comprising an exogenous protease cleavage site and an open reading frame encoding a protease that can proteolytically cleave the exogenous protease cleavage site located in the di-chain loop region. In further aspects, the single-chain protein comprising a di-chain loop region comprising an exogenous protease cleavage site can be, *e.g.*, a Clostridial toxin comprising a di-chain loop region comprising an exogenous protease cleavage site, a modified Clostridial toxin comprising a di-chain loop region comprising an exogenous protease cleavage site, or a single-chain protein comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a non-Clostridial toxin binding domain, and a di-chain loop region comprising an exogenous protease cleavage site as disclosed in the present specification.

[010] Yet other aspects of the present specification provide an intracellular method of converting a single-chain protein into its di-chain form, the method comprising the steps of a) growing a cell comprising a dual expression construct at a first temperature for a certain period of time in order to achieve maximal cell density, the dual expression construct comprising; i) an open reading frame encoding a single-chain protein comprising a di-chain loop region comprising an exogenous protease cleavage site; and ii) an open reading frame encoding a protease; wherein the protease can cleave the exogenous protease cleavage site located within the di-chain loop; b) growing the cell at a second temperature for a certain period of time in order to achieve maximal induction of protein expression from the open reading frame encoding the single-chain protein, wherein growth at step (b) induces expression of the single-chain protein and the protease from the dual expression construct; and wherein the produced protease cleaves the single-chain protein at the exogenous protease cleavage site located within the di-chain loop region, thereby converting the single-chain protein into its di-chain form.

[011] Still other aspects of the present specification provide an intracellular method of converting a single-chain Clostridial toxin into its di-chain form, the method comprising the steps of: a) growing a cell comprising a dual expression construct at 37 °C for about 2 to about 3.5 hours, the dual expression construct comprising; i) an open reading frame encoding a single-chain Clostridial toxin, the single-chain Clostridial toxin comprising an enzymatic domain, a translocation domain, a binding domain, and a di-chain loop region comprising an exogenous protease cleavage site; and ii) an open reading frame encoding a protease; wherein the protease can cleave the exogenous protease cleavage site located within the di-chain loop; b) growing the cell at 22 °C for about 16 to about 18 hours, wherein growth at step (b) induces expression of the single-chain Clostridial toxin and the protease from the dual expression construct; and wherein the produced protease cleaves the single-chain Clostridial toxin at the exogenous protease cleavage site located within the di-chain loop region, thereby converting the single-chain Clostridial toxin into its di-chain form.

[011a] In one aspect, there is provided an intracellular method of converting a single-chain Clostridial toxin comprising a di-chain loop region into its di-chain form, the method comprising the steps of:

a) growing a cell comprising a dual expression construct at 37 °C for about 3.5 hours, the dual expression construct comprising;

i) an open reading frame encoding a single-chain Clostridial toxin, the single-chain Clostridial toxin comprising an enzymatic domain, a translocation domain, a binding domain, and a di-chain loop region comprising a TEV protease cleavage site; and

ii) an open reading frame encoding a TEV protease;

b) growing the cell at 22 °C for about 16 to about 18 hours,

wherein growth at step (b) induces expression of the single-chain Clostridial toxin and the TEV protease from the dual expression construct; and

wherein the produced TEV protease cleaves the single-chain Clostridial toxin at the TEV protease cleavage site located within the di-chain loop region, thereby converting the single-chain Clostridial toxin into its di-chain form.

[011b] In one aspect, there is provided an intracellular method of converting a single-chain protein into its di-chain form, the method comprising the steps of

a) growing a cell comprising a dual expression construct at 37 °C for about 8 hours, the dual expression construct comprising;

i) an open reading frame encoding a single-chain protein, the single-chain protein comprising an enzymatic domain, a translocation domain, an integrated TEV protease cleavage site-opioid binding domain; and

ii) an open reading frame encoding a TEV protease;

b) growing the cell at about 12 to about 16 °C for about 16 to about 18 hours,

wherein growth at step (b) induces expression of the single-chain protein and the TEV protease from the dual expression construct; and

wherein the produced TEV protease cleaves the single-chain protein at the TEV protease cleavage site located within the integrated TEV cleavage site opioid binding domain, thereby converting the single-chain protein into its di-chain form.

[011c] In one aspect, there is provided an intracellular method of converting a single-chain protein into its di-chain form, the method comprising the steps of

a) growing a cell comprising a dual expression construct at 37 °C for about 8 hours, the dual expression construct comprising;

i) an open reading frame encoding a single-chain protein, the single-chain protein comprising an enzymatic domain, a translocation domain, a non-Clostridial toxin binding domain and a di-chain loop region comprising a TEV protease cleavage site; and

ii) an open reading frame encoding a TEV protease;

b) growing the cell at about 12 to about 16 °C for about 16 to about 18 hours,

wherein growth at step (b) induces expression of the single-chain protein and the TEV protease from the dual expression construct; and

wherein the produced TEV protease cleaves the single-chain protein at the TEV protease cleavage site located within the di-chain loop region, thereby converting the single-chain protein into its di-chain form.

[012] Further aspects of the present specification provide an intracellular method of converting a single-chain protein into its di-chain form, the method comprising the steps of a) growing a cell comprising a dual expression construct at 37 °C for about 2 to about 8 hours, the dual expression construct comprising; i) an open reading frame encoding a single-chain protein, the single-chain protein comprising an enzymatic domain, a translocation domain, and an integrated TEV protease cleavage site-opioid binding domain; and ii) an open reading frame encoding a TEV protease; b) growing the cell at about 12 to about 16 °C for about 16 to about 18 hours, wherein growth at step (b) induces expression of the single-chain protein and the TEV protease from the dual expression construct; and wherein the