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.