

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 ~~OF THE INVENTION~~

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 and the ROS detector compound without the FXN protein.
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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 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.
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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 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.
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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 Vi 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.

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 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 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.

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.

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.

Accordingly, in some ~~aspect~~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.

In some ~~aspect~~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.

In some ~~embodiment~~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 aspectexamples, 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 aspectexamples, 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 embodimentexamples, the method further comprises adjusting pH of the assay mixture to pH of greater than about 7.4. In some embodimentexamples, the method further comprises adjusting pH of the assay mixture to pH of about 7.9 or greater.

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

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

In some embodimentexamples, the method further comprises measuring the amount of ROS generated at regular time intervals. In some embodimentexamples, the method comprises measuring the amount of ROS generated about every 0.1 second to about every 10 minutes, *e.g.*,
30 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 after the start of incubation of the assay mixture.

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

In some aspectexamples, 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; 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 embodimentexamples, the method comprises measuring the amount of ROS generated at regular time intervals. In some embodimentexamples, 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 embodimentexamples, the method further comprises determining maximum initial velocity (V_i) of ROS generation in the assay mixture. In some embodimentexamples, the method further comprises determining time to maximum initial velocity (T_i). In some embodimentexamples, the method further comprises determining the unit activity of the FXN protein.

In some embodimentexamples, the reducing agent is an organic compound. In some embodimentexamples, the reducing agent is a reduced quinone compound. In some embodimentexamples, 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 [embodimentexamples](#), the reduced quinone compound is a reduced benzoquinone compound. In some [embodimentexamples](#), the reduced benzoquinone compound is hydroquinone.

In some [embodimentexamples](#), the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some [embodimentexamples](#), the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some [embodimentexample](#)s, 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 [embodimentexamples](#), the ROS detector compound is 2',7'-dichlorodihydrofluorescein diacetate (H2DCF).

In some [embodimentexamples](#), the reducing agent and the ROS detector compound are the same compound. In some [embodimentexample](#)s, the reducing agent and the ROS detector compound are different compounds. In one [embodimentexample](#), the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H2DCF).

In some [embodimentexamples](#), the FXN protein is frataxin (FXN). In some [embodimentexample](#)s, the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP). In some [embodimentexamples](#), the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some [embodimentexamples](#), 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 [embodimentexamples](#), the CPP comprises HIV-TAT or a variant or derivative thereof. In one [embodimentexample](#), the FXN fusion protein comprises SEQ ID NO: 12.

In some [aspectexamples](#), 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 [aspectexamples](#), 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 embodimentexamples, pH of the composition is about 7.9 or greater.

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

In some aspectexample, 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 metal ions.

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

In some embodimentexamples, the reducing agent is an organic compound. In some embodimentexamples, the reducing agent is a reduced quinone compound. In some embodimentexamples, 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 embodimentexamples, the reduced quinone compound is a reduced benzoquinone compound. In some embodimentexamples, the reduced benzoquinone compound is hydroquinone.

In some embodimentexamples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some embodimentexamples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some embodimentexamples, 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-Sulphophenyl)-2H-Tetrazolium-5-

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

In some [embodimentexamples](#), the ROS detector compound are the same compound. In some [embodimentexamples](#), the reducing agent and the ROS detector compound are different compounds. In one [embodimentexample](#), the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

In some [embodimentexamples](#), the FXN protein is frataxin (FXN). In some [embodimentexamples](#), the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP). In some [embodimentexamples](#), the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some [embodimentexamples](#), 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 [embodimentexamples](#), the CPP comprises HIV-TAT or a variant or derivative thereof. In some [embodimentexamples](#), the FXN fusion protein comprises SEQ ID NO: 12.

In some [aspectexamples](#), 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 [embodimentexamples](#), the specific activity of hydroquinone reduction is within the range of about 1200 mU/mg to about 6000 mU/mg. In some [embodimentexamples](#), the specific activity is within the range of about 1400 mU/mg to about 5900 mU/mg.

