

CLAIMS

What is claimed is:

1. A method for measuring activity of a Frataxin (FXN) protein, said method comprising:
 - 5 combining said FXN protein with 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, 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
10 FXN protein.
2. 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 about 7.4, thereby producing an assay mixture,

15 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.
3. The method of claim 2, comprising combining said FXN protein with a reducing
20 agent and a reactive oxygen species (ROS) detector compound at a pH of about 7.9 or greater.
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 metal ions, thereby producing an assay mixture,

wherein said FXN protein facilitates oxidation of the reducing agent and generation of
25 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. 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.
6. The method of claim 1, 4 or 5, further comprising adjusting pH of the assay mixture to pH of greater than about 7.4.
7. The method of claim 6, further comprising adjusting pH of the assay mixture to pH of about 7.9 or greater.
8. The method of any one of claims 1-3 and 6-7, further comprising adding a metal ion into the assay mixture.
9. The method of claim 8, wherein the metal ion is not Fe^{2+} .
10. The method of claim 8, wherein the metal ion is selected from the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
11. The method of claim 10, wherein the metal ion is Mn^{2+} .
12. The method of claim 10, wherein the metal is Fe^{3+} .
13. The method of any one of claims 1-12, further comprising incubating said assay mixture.
14. The method of any one of claims 1-13, further comprising measuring the amount of ROS generated in said assay mixture.
15. The method of claim 14, comprising measuring the amount of ROS generated in the assay mixture over time.

16. The method of claim 15, comprising measuring the amount of ROS generated at regular time intervals.
17. The method of claim 15 or claim 16, 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.
18. The method of any one of claims 15-17, further comprising determining maximum initial velocity (V_i) of ROS generation in the assay mixture.
19. The method of claim 18, further comprising determining time to maximum initial velocity (T_i).
20. The method of claim 19, further comprising determining the unit activity of the FXN protein at pH of greater than about 7.4.
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) 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.
22. The method of claim 21, comprising measuring the amount of ROS generated at regular time intervals.
23. The method of claim 21 or claim 22, comprising measuring the amount of ROS generated about every 0.1 second to about every 10 minutes.
24. The method of any one of claims 21-23, further comprising determining maximum initial velocity (V_i) of ROS generation in the assay mixture.

25. The method of claim 24, further comprising determining time to maximum initial velocity (Ti).
26. The method of claim 25, further comprising determining the unit activity of the FXN protein.
- 5 27. The method of any one of claims 1-26, wherein the reducing agent is an organic compound.
28. The method of claim 27, wherein the reducing agent is a reduced quinone compound.
29. The method of claim 28, wherein the reduced quinone compound is selected from the group consisting of a reduced benzoquinone compound, a reduced naphthoquinone
10 compound and reduced anthraquinone compound.
30. The method of claim 29, wherein the reduced quinone compound is a reduced benzoquinone compound.
31. The method of claim 30, wherein the reduced benzoquinone compound is hydroquinone.
- 15 32. The method of any one of claims 1-31, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
33. The method of any one of claims 1-31, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
34. The method of any one of claims 1-33, wherein the ROS detector compound is
20 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
25 variants and analogs thereof.
35. The method of claim 34, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF).

36. The method of any one of claims 1-35, wherein the reducing agent and the ROS detector compound are the same compound.
37. The method of any one of claims 1-36, wherein the reducing agent and the ROS detector compound are different compounds.
- 5 38. The method of claim 37, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
39. The method of any one of claims 1-38, wherein the FXN protein is frataxin (FXN).
40. The method of any one of claims 1-38, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
- 10 41. The method of claim 40, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
42. The method of claim 41, 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.
- 15 43. The fusion protein of claim 42, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
44. The method of any one of claims 40-43, wherein the FXN fusion protein comprises SEQ ID NO: 12.
45. 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 1-44.
- 20 46. 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 about 7.4.
- 25 47. The composition of claim 46, wherein pH of the composition is about 7.9 or greater.
48. The composition of claim 46 or 47, further comprising a metal ion.

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

62. The composition of any one of claims 46-61, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
63. The composition of any one of claims 46-61, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
- 5 64. The composition of claim 62 or 63, 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 65. The composition of claim 64, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
66. The composition of any one of claims 46-65, wherein the reducing agent and the ROS
15 detector compound are the same compound.
67. The composition of any one of claims 46-65, wherein the reducing agent and the ROS detector compound are different compounds.
68. The composition of claim 67, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
- 20 69. The composition of any one of claims 45-68, wherein the FXN protein is frataxin (FXN).
70. The composition of any one of claims 45-68, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
71. The composition of claim 70, wherein the CPP comprises a peptide selected from the
25 group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.

