

the subsequent processing into mature hFXN after translocation into the mitochondria. The FXN fusion protein is described in US Provisional Patent Application No. 62/891,029 and US Patent Application No. 16/942,276, the entire contents of each of which are hereby incorporated herein by reference.

5 To facilitate development of FXN replacement therapies, methods are needed to accurately and reliably quantify activity of FXN and FXN fusion proteins. For example, it is desirable to quantify activity of various batches of FXN fusion proteins, as a quality control measure of the manufacturing process for producing the FXN fusion proteins. It is also desirable to quantify activity of various batches of FXN fusion proteins to identify
10 appropriate storage conditions for the manufactured batches of FXN fusion proteins. The present invention addresses this unmet need.

SUMMARY

15 In one aspect, the present disclosure refers to a method for measuring activity of a Frataxin (FXN) protein, said method comprising combining said FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound at a pH of greater than 7.4, thereby producing an assay mixture, wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising the reducing agent
20 and the ROS detector compound without the FXN protein.

In another aspect, the present disclosure refers to a method for measuring activity of a Frataxin (FXN) protein, said method comprising combining said FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound in the absence of metal ions, thereby producing an assay mixture, and wherein said FXN protein facilitates
25 oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising the reducing agent and the ROS detector compound without the FXN protein.

In another aspect, the present disclosure refers to a method for measuring activity of a Frataxin (FXN) protein, said method comprising combining said FXN protein with a
30 reducing agent and a reactive oxygen species (ROS) detector compound in the absence of iron ions, thereby producing an assay mixture, wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising the reducing agent and the ROS detector compound without the FXN protein.

35 In another aspect, the present disclosure refers to a method for measuring activity of a Frataxin (FXN) protein, said method comprising combining said FXN protein with a

reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture; and measuring the amount of ROS generated in said assay mixture over time, wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising a reducing agent and an ROS detector compound without the FXN protein.

In another aspect, the present disclosure refers to a method for performing quality control on a sample comprising a Frataxin (FXN) protein, said method comprising measuring activity of an FXN protein in accordance with the method as disclosed herein.

In another aspect, the present disclosure refers to a composition for measuring activity of an FXN protein, said composition comprising an FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein pH of the composition is greater than 7.4.

In another aspect, the present disclosure refers to a composition for measuring activity of an FXN protein, said composition comprising an FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein the composition is substantially free of a metal ions.

In another aspect, the present disclosure refers to a composition for measuring activity of an FXN protein, said composition comprising an FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein the composition is substantially free of iron ions.

In another aspect, the present disclosure refers to a composition comprising an FXN protein, wherein the FXN protein exhibits a specific activity of hydroquinone reduction within the range of about 1000 mU/mg of FXN protein to about 6500 mU/mg of FXN protein as measured by the method as disclosed herein.

In another aspect, the present disclosure refers to a kit for determining activity of an FXN protein, said kit comprising a reducing agent, an ROS detector compound and instructions to combine the reducing agent, the ROS detector compound and an FXN protein at a pH of greater than about 7.4, thereby producing an assay mixture.

In another aspect, the present disclosure refers to a kit for determining activity of an FXN protein, said kit comprising a reducing agent, an ROS detector compound and instructions to combine the reducing agent, the ROS detector compound and an FXN protein, thereby producing an assay mixture; and determine V_i of the FXN protein in the assay mixture.

In another aspect, the present disclosure refers to a method for identifying a compound capable of modulating activity of a Frataxin (FXN) protein, said method comprising the steps of: (i) combining said FXN protein with a test compound, a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS; (ii) measuring the amount of ROS generated in the presence of the test compound; (iii) comparing the amount of ROS generated in the presence of the test compound with an amount of ROS generated in the absence of the test compound; and (iv) identifying the test

compound as a compound capable of modulating activity of the FXN protein when the amount of ROS generated in the presence of the test compound is different from the amount of ROS generated in the absence of the test compound.

5 In another aspect, the present disclosure refers to a method for identifying a compound capable of modulating activity of a Frataxin (FXN) protein, said method comprising the steps of: (i) combining said FXN protein with a test compound, a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS; (ii) measuring the amount of ROS generated in the presence of the test compound and determining V_i of the FXN protein (iii) comparing the V_i of the FXN protein in the presence of the test compound with V_i of the FXN protein in the absence of the test compound; and (iv) identifying the test compound as a compound capable of modulating activity of the FXN protein when V_i of the FXN protein in the presence of the test compound is different from V_i of the FXN protein in the absence of the test compound.

15 In another aspect, the present disclosure refers to a composition comprising an FXN protein, wherein activity of the FXN protein has been measured according to the method as disclosed herein.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising an FXN protein and a pharmaceutically acceptable excipient, wherein activity of the FXN protein has been measured according to the method as disclosed herein.

20 In yet another aspect, the present disclosure refers to a method of preparing a pharmaceutical composition comprising an FXN protein, the method comprising measuring activity of the FXN protein according to the method as disclosed herein, and formulating the FXN protein, thereby preparing the pharmaceutical composition.

