

GG-FXN (SEQ ID NO: 11) is described in US Provisional Patent Application No. 62/891,029, the entire contents of each of which are hereby incorporated herein by reference.

To facilitate development of FXN replacement therapies, new reliable methods are needed to accurately and precisely measure level of FXN and FXN fusion proteins in various biological fluids and tissues of a subject.

SUMMARY ~~OF THE INVENTION~~

In one aspect, the present disclosure refers to a method for determining the amount of unprocessed TAT-GG-FXN (SEQ ID NO: 11) in a sample, said method comprising determining the amount of at least one peptide in said sample by using MS, wherein said at least one peptide is selected from the group consisting of: a) a peptide comprising the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and b) a peptide comprising the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14); wherein said method comprises purifying by immunocapture the unprocessed TAT-GG-FXN from said sample prior to MS; wherein said immunocapture is carried out using a binding protein comprising an antigen binding domain, wherein said binding protein is capable of binding a TAT protein transduction domain.

In another aspect, the present disclosure refers to a method for determining the amount of FXN in a tissue sample, said method comprising determining the amount of at least one peptide derived from said FXN in said sample by using MS, wherein said at least one peptide is selected from the group consisting of: a) a peptide comprising the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and b) a peptide comprising the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14); wherein the method is characterized by at least one of the following: the method has a precision as measured by % coefficient of variation (%CV) of 20% or less; and the method has an accuracy of 80% to 120%.

In another aspect, the present disclosure refers to a method for determining the amount of FXN in a skin sample, said method comprising determining the amount of at least one peptide derived from said FXN in said skin sample by using MS, wherein said at least one peptide is selected from the group consisting of: a) a peptide comprising the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and b) a peptide comprising the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14).

In one aspectexample, the present disclosure provides a method for determining the amount of unprocessed frataxin (FXN) fusion protein in a sample, said method comprising determining the amount of a peptide in said sample by using mass spectrometry (MS),

wherein said peptide comprises, or consists of, the amino acid sequence GGMWTLGR (SEQ ID NO: 12); and

wherein said unprocessed FXN fusion protein comprises, or consists of, from N-terminus to C-terminus, a cell penetrating peptide (CPP); a mitochondrial targeting sequence (MTS) comprising, or consisting of, SEQ ID NO: 3; and FXN comprising, or consisting of, SEQ ID NO: 2.

In one embodimentexample, said sample is obtained from a subject being administered the unprocessed FXN fusion protein.

In one embodimentexample, said CPP comprises, or consists of, HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15).

In one embodimentexample, said unprocessed FXN fusion protein is TAT-GG-FXN (SEQ ID NO: 11).

In one embodimentexample, said method comprises purifying by immunocapture the unprocessed FXN fusion protein from said sample prior to MS.

In one embodimentexample, said method comprises the following steps:

a) purifying by immunocapture the unprocessed FXN fusion protein from said sample, thereby obtaining immunocaptured complexes comprising the unprocessed FXN fusion protein;

b) subjecting said immunocaptured complexes comprising the unprocessed FXN fusion protein to digestion by trypsin, thereby producing the peptide comprising, or consisting of, SEQ ID NO: 12; and

c) determining the amount of the peptide comprising, or consisting of, SEQ ID NO: 12 by liquid chromatography and tandem mass spectrometry (LC/MS-MS).

In one [embodimentexample](#), in part c) the liquid chromatography (LC) comprises reversed-phase chromatography.

In one [embodimentexample](#), tandem mass spectrometry (MS-MS) comprises monitoring the transition 439.2 → 632.3.

In one [embodimentexample](#), the tandem mass spectrometry (MS-MS) comprises monitoring the transition 439.2 → 446.3.

In one [embodimentexample](#), the immunocapture is carried out using a binding protein comprising an antigen binding domain that specifically binds to the FXN fusion protein.

In one [embodimentexample](#), said binding protein specifically binds to the FXN present in the FXN fusion protein.

In one [embodimentexample](#), said binding protein specifically binds to the CPP present in the FXN fusion protein.

In one [embodimentexample](#), the FXN fusion protein comprises HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15) and wherein said binding protein specifically binds to the HIV-TAT.

In one [embodimentexample](#), the sample is a tissue sample.