72. The composition of claim 71, 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. The composition of claim 72, wherein the CPP comprises HIV-TAT or a variant or
5 derivative thereof.
74. The composition of claim 73, wherein the FXN fusion protein comprises SEQ ID NO:
12.
75. 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
10 protein to about 6500 mU/mg of FXN protein.
76. The composition of claim 75, wherein the specific activity of hydroquinone reduction is within the range of about 1200 mU/mg to about 6000 mU/mg.
77. The composition of claim 76, wherein the specific activity is within the range of about 1400 mU/mg to about 5900 mU/mg.
- 15 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.
79. A kit for determining activity of an FXN protein, said kit comprising a reducing agent, an ROS detector compound and instructions to
20 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. The kit of claim 78 or 79, further comprising a metal ion compound.
81. The kit of claim 80, wherein the metal ion compound comprises an ion selected from
25 the group consisting of Mn^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} and Mg^{2+} .
82. The kit of claim 81, wherein the metal ion compound comprises Fe^{3+} .

83. The kit of claim 81, wherein the metal ion compound comprises Mn^{2+} .
84. The kit of any one of claims 78-83, wherein the reducing agent is an organic compound.
85. The kit of claim 84, wherein the reducing agent is a reduced quinone compound.
- 5 86. The kit of claim 85, 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.
87. The kit of claim 86, wherein the reduced quinone compound is a reduced benzoquinone compound.
- 10 88. The kit of claim 87, wherein the reduced benzoquinone compound is hydroquinone.
89. The kit of any one of claims 78-88, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
90. The kit of any one of claims 78-88, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
- 15 91. The kit of claim 89 or 90, 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-
- 20 Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof.
92. The kit of claim 91, wherein the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H_2DCF).
93. The kit of any one of claims 78-92, wherein the reducing agent and the ROS detector
- 25 compound are the same compound.
94. The kit of any one of claims 78-92, wherein the reducing agent and the ROS detector compound are different compounds.

95. The kit of claim 94, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).

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

- 5 (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
10 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.

97. The method of claim 96, further comprising incubating said assay mixture.

15 98. The method of claim 96 or 97, wherein step (ii) comprises measuring the amount of ROS generated in the assay mixture over time.

99. The method of claim 98, wherein step (ii) comprises measuring the amount of ROS generated at regular time intervals.

100. The method of claim 98 or claim 99, wherein step (ii) comprises measuring the
20 amount of ROS generated every 0.1 second to every 10 minutes.

101. The method of any one of claims 96-100, further comprising determining maximum initial velocity (V_i) of ROS generation in the assay mixture.

102. The method of claim 101, further comprising determining time to maximum initial velocity (T_i).

25 103. The method of claim 102, further comprising determining the unit activity of the FXN protein.

104. The method of claim 101, 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 protein in the absence of the test compound; and

identifying the test compound as a compound capable of modulating activity of the
5 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.

105. 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
10 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
15 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.

106. The method of any one of claims 96-105, further comprising adjusting pH of the
20 assay mixture to pH of greater than about 7.4.

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

108. The method of any one of claims 96-107, further comprising adding a metal ion to the assay mixture.

25 109. The method of claim 108, 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+} .
111. The method of claim 109, wherein the metal ion is Fe^{3+} .
112. The method of any one of claims 96-111, wherein the reducing agent is an organic compound.
- 5 113. The method of claim 112, wherein the reducing agent is a reduced quinone compound.
114. The method of claim 113, 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. The method of claim 114, wherein the reduced quinone compound is a reduced
10 benzoquinone compound.
116. The method of claim 115, wherein the reduced benzoquinone compound is hydroquinone.
117. The method of any one of claims 96-116, wherein the ROS detector compound is a fluorescent probe or a chemiluminescent probe.
- 15 118. The method of any one of claims 96-116, wherein the ROS detector compound is a superoxide anion detection reagent or a hydrogen peroxide detection reagent.
119. The method of any one of claims 96-118, 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),
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-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) and structural variants and analogs thereof.
120. The method of claim 119, wherein the ROS detector compound is 2',7'-
25 dichlorodihydrofluorescein (H_2DCF).
121. The method of any one of claims 96-120, wherein the reducing agent and the ROS detector compound are the same compound.

122. The method of any one of claims 96-120, wherein the reducing agent and the ROS detector compound are different compounds.
123. The method of claim 122, wherein the reducing agent is dihydroquinone and the ROS detector compound is 2',7'-dichlorodihydrofluorescein (H₂DCF).
- 5 124. The method of any one of claims 96-123, wherein the FXN protein is frataxin (FXN).
125. The method of any one of claim 96-123, wherein the FXN protein is an FXN fusion protein comprising full-length hFXN and a cell penetrating peptide (CPP).
126. The method of claim 125, wherein the CPP comprises a peptide selected from the group of CPPs listed in the Database of Cell-Penetrating Peptides CPPsite 2.0.
- 10 127. The method of claim 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. The method of claim 127, wherein the CPP comprises HIV-TAT or a variant or derivative thereof.
- 15 129. The method of claim 128, wherein the FXN fusion protein comprises SEQ ID NO: 12.
130. A composition comprising an FXN protein, wherein activity of the FXN protein has been measured according to the method of any one of claims 1-44.
131. A pharmaceutical composition comprising an FXN protein and a pharmaceutically acceptable excipient, wherein activity of the FXN protein has been measured according to the
- 20 method of any one of claims 1-44.
132. 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 1-44, and formulating the FXN protein, thereby preparing the pharmaceutical composition.