25 Accordingly, in some examples the present disclosure provides a method for measuring activity of a Frataxin (FXN) protein, the method comprising: combining the FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein the FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising the reducing agent and the ROS detector compound without the FXN protein.

30 In some examples, the present disclosure provides a method for measuring activity of a Frataxin (FXN) protein, the method comprising combining the FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound at a pH of greater than about 7.4, thereby producing an assay mixture, wherein the FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising the reducing agent and the ROS detector compound without the FXN protein.

35 In some examples, the method comprises combining the FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound at a pH of about 7.9 or greater.

In some examples, the present disclosure provides a method for measuring activity of a Frataxin (FXN) protein, the method comprising combining the FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound in the absence of metal ions, thereby producing an assay mixture, wherein the FXN protein facilitates oxidation of the
5 reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising the reducing agent and the ROS detector compound without the FXN protein.

In some examples, the present disclosure provides a method for measuring activity of a Frataxin (FXN) protein, the method comprising combining the FXN protein with a reducing
10 agent and a reactive oxygen species (ROS) detector compound in the absence of iron ions, thereby producing an assay mixture, wherein the FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising the reducing agent and the ROS detector compound without the FXN protein.

15 In some examples, the method further comprises adjusting pH of the assay mixture to pH of greater than about 7.4. In some examples, the method further comprises adjusting pH of the assay mixture to pH of about 7.9 or greater.

In some examples, the method further comprises adding a metal ion into the assay mixture. In some examples, the metal ion is not Fe^{2+} . In some examples, the metal ion is
20 selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} . In one example, the metal ion is Mn^{2+} . In another example, the metal is Fe^{3+} .

In some examples, the method further comprises incubating the assay mixture. In some examples, the method further comprises measuring the amount of ROS generated in the assay mixture. In some examples, the method further comprises measuring the amount of ROS
25 generated in the assay mixture over time.

In some examples, the method further comprises measuring the amount of ROS generated at regular time intervals. In some examples, the method comprises measuring the amount of ROS generated about every 0.1 second to about every 10 minutes, *e.g.*, about every 0.1 second to about every 2 seconds, about every 1 second to about every 10 seconds, about every 5 seconds to about
30 every 60 seconds, about every 30 seconds to about every 5

minutes or about every 2 minutes to about every 10 minutes after the start of incubation of the assay mixture.

In some examples, the method further comprises determining maximum initial velocity (Vi) of ROS generation in the assay mixture. In some examples, the method further comprises
5 determining time to maximum initial velocity (Ti). In some examples, the method further comprises determining the unit activity of the FXN protein at pH of greater than about 7.4.

In some examples, the present disclosure provides a method for measuring activity of a Frataxin (FXN) protein, the method comprising combining the FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay
10 mixture; and measuring the amount of ROS generated in the assay mixture over time, wherein the FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising a reducing agent and an ROS detector compound without the FXN protein.

In some examples, the method comprises measuring the amount of ROS generated at
15 regular time intervals. In some examples, the method comprises measuring the amount of ROS generated about every 0.1 second to about every 10 minutes, *e.g.*, about every 0.1 second to about every 2 seconds, about every 1 second to about every 10 seconds, about every 5 seconds to about every 60 seconds, about every 30 seconds to about every 5 minutes or about every 2 minutes to about every 10 minutes.

In some examples, the method further comprises determining maximum initial velocity
20 (Vi) of ROS generation in the assay mixture. In some examples, the method further comprises determining time to maximum initial velocity (Ti). In some examples, the method further comprises determining the unit activity of the FXN protein.

In some examples, the reducing agent is an organic compound. In some examples, the
25 reducing agent is a reduced quinone compound. In some examples, the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and reduced anthraquinone compound. In

some examples, the reduced quinone compound is a reduced benzoquinone compound. In some examples, the reduced benzoquinone compound is hydroquinone.

In some examples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some examples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some examples, the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H2DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof. In some examples, the ROS detector compound is 2',7'-dichlorodihydrofluorescein diacetate (H2DCF).

In some examples, the reducing agent and the ROS detector compound are the same compound. In some examples, the reducing agent and the ROS detector compound are different compounds. In one example, the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H2DCF).

In some examples, the FXN protein is frataxin (FXN). In some examples, the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP). In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof.

In some examples, the CPP comprises HIV-TAT or a variant or derivative thereof. In one example, the FXN fusion protein comprises SEQ ID NO: 12.

In some examples, the present disclosure provides a method for performing quality control on a sample comprising a Frataxin (FXN) protein, the method comprising measuring activity of an FXN protein in accordance with methods of the disclosure.

In some examples, the present disclosure provides a composition for measuring activity of an FXN protein, the composition comprising an FXN protein, a reducing agent and a

reactive oxygen species (ROS) detector compound, wherein pH of the composition is greater than about 7.4. In some examples, pH of the composition is about 7.9 or greater.