In one [embodimentexample](#), the tissue sample comprises a buccal swab.

In one [embodimentexample](#), the tissue sample comprises a skin biopsy.

In one [embodimentexample](#), the sample is derived from the blood of the subject.

In one [embodimentexample](#), the sample comprises platelets.

In one [embodimentexample](#), the sample is a plasma sample.

In one [embodimentexample](#), the method has a precision as measured by % coefficient of variation (%CV) of about 20% or less.

In one [embodimentexample](#), the method has a precision as measured by %CV of about 15% or less, about 14% or less, about 13% or less, about 12% or less, about 11% or less, about 10% or less, about 9% or less, about 8% or less, about 7% or less, about 6% or less, about 5% or less, about 4% or less, about 3% or less, about 2% or less or about 1% or less.

In one [embodimentexample](#), the method has an accuracy of about 80% to about 120%.

In one [embodimentexample](#), the method is linear for concentration of unprocessed FXN fusion protein ranging from about 0.250 ng/mL to about 25.000 ng/mL.

In another [aspectexample](#), the present disclosure provides a method of monitoring processing of unprocessed FXN fusion protein in a sample, said method comprising:

obtaining at least two temporally separated samples from said subject; and

determining the amount of unprocessed FXN fusion protein in said temporally separated samples using the method of any one of claims 1-23.

In one [embodimentexample](#), the sample is derived from a subject being administered the unprocessed FXN fusion protein.

In yet another [aspectexample](#), the present disclosure provides a method of adjusting a dosing schedule for administering unprocessed FXN fusion protein, said method comprising:

obtaining at least two temporally separated samples from the subject being administered unprocessed FXN fusion protein;

determining the amount of unprocessed FXN fusion protein in said temporally separated samples using the method of any one of claims 1-23; and

adjusting the dose of unprocessed FXN fusion protein being administered to the subject and/or dosing frequency of the unprocessed FXN fusion protein being administered to the subject based on the temporal changes in the amount of unprocessed FXN fusion protein observed in the subject.

In still another [aspect example](#), the present disclosure provides a method for determining the amount of unprocessed FXN fusion protein, *e.g.*, TAT-GG-FXN (SEQ ID NO: 11), in a sample, said method comprising determining the amount of at least one peptide in said sample by using MS,

wherein said at least one peptide is selected from the group consisting of:

- a) a peptide comprising, or consisting of, the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and
- b) a peptide comprising, or consisting of, the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14);

wherein said method comprises purifying by immunocapture the unprocessed TAT-GG-FXN from said sample prior to MS;

wherein said immunocapture is carried out using a binding protein comprising an antigen binding domain, wherein said binding protein is capable of binding a TAT protein transduction domain.

In one [embodiment example](#), said sample is obtained from a subject being administered the unprocessed TAT-GG-FXN (SEQ NO: 11).

In one [embodiment example](#), said method comprises the following steps:

- a) purifying by immunocapture the unprocessed FXN fusion protein, *e.g.*, TAT-GG-FXN from said sample, thereby obtaining immunocaptured complexes comprising the unprocessed FXN fusion protein, *e.g.*, TAT-GG-FXN;
- b) subjecting said immunocaptured complexes comprising the unprocessed FXN fusion protein, *e.g.*, TAT-GG-FXN to digestion by a protease, *e.g.*, trypsin, thereby producing

the peptide comprising, or consisting of, SEQ ID NO: 13 and/or the peptide comprising, or consisting of, SEQ ID NO: 14; and

c) determining the amount of the peptide comprising, or consisting of, SEQ ID NO: 13 and/or the peptide comprising, or consisting of, SEQ ID NO: 14 by LC/MS-MS.

In one [embodimentexample](#), in part c) the LC comprises reversed-phase chromatography.

In one [embodimentexample](#), the MS-MS comprises monitoring the transition 607.3 → 669.3 for the peptide comprising, or consisting of, SEQ ID NO: 13 and the transition 625.3 → 794.4 for the peptide comprising, or consisting of, SEQ ID NO: 14.

In one [embodimentexample](#), the sample is a tissue sample.

In one [embodimentexample](#), the tissue sample comprises a buccal swab.

In one [embodimentexample](#), the tissue sample comprises a skin biopsy.

In one [embodimentexample](#), the sample is derived from the blood of the subject.

