *C. botulinum* neurotoxin (BoNT) cleavage site, and/or a Tetanus neurotoxin cleavage site; and

(c) a C-terminal fragment of the reporter protein;

in which the N-terminal and the C-terminal fragments collectively comprise a functional reporter protein sequence; and

in which the linker functionally joins the N-terminal fragment and the C-terminal fragment to generate a functional reporter fusion protein.

2. A single chain polypeptide according to Claim 1, in which the reporter protein is a luciferase protein.

3. A single chain polypeptide according to Claim 1, in which the N-terminal fragment has a sequence of amino acids 1-159 of luciferase of SEQ ID NO: 1 (N-nano$_{1-159}$), and the C-terminal fragment has a sequence of amino acids 160-170 of luciferase of SEQ ID NO: 1 (C-nano$_{160-170}$).

4. A single chain polypeptide according to Claim 3, in which the N-terminal fragment comprises a sequence with at least 90% sequence identity to amino acids 1-159 of luciferase of SEQ ID NO: 1 (N-nano$_{1-159}$), and the C-terminal fragment comprises a sequence with at least 90% sequence identity amino acids 160-170 of luciferase of SEQ ID NO: 1 (C-nano$_{160-170}$).

5. A single chain polypeptide according to Claim 3, in which the N-terminal fragment comprises a sequence having less than 10 amino acid residues substitutions, deletions, or additions relative to amino acids 1-159 of luciferase of SEQ ID NO: 1 (N-nano$_{1-159}$), and the C-terminal fragment comprises a sequence having less than 3 amino acid residues substitutions, deletions, or additions relative to amino acids 160-170 of luciferase of SEQ ID NO: 1 (C-nano$_{160-170}$).

6. A single chain polypeptide according to Claim 1, in which the BoNT cleavage site is recognized by BoNT of serotype A, E, C, B, D, F, or G.

7. A single chain polypeptide according to Claim 1, in which the BoNT cleavage site is from a SNARE protein.

8. A single chain polypeptide according to Claim 7, in which the SNARE protein is SNAP-25, synaptobrevin (VAMP), or syntaxin.

9. A single chain polypeptide according to Claim 7, in which the SNARE protein is amino acids 141-206 of human SNAP-25b.

10. A single chain polypeptide according to Claim 7, in which the SNARE protein is amino acids 35-96 of human VAMP1.

11. A single chain polypeptide according to Claim 7, in which the SNARE protein is amino acids 1-206 of human SNAP25b.

12. A single chain polypeptide according to Claim 1, in which the neurotoxin binding fragment is located between the N-terminal fragment and the neurotoxin cleavage site.

13. A single chain polypeptide according to Claim 1, in which the neurotoxin binding fragment is a human, mouse, rat, or primate neurotoxin receptor.

14. A single chain polypeptide according to Claim 13, in which the neurotoxin receptor is SV2C.

15. A single chain polypeptide according to Claim 13, in which the neurotoxin receptor comprises amino acids 529-566 of human SV2C.

16. A single chain polypeptide according to Claim 13, in which the neurotoxin receptor is SYTI.

17. A single chain polypeptide according to Claim 13, in which the neurotoxin receptor comprises amino acids 32-52 of SYTI.

18. A single chain polypeptide according to Claim 13, in which the neurotoxin receptor is SYT2.

19. A single chain polypeptide according to Claim 13, in which the neurotoxin receptor comprises amino acids 40-60 of SYT2.

20. A single chain polypeptide according to Claim 2, in which the linker further comprises an intact second reporter polypeptide located between the N-terminal fragment and the neurotoxin cleavage site.

21. A single chain polypeptide according to Claim 20, in which the second reporter polypeptide is firefly luciferase (e.g., *Photinus pyralis*), bacterial luciferase (e.g., *Vibrio fischeri*, *Vibrio harveyi*), sea pansy luciferase (*Renilla reniformis*), dinoflagellate luciferase, *Gaussia* luciferase, or copepod luciferase.

22. A single chain polypeptide according to Claim 2, further comprising a polyhistidine affinity tag.

23. A single chain polypeptide according to Claim 2, in which the linker further comprises one or more spacers located between the neurotoxin cleavage site and the N-terminal fragment, and one or more spacers located between the neurotoxin cleavage site and the C-terminal fragment, or a combination thereof.

24. A single chain polypeptide according to Claim 12, in which the linker further comprises one or more spacers located between the neurotoxin binding fragment and the N-terminal fragment.

25. A single chain polypeptide according to Claim 12, in which the linker further comprises one or more spacers located between the neurotoxin binding fragment and the neurotoxin cleavage site.

26. A single chain polypeptide according to Claim 20, in which the linker further comprises one or more spacers located between the second reporter polypeptide and the N-terminal fragment.

27. A single chain polypeptide according to Claim 20, in which the linker further comprises one or more spacers located between the second reporter polypeptide and the neurotoxin cleavage site.

28. A nucleic acid comprising a nucleotide sequence that encodes the single chain polypeptide according to Claim 2.

29. A nucleic acid vector comprising a nucleic acid according to Claim 28.

30. A cell comprising a nucleic acid according to Claim 28.

31. A method for determining potency of *C. botulinum neurotoxin* (BoNT), comprising:

(a) contacting the BoNT to a single chain polypeptide under conditions appropriate for BoNT activity, in which the single chain polypeptide comprises:

(i) an N-terminal fragment of a reporter protein;

(ii) a linker comprising a binding fragment of a receptor for the BoNT and a BoNT cleavage site; and

(iii) a C-terminal fragment of the reporter protein; and

(b) determining reporter activity of the polypeptide, as compared to a reference, thereby determining potency, in which the BoNT is determined to be more potent than the reference if it decreases reporter activity compared to the reference.

32. A method for detecting *C. botulinum neurotoxin* (BoNT) activity in a sample, comprising:

(a) contacting the sample to a single chain polypeptide under conditions appropriate for BoNT activity, in which the single chain polypeptide comprises:

(i) an N-terminal fragment of a reporter protein;

(ii) a linker comprising a binding fragment of a receptor for the BoNT and a BoNT cleavage site; and

(iii) a C-terminal fragment of the reporter protein; and

(b) determining reporter activity of the polypeptide, as compared to reporter activity of the polypeptide in the absence of the sample, in which a decrease of reporter activity indicates neurotoxin activity in the sample.

33. A method according to Claim 33, in which the step of contacting comprises contacting the BoNT to neuronal cells that express the single chain polypeptide and the method further comprises between the contacting step:

incubating the neuronal cells for a period from about 12 hours to about 60 hours, and harvesting lysate from the neuronal cells.

34. A kit comprising:

(a) one or more single chain polypeptides described in Claim 2, with each or a combination of the single chain polypeptides packaged into a separate container;

(b) one or more nucleic acids or nucleic acid vectors comprising a nucleotide sequence that encodes the one or more single chain polypeptide of (a), with each or a combination of the nucleic acids or nucleic acid vectors packaged into a separate container; and/or

(c) one or more cells that express the one or more single chain polypeptide of (a), with each or a combination of the cells packaged into a separate container.