In some examples, the composition further comprises a metal ion. In some examples, the metal ion is not Fe^{2+} . In some examples, the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} . In one example, the metal ion is Fe^{3+} . In another example, the metal ion is Mn^{2+} .

In some examples, the present disclosure also provides a composition for measuring activity of an FXN protein, the composition comprising an FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein the composition is substantially free of a metal ions.

In some examples, the present disclosure also provides a composition for measuring activity of an FXN protein, the composition comprising an FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein the composition is substantially free of iron ions.

In some examples, pH of the composition is greater than about 7.4. In some examples, pH of the composition is about 7.9 or greater.

In some examples, the reducing agent is an organic compound. In some examples, the reducing agent is a reduced quinone compound. In some examples, the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and a reduced anthraquinone compound. In some examples, the reduced quinone compound is a reduced benzoquinone compound. In some examples, the reduced benzoquinone compound is hydroquinone.

In some examples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some examples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some examples, the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H2DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfo-phenyl)-2H-Tetrazolium-5-

Carboxanilide (XTT) and structural variants and analogs thereof. In some examples, the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

5 In some examples, the ROS detector compound are the same compound. In some examples, the reducing agent and the ROS detector compound are different compounds. In one example, the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

10 In some examples, the FXN protein is frataxin (FXN). In some examples, the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP). In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof.

In some examples, the CPP comprises HIV-TAT or a variant or derivative thereof. In some examples, the FXN fusion protein comprises SEQ ID NO: 12.

15 In some examples, the present disclosure also provides a composition comprising an FXN protein, wherein the FXN protein exhibits a specific activity of hydroquinone reduction within the range of about 1000 mU/mg of FXN protein to about 6500 mU/mg of FXN protein. In some examples, the specific activity of hydroquinone reduction is within the range of about 1200 mU/mg to about 6000 mU/mg. In some examples, the specific activity is within the range of
20 about 1400 mU/mg to about 5900 mU/mg.

In some examples, the present disclosure also provides a kit for determining activity of an FXN protein, the kit comprising a reducing agent, an ROS detector compound and instructions to combine the reducing agent, the ROS detector compound and an FXN protein, thereby producing an assay mixture.

25 In some examples, the present disclosure also provides a kit for determining activity of an FXN protein, the kit comprising a reducing agent, an ROS detector compound and instructions to combine the reducing agent, the ROS detector compound and an FXN protein, thereby producing an assay mixture; and determine V_i of the FXN protein in the assay mixture.

In some examples, the kit further comprises a metal ion compound. In some examples, the metal ion compound comprises an ion selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} . In one example, the metal ion compound comprises Fe^{3+} . In another example, the metal ion compound comprises Mn^{2+} .

5 In some examples, the reducing agent is an organic compound. In some examples, the reducing agent is a reduced quinone compound. In some examples, the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and a reduced anthraquinone compound. In some examples, the reduced quinone compound is a reduced benzoquinone compound. In some examples, the reduced benzoquinone
10 compound is hydroquinone.

In some examples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some examples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some examples, the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein
15 (H_2DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfo-phenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof. In some examples, the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H_2DCF).

20 In some examples, the reducing agent and the ROS detector compound are the same compound. In some examples, the reducing agent and the ROS detector compound are different compounds. In some examples, the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H_2DCF).

In some examples, the present disclosure also provides a method for identifying a compound
25 capable of modulating activity of a Frataxin (FXN) protein, the method comprising the steps of: (i) combining the FXN protein with a test compound, a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein the FXN protein facilitates oxidation of the reducing agent and generation of ROS; (ii) measuring the amount of ROS generated in the presence of the test compound; (iii) comparing the amount of ROS generated in the presence of
30 the test compound with an

amount of ROS generated in the absence of the test compound; and (iv) identifying the test compound as a compound capable of modulating activity of the FXN protein when the amount of ROS generated in the presence of the test compound is different from the amount of ROS generated in the absence of the test compound.

5 In some examples, the method further comprises incubating the assay mixture. In some examples, step (ii) comprises measuring the amount of ROS generated in the assay mixture over time. In some examples, step (ii) comprises measuring the amount of ROS generated at regular time intervals. In some examples, step (ii) comprises measuring the amount of ROS generated every 0.1 second to every 10 minutes, *e.g.*, about every 0.1 second to about every 2 seconds,
10 about every 1 second to about every 10 seconds, about every 5 seconds to about every 60 seconds, about every 30 seconds to about every 5 minutes or about every 2 minutes to about every 10 minutes.

 In some examples, the method further comprises determining maximum initial velocity (Vi) of ROS generation in the assay mixture. In some examples, the method further comprises
15 determining time to maximum initial velocity (Ti). In some examples, the method further comprises determining the unit activity of the FXN protein.

 In some examples, the method further comprises the steps of: comparing Vi of the FXN protein in the presence of the test compound with Vi of the FXN protein in the absence of the test compound; and identifying the test compound as a compound capable of modulating activity of
20 the FXN protein when the Vi of the FXN protein in the presence of the test compound is different from the Vi of the FXN protein in the absence of the test compound.