In some [aspectexamples](#), 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.

In some [aspectexamples](#), 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 embodimentexamples, the kit further comprises a metal ion compound. In some embodimentexamples, 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 embodimentexample, the metal ion compound comprises Fe^{3+} . In another embodimentexample, the metal ion compound comprises Mn^{2+} .

5 In some embodimentexamples, the reducing agent is an organic compound. In some embodimentexamples, the reducing agent is a reduced quinone compound. In some embodimentexamples, 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 embodimentexamples, the reduced quinone compound is a reduced
10 benzoquinone compound. In some embodimentexamples, the reduced benzoquinone compound is hydroquinone.

In some embodimentexamples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some embodimentexamples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some embodimentexamples, the
15 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
20 some embodimentexamples, the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

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

25 In some aspectexamples, the present disclosure also 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 facilitates oxidation of the reducing agent and generation of ROS; (ii) measuring the amount of ROS
30 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 some embodimentexamples, the method further comprises incubating the assay mixture. In some embodimentexamples, step (ii) comprises measuring the amount of ROS generated in the assay mixture over time. In some embodimentexamples, step (ii) comprises measuring the amount of ROS generated at regular time intervals. In some embodimentexamples, step (ii) comprises measuring the amount of ROS generated every 0.1 second to every 10 minutes,
10 *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 embodimentexamples, the method further comprises determining maximum initial velocity (V_i) of ROS generation in the assay mixture. In some embodimentexamples, the
15 method further comprises determining time to maximum initial velocity (T_i). In some embodimentexample, the method further comprises determining the unit activity of the FXN protein.

In some embodimentexamples, the method further comprises the steps of: comparing V_i of the FXN protein in the presence of the test compound with V_i of the FXN protein in the
20 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.

In some aspectexamples, the present disclosure comprises identifying a compound capable of modulating activity of a Frataxin (FXN) protein, the method comprising the steps of:
25 (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 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
30 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 Vi of the FXN protein in the absence of the test compound.

5 In some [embodimentexamples](#), the method further comprises adjusting pH of the assay mixture to pH of greater than about 7.4. In some [embodimentexamples](#), the method further comprises adjusting pH of the assay mixture to pH of about 7.9 or greater.

In some [embodimentexamples](#), the method further comprises adding a metal ion to the assay mixture. In some [embodimentexample](#), the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} . In one [embodimentexample](#), the metal ion is Mn^{2+} . In another [embodimentexample](#), the metal ion is Fe^{3+} .

10 In some [embodimentexamples](#), the reducing agent is an organic compound. In some [embodimentexamples](#), the reducing agent is a reduced quinone compound. In some [embodimentexamples](#), 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 [embodimentexamples](#), the reduced quinone compound is a
15 reduced benzoquinone compound. In some [embodimentexamples](#), the reduced benzoquinone compound is hydroquinone.

In some [embodimentexamples](#), the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some [embodimentexamples](#), the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some
20 [embodimentexample](#), 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-
25 Carboxanilide (XTT) and structural variants and analogs thereof. In some [embodimentexamples](#), the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

In some [embodimentexamples](#), the reducing agent and the ROS detector compound are the same compound. In some [embodimentexamples](#), the reducing agent and the ROS detector compound are different compounds. In one [embodimentexample](#), the reducing agent is
30 dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

In some embodimentexamples, the FXN protein is frataxin (FXN). In some embodimentexamples, the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP). In some embodimentexamples, the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some embodimentexamples, 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 embodimentexamples, the CPP comprises HIV-TAT or a variant or derivative thereof. In some embodimentexamples, the FXN fusion protein comprises SEQ ID NO: 12.

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

In some [embodimentexamples](#), the method further comprises incubating the assay mixture.
In some [embodimentexamples](#), the method further comprises measuring the amount of ROS generated.
In some [embodimentexamples](#), 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 [aspectexamples](#), the reducing agent is an organic compound. In some [aspectexamples](#), the reducing agent is a reduced quinone compound. In some [aspectexamples](#), 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 [embodimentexamples](#), the reduced quinone compound is a reduced benzoquinone compound. In one [embodimentexample](#), the reduced benzoquinone compound is hydroquinone.