In one [embodimentexample](#), the sample comprises platelets.

In one [embodimentexample](#), the sample is a plasma sample.

In one [embodimentexample](#), the method has a precision as measured by % coefficient of variation (%CV) of 20% or less;

In one [embodimentexample](#), the method has an accuracy of about 80% to about 120%.

In one [embodimentexample](#), the method is linear for concentrations of unprocessed TAT-GG-FXN ranging from 0.250 ng/mL to 25.000 ng/mL.

In another [aspectexample](#), the present disclosure provides a method for determining the amount of FXN in a tissue sample, said method comprising determining the amount of at least one peptide derived from said FXN in said sample by using MS,

wherein said at least one peptide is selected from the group consisting of:

a) a peptide comprising, or consisting of, the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and

b) a peptide comprising, or consisting of, the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14);

wherein the method is characterized by at least one of the following:

the method has a precision as measured by % coefficient of variation (%CV) of 20% or less; and

the method has an accuracy of about 80% to about 120%.

In one [embodimentexample](#), said sample is obtained from a subject.

In one [embodimentexample](#), said subject is being administered unprocessed FXN fusion protein.

In one [embodimentexample](#), said unprocessed FXN fusion protein comprises, or consists of, from N-terminus to C-terminus, a cell penetrating peptide (CPP), a mitochondrial targeting sequence (MTS) of SEQ ID NO: 3 and FXN.

In one [embodimentexample](#), said CPP comprises, or consists of, HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15).

In one [embodimentexample](#), said unprocessed FXN fusion protein is TAT-GG-FXN (SEQ ID NO: 11).

In one [embodimentexample](#), said method comprises purifying by immunocapture the FXN from said sample prior to MS.

In one [embodimentexample](#), said method comprises the following steps:

a) purifying by immunocapture the FXN from said sample, thereby obtaining immunocaptured complexes comprising FXN;

b) subjecting said immunocaptured complexes comprising FXN to digestion by a protease, *e.g.*, trypsin, thereby producing the peptide comprising, or consisting of, SEQ ID NO: 13 and/or the peptide comprising, or consisting of, SEQ ID NO: 14; and

c) determining the amount of at least one peptide selected from the group consisting of the peptide comprising, or consisting of, SEQ ID NO: 13 and/or the peptide comprising, or consisting of, SEQ ID NO: 14 by LC/MS-MS.

In one [embodimentexample](#), in part c) the LC comprises reversed-phase chromatography.

In one [embodimentexample](#), the tandem mass spectrometry (MS-MS) comprises monitoring the transition 607.3 → 669.3 for the peptide comprising, or consisting of, SEQ ID NO: 13 and the transition 625.3 → 794.4 for the peptide comprising, or consisting of, SEQ ID NO: 14.

In one [embodimentexample](#), the immunocapture is carried out using a binding protein comprising an antigen binding domain that specifically binds FXN.

In one [embodimentexample](#), said FXN is a part of an FXN fusion protein also comprising HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15) and wherein said binding protein specifically binds the HIV-TAT.

In one [embodimentexample](#), the tissue sample comprises a buccal swab.

In one [embodimentexample](#), the tissue sample comprises a skin biopsy.

In another [aspectexample](#), the present disclosure provides a method for determining the amount of FXN in a skin sample, said method comprising determining the amount of at least one peptide derived from said FXN in said skin sample by using MS,

wherein said at least one peptide is selected from the group consisting of:

a) a peptide comprising, or consisting of, the amino acid sequence GGMWTLGR (SEQ ID NO: 12);

b) a peptide comprising, or consisting of, the amino acid sequence SGTLGHPGSLDETTYER (SEQ ID NO: 13); and

c) a peptide comprising, or consisting of, the amino acid sequence LGGDLGTYVINK (SEQ ID NO: 14).

In one [embodimentexample](#), said skin sample is obtained from a subject.

In one [embodimentexample](#), said subject is being administered unprocessed FXN fusion protein.

In one [embodimentexample](#), said unprocessed FXN fusion protein comprises, or consists of, from N-terminus to C-terminus, a cell penetrating peptide (CPP), a mitochondrial targeting sequence (MTS) of SEQ ID NO: 3 and FXN, *e.g.*, FXN of SEQ ID NO: 2..