 In some examples, the present disclosure comprises identifying a compound capable of modulating activity of a Frataxin (FXN) protein, the method comprising the steps of: (i) combining the FXN protein with a test compound, a reducing agent and a reactive oxygen species
25 (ROS) detector compound, thereby producing an assay mixture, wherein the FXN protein facilitates oxidation of the reducing agent and generation of ROS; (ii) measuring the amount of ROS generated in the presence of the test compound and determining Vi of the FXN protein; (iii) comparing the Vi of the FXN protein in the presence of the test compound with Vi of the FXN protein in the absence of the test compound; and (iv) identifying the test compound as a
30 compound capable of modulating activity of the FXN protein when Vi of the

FXN protein in the presence of the test compound is different from Vi of the FXN protein in the absence of the test compound.

5 In some examples, the method further comprises adjusting pH of the assay mixture to pH of greater than about 7.4. In some examples, the method further comprises adjusting pH of the assay mixture to pH of about 7.9 or greater.

In some examples, the method further comprises adding a metal ion to the assay mixture. In some examples, the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} . In one example, the metal ion is Mn^{2+} . In another example, the metal ion is Fe^{3+} .

10 In some examples, the reducing agent is an organic compound. In some examples, the reducing agent is a reduced quinone compound. In some examples, the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and reduced anthraquinone compound. In some examples, the reduced quinone compound is a reduced benzoquinone compound. In some examples, the reduced benzoquinone compound is hydroquinone.

15 In some examples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some examples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some examples, the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H₂DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA),
20 Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulphophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof. In some examples, the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

25 In some examples, the reducing agent and the ROS detector compound are the same compound. In some examples, the reducing agent and the ROS detector compound are different compounds. In one example, the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

In some examples, the FXN protein is frataxin (FXN). In some examples, the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP). In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof.

In some examples, the CPP comprises HIV-TAT or a variant or derivative thereof. In some examples, the FXN fusion protein comprises SEQ ID NO: 12.

In some examples, the present disclosure also provides a composition comprising an FXN protein, wherein activity of the FXN protein has been measured according to the methods of the present disclosure.

In some examples, the present disclosure provides a pharmaceutical composition comprising an FXN protein and a pharmaceutically acceptable excipient, wherein activity of the FXN protein has been measured according to the method of the present disclosure.

In some examples, the present disclosure provides a method of preparing a pharmaceutical composition comprising an FXN protein, the method comprising measuring activity of the FXN protein according to the method of the present disclosure, and formulating the FXN protein, thereby preparing the pharmaceutical composition.

In some examples, the present disclosure provides a method for measuring activity of a Frataxin (FXN) protein, the method comprising combining the FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein the FXN protein facilitates oxidation of the reducing agent and generation of ROS.

In some examples, the method further comprises adding a metal ion into the assay mixture. In further examples, the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} . In one further example, the metal ion is Fe^{2+} . In another further example, the metal ion is Mn^{2+} .

In some examples, the method further comprises incubating the assay mixture. In some examples, the method further comprises measuring the amount of ROS generated. In some examples, the method further comprises correlating the amount of ROS generated with activity of the FXN protein, thereby determining the activity the FXN protein.

5 In some examples, the reducing agent is an organic compound. In some examples, the reducing agent is a reduced quinone compound. In some examples, the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and reduced anthraquinone compound. In some examples, the reduced quinone compound is a reduced benzoquinone compound. In one example, the reduced benzoquinone compound is
10 hydroquinone.

In some examples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some examples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some examples, the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein
15 (H₂DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfo-phenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof. In one specific example, the ROS detector compound is 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF).

20 In some examples, the reducing agent and the ROS detector compound are the same compound. In other examples, the reducing agent and the ROS detector compound are different compounds.

In some examples, the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

25 In some examples, the FXN protein is frataxin (FXN). In some examples, the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).

In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, the

CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof. In one example, the CPP comprises HIV-TAT or a variant or derivative thereof.

In some examples, the FXN fusion protein comprises, or consists of, SEQ ID NO: 12.

5 In some examples, the present disclosure provides a method of performing quality control on a sample comprising a Frataxin (FXN) protein, the method comprising measuring activity of an FXN protein in accordance with the methods of the present disclosure.

In some examples, the present disclosure provides a composition for measuring activity of an FXN protein, the composition comprising an FXN protein, a reducing agent and a reactive
10 oxygen species (ROS) detector compound.

In some examples, the composition further comprises a metal ion. In some examples, the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} . In one further example, the metal ion is Fe^{2+} . In another further example, the metal ion is Mn^{2+} .

In some examples, the reducing agent is an organic compound. In some examples, the
15 reducing agent is a reduced quinone compound. In some examples, the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and a reduced anthraquinone compound.

In some examples, the reduced quinone compound is a reduced benzoquinone compound. In one example, the reduced benzoquinone compound is hydroquinone.