In some [aspectexamples](#), the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some [aspectexamples](#), the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some [embodimentexamples](#), 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-Sulphophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof. In one specific [embodimentexample](#), the ROS detector compound is 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF).

In some [embodimentexamples](#), the reducing agent and the ROS detector compound are the same compound. In other [embodimentexamples](#), the reducing agent and the ROS detector compound are different compounds.

25 In some [embodimentexamples](#), the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

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

30 In some [embodimentexamples](#), the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0. In some [embodimentexamples](#), 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 aspectexample, the CPP comprises HIV-TAT or a variant or derivative thereof.

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

In some embodimentexamples, 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 embodimentexamples, the present inventiondisclosure 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.

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

In some embodimentexamples, the reducing agent is an organic compound. In some embodimentexamples, the reducing agent is a reduced quinone compound. In some aspectexamples, 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 aspectexamples, the reduced quinone compound is a reduced benzoquinone compound. In one aspectexample, the reduced benzoquinone compound is hydroquinone.

In some embodimentexamples, the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some embodimentexamples, the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some embodimentexamples, 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-Sulphophenyl)-2H-Tetrazolium-5-

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

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

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

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

In some embodimentexamples, 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 embodimentexamples, the specific activity of hydroquinone reduction is within the range of about 1200 mU/mg to about 6000 mU/mg. In some embodimentexamples, the specific activity is within the range of about 1400 mU/mg to about 5900 mU/mg.

In some embodimentexamples, 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 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 [aspectexamples](#), the method further comprises incubating the assay mixture. In some [aspectexample](#), 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 [embodimentexamples](#), the reducing agent is an organic compound. In some [embodimentexample](#), the reducing agent is a reduced quinone compound. In some [embodimentexamples](#), 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 [aspectexample](#), the reduced quinone compound is a reduced benzoquinone compound. In one [aspectexample](#), the reduced benzoquinone compound is hydroquinone.

In some [embodimentexamples](#), the ROS detector compound is a fluorescent probe or a chemiluminescent probe. In some [embodimentexamples](#), the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent. In some [embodimentexample](#), 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 [embodimentexample](#), the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

In some [aspectexamples](#), the reducing agent and the ROS detector compound are the same compound. In other [aspectexamples](#), the ROS detector compound are different compounds.

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

In some [embodimentexamples](#), the FXN protein is frataxin (FXN).

In some [aspectexamples](#), 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 aspeetexamples, 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 aspeetexamples, 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.

10 BRIEF DESCRIPTION OF THE DRAWINGS

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
15 of the disclosure in a figure may be used to reference the given feature. Dimensions of 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
25 protein and metal ions, such as Mn^{2+} and Fe^{3+} , in accordance with an embodiment of the 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
30 (small black rectangles).

CLAIMS

What is claimed is:

21. 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.
32. The method of claim 21, 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.
43. 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.
54. 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.
65. The method of claim 43 or 54, further comprising adjusting pH of the assay mixture to pH of
greater than 7.4.
76. The method of claim 65, further comprising adjusting pH of the assay mixture to pH of about
25 7.9 or greater.