In one [embodimentexample](#), said CPP comprises, or consists of, HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15).

In one [embodimentexample](#), said unprocessed FXN fusion protein is TAT-GG-FXN (SEQ ID NO: 11).

In one [embodimentexample](#), said method comprises purifying by immunocapture the FXN from said skin sample prior to MS.

In one [embodimentexample](#), said method comprises the following steps:

a) purifying by immunocapture FXN from said skin sample, thereby obtaining immunocaptured complexes comprising FXN;

b) subjecting said immunocaptured complexes comprising FXN to digestion by a protease, *e.g.*, trypsin, thereby producing the peptide comprising, or consisting of, SEQ ID NO: 12 and/or the peptide comprising, or consisting of, SEQ ID NO: 13 and/or the peptide comprising, or consisting of, SEQ ID NO: 14; and

c) determining the amount of at least one peptide selected from the group consisting of the peptide comprising, or consisting of, SEQ ID NO: 12 and/or the peptide comprising, or consisting of, SEQ ID NO: 13 and/or the peptide comprising, or consisting of, SEQ ID NO: 14 by LC/MS-MS.

In one [embodimentexample](#), said immunocapture is carried out using a binding protein comprising an antigen binding domain that specifically binds FXN.

In one [embodiment example](#), said FXN is a part of an FXN fusion protein also comprising HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15) and wherein said binding protein specifically binds the HIV-TAT.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration of the procedure used for the preparation of biological samples for quantifying TAT-GG-FXN (SEQ ID NO: 11).

Figure 2 is an illustration of sample processing for hybrid LC-MS/MS analysis. Figure 2 discloses SEQ ID NOS 12-14, respectively, in order of appearance.

Figure 3 is a series of graphs showing the concentration of TAT-GG-FXN (SEQ ID NO: 11) levels in monkey tissues (N=6) following repeated subcutaneous (SC) administration at 15 mg/kg BID for 14 days. Data is expressed at pg of TAT-GG-FXN per μ g of total protein.

Figure 4 is a series of representative chromatograms of TAT-GG-FXN tryptic peptides in monkey tissues prior to dosing (Day 1) and following repeated SC administration at 15 mg/kg BID for 14 days.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides methods for determining the amount of frataxin (FXN) or an FXN fusion protein in a sample using mass spectrometry (MS). FXN is a protein which is associated with the disorder Friedreich's Ataxia (FRDA). FRDA is a genetic, progressive neurodegenerative disorder caused by a mutation in a gene encoding FXN. Methods of the present disclosure may be used, *e.g.*, to monitor the levels of FXN in a subject, *e.g.*, a subject who is being administered FXN as a protein replacement therapy for treating FRDA. Methods of the present invention may also be used to monitor processing of an FXN fusion protein, *e.g.*, TAT-GG-FXN (SEQ ID NO: 11), after the FXN fusion protein is added to an in vitro sample or is administered to a subject. Methods of the present disclosure may also be used to monitor levels of FXN or an FXN fusion protein in a subject, *e.g.*, subject

CLAIMS

1. ~~—A method for determining the amount of unprocessed frataxin (FXN) fusion protein in a sample, said method comprising determining the amount of a peptide in said sample by using mass spectrometry (MS);~~

~~—wherein said peptide comprises the amino acid sequence GGMWTLGR (SEQ ID NO: 12); and~~

~~—wherein said unprocessed FXN fusion protein comprises, from N terminus to C terminus, a cell penetrating peptide (CPP); a mitochondrial targeting sequence (MTS) comprising SEQ ID NO: 3; and FXN comprising SEQ ID NO: 2.~~

2. ~~—The method of claim 1, wherein said sample is obtained from a subject being administered the unprocessed FXN fusion protein.~~

3. ~~—The method of claim 1, wherein said CPP comprises HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15).~~

4. ~~—The method of claim 2, wherein said unprocessed FXN fusion protein is TAT-GG-FXN (SEQ ID NO: 11).~~

5. ~~—The method of any one of claims 1-4, wherein said method comprises purifying by immunocapture the unprocessed FXN fusion protein from said sample prior to MS.~~

6. ~~—The method of any one of claims 1-5, wherein said method comprises the following steps:~~