20 In some examples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some examples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some examples, the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H_2DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA),
25 Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-

Carboxanilide (XTT) and structural variants and analogs thereof. In one example, the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

5 In some examples, the reducing agent and the ROS detector compound are the same compound. In other examples, the reducing agent and the ROS detector compound are different compounds. In one example, the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

10 In some examples, the FXN protein is frataxin (FXN). In some examples, the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP). In some examples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some examples, the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof. In one example, the CPP comprises HIV-TAT or a variant or derivative thereof.

In one example, the FXN fusion protein comprises, or consists of, SEQ ID NO: 12.

15 In some examples, the present disclosure provides a composition comprising an FXN protein, wherein the FXN protein exhibits a specific activity of hydroquinone reduction within the range of about 1000 mU/mg of FXN protein to about 6500 mU/mg of FXN protein. In some examples, the specific activity of hydroquinone reduction is within the range of about 1200 mU/mg to about 6000 mU/mg. In some examples, the specific activity is within the range of
20 about 1400 mU/mg to about 5900 mU/mg.

In some examples, the present disclosure provides a method for identifying a compound capable of modulating activity of a Frataxin (FXN) protein, the method comprising the steps of:
(i) combining the FXN protein with a test compound, a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein the FXN protein
25 facilitates oxidation of the reducing agent and generation of ROS; (ii) measuring the amount of ROS generated in the presence of the test compound; (iii) comparing the amount of ROS generated in the presence of the test compound with an amount of ROS generated in the absence of the test compound; and (iv) identifying the test compound as a compound capable of modulating activity of the FXN protein when the

amount of ROS generated in the presence of the test compound is different from the amount of ROS generated in the absence of the test compound.

In some examples, the method further comprises incubating the assay mixture. In some examples, the method further comprises correlating the amount of ROS generated with activity of the FXN protein, thereby determining the activity the FXN protein.

In some examples, the reducing agent is an organic compound. In some examples, the reducing agent is a reduced quinone compound. In some examples, the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and reduced anthraquinone compound. In some examples, the reduced quinone compound is a reduced benzoquinone compound. In one example, the reduced benzoquinone compound is hydroquinone.

In some examples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some examples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some examples, the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H₂DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof. In one example, the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

In some examples, the reducing agent and the ROS detector compound are the same compound. In other examples, the ROS detector compound are different compounds.

In one specific example, the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

In some examples, the FXN protein is frataxin (FXN).

In some examples, the present disclosure also provides a composition comprising an FXN protein, wherein activity of the FXN protein has been measured according to methods provided by the present disclosure.

In some examples, the present disclosure also provides a pharmaceutical composition comprising an FXN protein and a pharmaceutically acceptable excipient, wherein activity of the FXN protein has been measured according to methods provided of the present disclosure.

5 In some examples, the present disclosure also provides a method of preparing a pharmaceutical composition comprising an FXN protein, the method comprising measuring activity of the FXN protein according to methods provided by the present disclosure, and formulating the FXN protein, thereby preparing the pharmaceutical composition.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Non-limiting examples of embodiments of the disclosure are described below with reference to figures attached hereto that are listed following this paragraph. Identical features that appear in more than one figure are generally labeled with a same label in all the figures in which they appear. A label labeling an icon representing a given feature of an embodiment of the disclosure in a figure may be used to reference the given feature. Dimensions of
15 features shown in the figures are chosen for convenience and clarity of presentation and are not necessarily shown to scale.

Figure 1 shows the kinetics of hydroquinone (HQ) reduction in the presence of an exemplary FXN fusion protein, as measured by the amount of reactive oxygen species (ROS) generated over time. The amount of ROS is expressed as fluorescence, measured in arbitrary
20 fluorescence units (AFU). **Panel A of Figure 1** is a graph showing the kinetics of superoxide ($O_2^{\bullet-}$) generation *via* oxidation of hydroquinone (HQ) in the presence or absence of the FXN fusion protein, in accordance with an embodiment of the disclosure. **Panel B of Figure 1** is a graph showing the kinetics of superoxide generation in the presence of the FXN fusion protein and metal ions, such as Mn^{2+} and Fe^{3+} , in accordance with an embodiment of the
25 disclosure. **Panel C of Figure 1** is a graph showing the kinetics of superoxide generation in the presence of the FXN fusion protein and Fe^{3+} (gray squares); in the presence of the FXN fusion protein and in the absence of Fe^{3+} (gray circles); in the presence of the FXN fusion protein, Fe^{3+} and deferoxamine (black circles); and in the absence of the FXN fusion protein (small black rectangles).