- ~~87~~. The method of any one of claims ~~21-32~~ and ~~65-76~~, further comprising adding a metal ion into the assay mixture.
- ~~98~~. The method of claim ~~87~~, wherein the metal ion is not Fe^{2+} .
- ~~109~~. The method of claim ~~87~~, wherein the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
- ~~110~~. The method of claim ~~109~~, wherein the metal ion is Mn^{2+} .
- ~~1211~~. The method of claim ~~109~~, wherein the metal is Fe^{3+} .
- ~~1312~~. The method of any one of claims ~~21-1211~~, further comprising incubating said assay mixture.
- ~~1413~~. The method of any one of claims ~~21-1211~~, further comprising measuring the amount of ROS generated in said assay mixture.
- ~~1514~~. The method of claim ~~1413~~, comprising measuring the amount of ROS generated in the assay mixture over time.
- ~~1615~~. The method of claim ~~1514~~, comprising measuring the amount of ROS generated at regular time intervals.
- ~~1716~~. The method of claim ~~15-14~~ or claim ~~1615~~, 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.
- ~~1817~~. The method of any one of claims ~~1514-1716~~, further comprising determining maximum initial velocity (V_i) of ROS generation in the assay mixture.
- ~~1918~~. The method of claim ~~1817~~, further comprising determining time to maximum initial velocity (T_i).
- ~~2019~~. The method of claim ~~1918~~, further comprising determining the unit activity of the FXN protein at pH of greater than 7.4.
- ~~2120~~. 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 ~~22~~21. The method of claim ~~24~~20, comprising measuring the amount of ROS generated at regular time intervals.
- ~~23~~22. The method of claim ~~24~~20 or claim ~~22~~21, comprising measuring the amount of ROS generated about every 0.1 second to about every 10 minutes.
- 10 ~~24~~23. The method of any one of claims ~~24~~20-~~23~~22, further comprising determining maximum initial velocity (Vi) of ROS generation in the assay mixture.
- ~~25~~24. The method of claim ~~24~~23, further comprising determining time to maximum initial velocity (Ti).
- ~~26~~25. The method of claim ~~25~~24, further comprising determining the unit activity of the FXN protein.
- 15 ~~27~~26. The method of any one of claims ~~21~~-~~26~~25, wherein the reducing agent is an organic compound.
- ~~28~~27. The method of claim ~~27~~26, wherein the reducing agent is a reduced quinone compound.
- ~~29~~28. The method of claim ~~28~~27, wherein the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone compound and
20 reduced anthraquinone compound.
- ~~30~~29. The method of claim ~~29~~28, wherein the reduced quinone compound is a reduced benzoquinone compound.
- ~~31~~30. The method of claim ~~30~~29, wherein the reduced benzoquinone compound is hydroquinone.
- ~~32~~31. The method of any one of claims ~~21~~-~~31~~30, wherein the ROS detector compound is a
25 fluorescent probe or a chemiluminescent probe.
- ~~33~~32. The method of any one of claims ~~21~~-~~31~~30, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.

3433. The method of any one of claims 21-3332, 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 structural variants and analogs thereof.
3534. The method of claim 3433, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF).
3635. The method of any one of claims 21-3534, wherein the reducing agent and the ROS detector compound are the same compound.
3736. The method of any one of claims 21-3534, wherein the reducing agent and the ROS detector compound are different compounds.
3837. The method of claim 3736, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
3938. The method of any one of claims 21-3837, wherein the FXN protein is frataxin (FXN).
4039. The method of any one of claims 21-3837, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
4041. The method of claim 4039, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
4241. The method of claim 4140, 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.
4342. The fusion protein of claim 4241, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
4443. The method of any one of claims 4039-4342, wherein the FXN fusion protein comprises SEQ ID NO: 12.
4544. 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 one of claims 21-4443.

- ~~46~~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.
- ~~47~~46. The composition of claim ~~46~~45, wherein pH of the composition is about 7.9 or greater.
- 5 ~~48~~47. The composition of claim ~~46~~45 or ~~47~~46, further comprising a metal ion.
- ~~49~~48. The composition of claim ~~48~~47, wherein the metal ion is not Fe^{2+} .
- ~~50~~49. The composition of claim ~~48~~47, wherein the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
- ~~51~~50. The composition of claim ~~50~~49, wherein the metal ion is Fe^{3+} .
- 10 ~~52~~51. The composition of claim ~~50~~49, wherein the metal ion is Mn^{2+} .
- ~~53~~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 composition is substantially free of a metal ions.
- 15 ~~54~~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.
- ~~55~~54. The composition of claim ~~53~~52 or ~~54~~53, wherein pH of the composition is greater than 7.4.
- ~~56~~55. The composition of claim ~~55~~54 wherein pH of the composition is about 7.9 or greater.
- 20 ~~57~~56. The composition of any one of claims ~~46~~45-~~56~~55, wherein the reducing agent is an organic compound.
- ~~58~~57. The composition of claim ~~57~~56, wherein the reducing agent is a reduced quinone compound.
- ~~59~~58. The composition of claim ~~58~~57, 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.
- 25 ~~60~~59. The composition of claim ~~59~~58, wherein the reduced quinone compound is a reduced benzoquinone compound.