CLAIMS

What is claimed is:

1. A method for measuring activity of a Frataxin (FXN) protein, said method comprising
combining said FXN protein with a reducing agent and a reactive oxygen species (ROS)
5 detector compound at a pH of greater than 7.4, thereby producing an assay mixture,
wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS,
such that the amount of ROS generated is higher than the amount of ROS generated in an assay
mixture comprising the reducing agent and the ROS detector compound without the FXN protein.
2. The method of claim 1, comprising combining said FXN protein with a reducing agent and a
10 reactive oxygen species (ROS) detector compound at a pH of about 7.9 or greater.
3. A method for measuring activity of a Frataxin (FXN) protein, said method comprising
combining said FXN protein with a reducing agent and a reactive oxygen species (ROS)
detector compound in the absence of metal ions, thereby producing an assay mixture, and
wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS,
15 such that the amount of ROS generated is higher than the amount of ROS generated in an assay
mixture comprising the reducing agent and the ROS detector compound without the FXN protein.
4. A method for measuring activity of a Frataxin (FXN) protein, said method comprising
combining said FXN protein with a reducing agent and a reactive oxygen species (ROS)
detector compound in the absence of iron ions, thereby producing an assay mixture,
20 wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS,
such that the amount of ROS generated is higher than the amount of ROS generated in an assay
mixture comprising the reducing agent and the ROS detector compound without the FXN protein.
5. The method of claim 3 or 4, further comprising adjusting pH of the assay mixture to pH of
greater than 7.4.
- 25 6. The method of claim 5, further comprising adjusting pH of the assay mixture to pH of about
7.9 or greater.

7. The method of any one of claims 1-2 and 5-6, further comprising adding a metal ion into the assay mixture.
8. The method of claim 7, wherein the metal ion is not Fe^{2+} .
9. The method of claim 7, wherein the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
10. The method of claim 9, wherein the metal ion is Mn^{2+} .
11. The method of claim 9, wherein the metal is Fe^{3+} .
12. The method of any one of claims 1-11, further comprising incubating said assay mixture.
13. The method of any one of claims 1-11, further comprising measuring the amount of ROS generated in said assay mixture.
14. The method of claim 13, comprising measuring the amount of ROS generated in the assay mixture over time.
15. The method of claim 14, comprising measuring the amount of ROS generated at regular time intervals.
16. The method of claim 14 or claim 15, comprising measuring the amount of ROS generated about every 0.1 second to about every 10 minutes after the start of incubation of the assay mixture.
17. The method of any one of claims 14-16, further comprising determining maximum initial velocity (V_i) of ROS generation in the assay mixture.
18. The method of claim 17, further comprising determining time to maximum initial velocity (T_i).
19. The method of claim 18, further comprising determining the unit activity of the FXN protein at pH of greater than 7.4.
20. A method for measuring activity of a Frataxin (FXN) protein, said method comprising combining said FXN protein with a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture; and measuring the amount of ROS generated in said assay mixture over time,

wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS, such that the amount of ROS generated is higher than the amount of ROS generated in an assay mixture comprising a reducing agent and an ROS detector compound without the FXN protein.

- 5 21. The method of claim 20, comprising measuring the amount of ROS generated at regular time intervals.
22. The method of claim 20 or claim 21, comprising measuring the amount of ROS generated about every 0.1 second to about every 10 minutes.
23. The method of any one of claims 20-22, further comprising determining maximum initial velocity (V_i) of ROS generation in the assay mixture.
- 10 24. The method of claim 23, further comprising determining time to maximum initial velocity (T_i).
25. The method of claim 24, further comprising determining the unit activity of the FXN protein.
26. The method of any one of claims 1-25, wherein the reducing agent is an organic compound.
27. The method of claim 26, wherein the reducing agent is a reduced quinone compound.
- 15 28. The method of claim 27, wherein the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and reduced anthraquinone compound.
29. The method of claim 28, wherein the reduced quinone compound is a reduced benzoquinone compound.
- 20 30. The method of claim 29, wherein the reduced benzoquinone compound is hydroquinone.
31. The method of any one of claims 1-30, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
32. The method of any one of claims 1-30, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
- 25 33. The method of any one of claims 1-32, wherein the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H_2DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT),

RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfo-phenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof.

34. The method of claim 33, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF).
- 5 35. The method of any one of claims 1-34, wherein the reducing agent and the ROS detector compound are the same compound.
36. The method of any one of claims 1-34, wherein the reducing agent and the ROS detector compound are different compounds.
37. The method of claim 36, wherein the reducing agent is dihydroquinone and the ROS detector
10 compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
38. The method of any one of claims 1-37, wherein the FXN protein is frataxin (FXN).
39. The method of any one of claims 1-37, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
40. The method of claim 39, wherein the CPP comprises a peptide selected from the group of
15 CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
41. The method of claim 40, wherein the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof.
42. The fusion protein of claim 41, wherein the CPP comprises HIV-TAT or a variant or
20 derivative thereof.
43. The method of any one of claims 39-42, wherein the FXN fusion protein comprises SEQ ID NO: 12.
44. A method for performing quality control on a sample comprising a Frataxin (FXN) protein, said method comprising measuring activity of an FXN protein in accordance with the method of any
25 one of claims 1-43.
45. A composition for measuring activity of an FXN protein, said composition comprising an FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein pH of the composition is greater than 7.4.

46. The composition of claim 45, wherein pH of the composition is about 7.9 or greater.
47. The composition of claim 45 or 46, further comprising a metal ion.
48. The composition of claim 47, wherein the metal ion is not Fe^{2+} .
49. The composition of claim 47, wherein the metal ion is selected from the group consisting of
5 Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
50. The composition of claim 49, wherein the metal ion is Fe^{3+} .
51. The composition of claim 49, wherein the metal ion is Mn^{2+} .
52. A composition for measuring activity of an FXN protein, said composition comprising an
FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein the
10 composition is substantially free of a metal ions.
53. A composition for measuring activity of an FXN protein, said composition comprising an
FXN protein, a reducing agent and a reactive oxygen species (ROS) detector compound, wherein the
composition is substantially free of iron ions.
54. The composition of claim 52 or 53, wherein pH of the composition is greater than 7.4.
- 15 55. The composition of claim 54 wherein pH of the composition is about 7.9 or greater.
56. The composition of any one of claims 45-55, wherein the reducing agent is an organic
compound.
57. The composition of claim 56, wherein the reducing agent is a reduced quinone compound.
58. The composition of claim 57, wherein the reduced quinone compound is selected from the
20 group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and a
reduced anthraquinone compound.
59. The composition of claim 58, wherein the reduced quinone compound is a reduced
benzoquinone compound.
60. The composition of claim 59, wherein the reduced benzoquinone compound is hydroquinone.
- 25 61. The composition of any one of claims 45-60, wherein the ROS detector compound is a
fluorescent probe or a chemiluminescent probe.

62. The composition of any one of claims 45-60, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
63. The composition of claim 61 or 62, wherein the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H₂DCF),
5 lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof.
64. The composition of claim 63, wherein the ROS detector compound is 2',7'-
10 dichlorodihydrofluorescein (H₂DCF).
65. The composition of any one of claims 45-64, wherein the reducing agent and the ROS detector compound are the same compound.
66. The composition of any one of claims 45-64, wherein the reducing agent and the ROS detector compound are different compounds.
67. The composition of claim 66, wherein the reducing agent is dihydroquinone and the ROS
15 detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
68. The composition of any one of claims 45-67, wherein the FXN protein is frataxin (FXN).
69. The composition of any one of claims 45-67, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
70. The composition of claim 69, wherein the CPP comprises a peptide selected from the group
20 of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
71. The composition of claim 70, wherein the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a variant or derivative thereof.
72. The composition of claim 71, wherein the CPP comprises HIV-TAT or a variant or derivative
25 thereof.
73. The composition of claim 72, wherein the FXN fusion protein comprises SEQ ID NO: 12.

74. A composition comprising an FXN protein, wherein the FXN protein exhibits a specific activity of hydroquinone reduction within the range of about 1000 mU/mg of FXN protein to about 6500 mU/mg of FXN protein as measured by the method of any one of claims 1, 3, 4 and 20.
75. The composition of claim 74, wherein the specific activity of hydroquinone reduction is within the range of about 1200 mU/mg to about 6000 mU/mg.
76. The composition of claim 75, wherein the specific activity is within the range of about 1400 mU/mg to about 5900 mU/mg.
77. A kit for determining activity of an FXN protein, said kit comprising a reducing agent, an ROS detector compound and instructions to combine the reducing agent, the ROS detector compound and an FXN protein at a pH of greater than about 7.4, thereby producing an assay mixture.
78. A kit for determining activity of an FXN protein, said kit comprising a reducing agent, an ROS detector compound and instructions to combine the reducing agent, the ROS detector compound and an FXN protein, thereby producing an assay mixture; and
determine V_i of the FXN protein in the assay mixture.
79. The kit of claim 77 or 78, further comprising a metal ion compound.
80. The kit of claim 79, wherein the metal ion compound comprises an ion selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
81. The kit of claim 80, wherein the metal ion compound comprises Fe^{3+} .
82. The kit of claim 80, wherein the metal ion compound comprises Mn^{2+} .
83. The kit of any one of claims 77-82, wherein the reducing agent is an organic compound.
84. The kit of claim 83, wherein the reducing agent is a reduced quinone compound.
85. The kit of claim 84, wherein the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and a reduced anthraquinone compound.
86. The kit of claim 85, wherein the reduced quinone compound is a reduced benzoquinone compound.
87. The kit of claim 86, wherein the reduced benzoquinone compound is hydroquinone.

88. The kit of any one of claims 77-87, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
89. The kit of any one of claims 77-87, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
- 5 90. The kit of claim 88 or 89, wherein the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein (H₂DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and
10 structural variants and analogs thereof.
91. The kit of claim 90, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
92. The kit of any one of claims 77-91, wherein the reducing agent and the ROS detector compound are the same compound.
- 15 93. The kit of any one of claims 77-91, wherein the reducing agent and the ROS detector compound are different compounds.
94. The kit of claim 93, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
95. A method for identifying a compound capable of modulating activity of a Frataxin (FXN)
20 protein, said method comprising the steps of:
- (i) combining said FXN protein with a test compound, a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS;
 - (ii) measuring the amount of ROS generated in the presence of the test compound;
 - 25 (iii) comparing the amount of ROS generated in the presence of the test compound with an amount of ROS generated in the absence of the test compound; and
 - (iv) identifying the test compound as a compound capable of modulating activity of the FXN protein when the amount of ROS generated in the presence of the test compound is different from the amount of ROS generated in the absence of the test compound.