- ~~6160~~. The composition of claim ~~6059~~, wherein the reduced benzoquinone compound is hydroquinone.
- ~~6261~~. The composition of any one of claims ~~4645-6160~~, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
- 5 ~~6362~~. The composition of any one of claims ~~4645-6160~~, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
- ~~6463~~. The composition of claim ~~6261~~ or ~~6362~~, 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-Sulphophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof.
- 10 ~~6564~~. The composition of claim ~~6463~~, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
- 15 ~~6665~~. The composition of any one of claims ~~4645-6564~~, wherein the reducing agent and the ROS detector compound are the same compound.
- ~~6766~~. The composition of any one of claims ~~4645-6564~~, wherein the reducing agent and the ROS detector compound are different compounds.
- ~~6867~~. The composition of claim ~~6766~~, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
- 20 ~~6968~~. The composition of any one of claims ~~4645-6867~~, wherein the FXN protein is frataxin (FXN).
- ~~7069~~. The composition of any one of claims ~~4645-6867~~, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
- ~~7170~~. The composition of claim ~~7069~~, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
- 25 ~~7271~~. The composition of claim ~~7170~~, 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.

- ~~73~~72. The composition of claim ~~72~~71, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
- ~~74~~73. The composition of claim ~~73~~72, wherein the FXN fusion protein comprises SEQ ID NO: 12.
- ~~75~~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 ~~21~~, ~~43~~, ~~54~~ and ~~24~~20.
- ~~76~~75. The composition of claim ~~75~~74, wherein the specific activity of hydroquinone reduction is within the range of about 1200 mU/mg to about 6000 mU/mg.
- ~~77~~76. The composition of claim ~~76~~75, wherein the specific activity is within the range of about 1400 mU/mg to about 5900 mU/mg.
- ~~78~~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.
- ~~79~~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.
- ~~80~~79. The kit of claim ~~78~~77 or ~~79~~78, further comprising a metal ion compound.
- ~~81~~80. The kit of claim ~~80~~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+} .
- ~~82~~81. The kit of claim ~~81~~80, wherein the metal ion compound comprises Fe^{3+} .
- ~~83~~82. The kit of claim ~~81~~80, wherein the metal ion compound comprises Mn^{2+} .
- ~~84~~83. The kit of any one of claims ~~78~~77-~~83~~82, wherein the reducing agent is an organic compound.
- ~~85~~84. The kit of claim ~~84~~83, wherein the reducing agent is a reduced quinone compound.
- ~~86~~85. The kit of claim ~~85~~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.

8786. The kit of claim 8685, wherein the reduced quinone compound is a reduced benzoquinone compound.

8887. The kit of claim 8786, wherein the reduced benzoquinone compound is hydroquinone.

5 8988. The kit of any one of claims 7877-8887, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.

9089. The kit of any one of claims 7877-8887, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.

10 9190. The kit of claim 89-88 or 9089, 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 structural variants and analogs thereof.

15 9291. The kit of claim 9190, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

9392. The kit of any one of claims 7877-9291, wherein the reducing agent and the ROS detector compound are the same compound.

9493. The kit of any one of claims 7877-9291, wherein the reducing agent and the ROS detector compound are different compounds.