96. The method of claim 95, further comprising incubating said assay mixture.
97. The method of claim 95 or 96, wherein step (ii) comprises measuring the amount of ROS generated in the assay mixture over time.
98. The method of claim 97, wherein step (ii) comprises measuring the amount of ROS generated
5 at regular time intervals.
99. The method of claim 97 or claim 98, wherein step (ii) comprises measuring the amount of ROS generated every 0.1 second to every 10 minutes.
100. The method of any one of claims 95-99, further comprising determining maximum initial velocity (V_i) of ROS generation in the assay mixture.
101. The method of claim 100, further comprising determining time to maximum initial velocity (T_i).
10
102. The method of claim 101, further comprising determining the unit activity of the FXN protein.
103. The method of claim 100, further comprising the steps of:
- comparing V_i of the FXN protein in the presence of the test compound with V_i of the FXN
15 protein in the absence of the test compound; and
- identifying the test compound as a compound capable of modulating activity of the FXN protein when the V_i of the FXN protein in the presence of the test compound is different from the V_i of the FXN protein in the absence of the test compound.
104. A method for identifying a compound capable of modulating activity of a Frataxin (FXN)
20 protein, said method comprising the steps of:
- (i) combining said FXN protein with a test compound, a reducing agent and a reactive oxygen species (ROS) detector compound, thereby producing an assay mixture, wherein said FXN protein facilitates oxidation of the reducing agent and generation of ROS;
- (ii) measuring the amount of ROS generated in the presence of the test compound and
25 determining V_i of the FXN protein
- (iii) comparing the V_i of the FXN protein in the presence of the test compound with V_i of the FXN protein in the absence of the test compound; and

- (iv) identifying the test compound as a compound capable of modulating activity of the FXN protein when Vi of the FXN protein in the presence of the test compound is different from Vi of the FXN protein in the absence of the test compound.
105. The method of any one of claims 95-104, further comprising adjusting pH of the assay mixture to pH of greater than about 7.4.
106. The method of claim 105, further comprising adjusting pH of the assay mixture to pH of about 7.9 or greater.
107. The method of any one of claims 95-106, further comprising adding a metal ion to the assay mixture.
108. The method of claim 107, wherein the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
109. The method of claim 108, wherein the metal ion is Mn^{2+} .
110. The method of claim 108, wherein the metal ion is Fe^{3+} .
111. The method of any one of claims 95-110, wherein the reducing agent is an organic compound.
112. The method of claim 111, wherein the reducing agent is a reduced quinone compound.
113. The method of claim 112, wherein the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and reduced anthraquinone compound.
114. The method of claim 113, wherein the reduced quinone compound is a reduced benzoquinone compound.
115. The method of claim 114, wherein the reduced benzoquinone compound is hydroquinone.
116. The method of any one of claims 95-115, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
117. The method of any one of claims 95-115, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
118. The method of any one of claims 95-117, wherein the ROS detector compound is selected from the group consisting of coelenterazine; dihydroethidium; 2',7'-dichlorodihydrofluorescein

(H₂DCF), lucigenin, luminol, Cypridina Luciferin Analog (CLA), Cypridina Luciferin methoxy-analogue (MCLA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), p-nitrotetrazolium blue (NBT), RedoxSensor Red CC-1, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfo-phenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof.

- 5 119. The method of claim 118, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
120. The method of any one of claims 95-119, wherein the reducing agent and the ROS detector compound are the same compound.
121. The method of any one of claims 95-119, wherein the reducing agent and the ROS detector
10 compound are different compounds.
122. The method of claim 121, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
123. The method of any one of claims 95-122, wherein the FXN protein is frataxin (FXN).
124. The method of any one of claim 95-122, wherein the FXN protein is an FXN fusion protein
15 comprising full-length hFXN and a cell penetrating peptide (CPP).
125. The method of claim 124, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
126. The method of claim 124, wherein the CPP comprises a peptide selected from the group consisting of HIV-TAT, galanin, mastoparan, transportan, penetratin, polyarginine, VP22, and a
20 variant or derivative thereof.
127. The method of claim 126, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
128. The method of claim 127, wherein the FXN fusion protein comprises SEQ ID NO: 12.
129. A composition comprising an FXN protein, wherein activity of the FXN protein has been
25 measured according to the method of any one of claims 1-43.
130. A pharmaceutical composition comprising an FXN protein and a pharmaceutically acceptable excipient, wherein activity of the FXN protein has been measured according to the method of any one of claims 1-43.

131. A method of preparing a pharmaceutical composition comprising an FXN protein, the method comprising measuring activity of the FXN protein according to the method of any one of claims 1-43, and formulating the FXN protein, thereby preparing the pharmaceutical composition.