20 9594. The kit of claim 9493, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

9695. A method for identifying a compound capable of modulating activity of a Frataxin (FXN) protein, said method comprising the steps of:

25 (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
5 from the amount of ROS generated in the absence of the test compound.

~~9796~~. The method of claim ~~9695~~, further comprising incubating said assay mixture.

~~9897~~. The method of claim ~~96-95~~ or ~~9796~~, wherein step (ii) comprises measuring the amount of ROS generated in the assay mixture over time.

~~9998~~. The method of claim ~~9897~~, wherein step (ii) comprises measuring the amount of ROS
10 generated at regular time intervals.

~~10099~~. The method of claim ~~98-97~~ or claim ~~9998~~, wherein step (ii) comprises measuring the amount of ROS generated every 0.1 second to every 10 minutes.

~~101100~~. The method of any one of claims ~~9695-10099~~, further comprising determining maximum initial velocity (Vi) of ROS generation in the assay mixture.

~~101102~~. The method of claim ~~101100~~, further comprising determining time to maximum initial velocity (Ti).
15

~~103102~~. The method of claim ~~102101~~, further comprising determining the unit activity of the FXN protein.

~~104103~~. The method of claim ~~104100~~, further comprising the steps of:

20 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 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.

25 ~~105104~~. 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;

5 (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

10 (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.

~~106~~105. The method of any one of claims ~~9695-105~~104, further comprising adjusting pH of the assay mixture to pH of greater than about 7.4.

~~107~~106. The method of claim ~~106~~105, further comprising adjusting pH of the assay mixture to pH of about 7.9 or greater.

15 ~~108~~107. The method of any one of claims ~~9695-107~~106, further comprising adding a metal ion to the assay mixture.

~~109~~108. The method of claim ~~108~~107, wherein the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .

~~110~~109. The method of claim ~~109~~108, wherein the metal ion is Mn^{2+} .

20 ~~111~~110. The method of claim ~~109~~108, wherein the metal ion is Fe^{3+} .

~~112~~111. The method of any one of claims ~~9695-111~~110, wherein the reducing agent is an organic compound.

~~113~~112. The method of claim ~~112~~111, wherein the reducing agent is a reduced quinone compound.

25 ~~114~~113. The method of claim ~~113~~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.

~~115~~114. The method of claim ~~114~~113, wherein the reduced quinone compound is a reduced benzoquinone compound.

- ~~116~~115. The method of claim ~~115~~114, wherein the reduced benzoquinone compound is hydroquinone.
- ~~117~~116. The method of any one of claims ~~9695-116~~115, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
- 5 ~~118~~117. The method of any one of claims ~~9695-118~~115, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
- ~~119~~118. The method of any one of claims ~~9695-119~~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.
- 10 ~~120~~119. The method of claim ~~119~~118, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
- ~~121~~120. The method of any one of claims ~~9695-121~~119, wherein the reducing agent and the ROS detector compound are the same compound.
- 15 ~~122~~121. The method of any one of claims ~~9695-122~~119, wherein the reducing agent and the ROS detector compound are different compounds.
- ~~123~~122. The method of claim ~~122~~121, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
- 20 ~~124~~123. The method of any one of claims ~~9695-124~~122, wherein the FXN protein is frataxin (FXN).
- ~~125~~124. The method of any one of claim ~~9695-125~~122, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
- ~~126~~125. The method of claim ~~125~~124, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
- 25 ~~127~~126. The method of claim ~~126~~125, 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.

~~128~~127. The method of claim ~~127~~126, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.

~~129~~128. The method of claim ~~128~~127, wherein the FXN fusion protein comprises SEQ ID NO: 12.

5 ~~130~~129. A composition comprising an FXN protein, wherein activity of the FXN protein has been measured according to the method of any one of claims ~~21~~21-~~443~~443.

~~131~~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 ~~21~~21-~~443~~443.

10 ~~132~~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 ~~21~~21-~~443~~443, and formulating the FXN protein, thereby preparing the pharmaceutical composition.