~~—a) —purifying by immunocapture the unprocessed FXN fusion protein from said sample, thereby obtaining immunocaptured complexes comprising the unprocessed FXN fusion protein;~~

~~—b) —subjecting said immunocaptured complexes comprising the unprocessed FXN fusion protein to digestion by trypsin, thereby producing the peptide comprising SEQ ID NO: 12; and~~

- ~~—— c) —— determining the amount of the peptide comprising SEQ ID NO: 12 by liquid chromatography and tandem mass spectrometry (LC/MS-MS).~~
- ~~7. —— The method of claim 6, wherein in part c) the liquid chromatography (LC) comprises reversed phase chromatography.~~
- ~~8. —— The method of claim 6 or 7, wherein tandem mass spectrometry (MS-MS) comprises monitoring the transition 439.2 → 632.3.~~
- ~~9. —— The method of claim 6 or 7, wherein tandem mass spectrometry (MS-MS) comprises monitoring the transition 439.2 → 446.3.~~
- ~~10. —— The method of any one of claims 5-9, wherein the immunocapture is carried out using a binding protein comprising an antigen binding domain that specifically binds to the FXN fusion protein.~~
- ~~11. —— The method of claim 10, wherein said binding protein specifically binds to the FXN present in the FXN fusion protein.~~
- ~~12. —— The method of claim 10, wherein said binding protein specifically binds to the CPP present in the FXN fusion protein.~~
- ~~13. —— The method of claim 12, wherein the FXN fusion protein comprises HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15) and wherein said binding protein specifically binds to the HIV-TAT.~~
- ~~14. —— The method of any one of claims 1-13, wherein the sample is a tissue sample.~~
- ~~15. —— The method of claim 14, wherein the tissue sample comprises a buccal swab.~~
- ~~16. —— The method of claim 14, wherein the tissue sample comprises a skin biopsy.~~
- ~~17. —— The method of any one of claims 1-12, wherein the sample is derived from the blood of the subject.~~
- ~~18. —— The method of claim 16, wherein the sample comprises platelets.~~
- ~~19. —— The method of claim 16, wherein the sample is a plasma sample.~~

~~20.—The method of any one of claims 1-17, wherein the method has a precision as measured by % coefficient of variation (%CV) of about 20% or less.~~

~~21.—The method of claim 20, wherein the method has a precision as measured by %CV of about 15% or less, about 14% or less, about 13% or less, about 12% or less, about 11% or less, about 10% or less, about 9% or less, about 8% or less, about 7% or less, about 6% or less, about 5% or less, about 4% or less, about 3% or less, about 2% or less or about 1% or less.~~

~~22.—The method of any one of claims 1-21, wherein the method has an accuracy of about 80% to about 120%.~~

~~23.—The method of any one of claims 1-22, wherein the method is linear for concentration of unprocessed FXN fusion protein ranging from about 0.250 ng/mL to about 25.000 ng/mL.~~

~~24.—A method of monitoring processing of unprocessed FXN fusion protein in a sample, said method comprising:~~

~~——obtaining at least two temporally separated samples from said subject; and~~

~~——determining the amount of unprocessed FXN fusion protein in said temporally separated samples using the method of any one of claims 1-23.~~

~~25.—The method of claim 24, wherein the sample is derived from a subject being administered the unprocessed FXN fusion protein.~~

~~26.—A method of adjusting a dosing schedule for administering unprocessed FXN fusion protein, said method comprising:~~

~~——obtaining at least two temporally separated samples from the subject being administered unprocessed FXN fusion protein;~~

~~——determining the amount of unprocessed FXN fusion protein in said temporally separated samples using the method of any one of claims 1-23; and~~

~~——adjusting the dose of unprocessed FXN fusion protein being administered to the subject and/or dosing frequency of the unprocessed FXN fusion protein being administered to~~

~~the subject based on the temporal changes in the amount of unprocessed FXN fusion protein observed in the subject.~~

271. A method for determining the amount of unprocessed TAT-GG-FXN (SEQ ID NO: 11) in a sample, said method comprising determining the amount of at least one peptide in said sample by using MS,

wherein said at least one peptide is selected from the group consisting of:

a) a peptide comprising the amino acid sequence
SGTLGHPGSLDETTYER (SEQ ID NO: 13); and

b) a peptide comprising the amino acid sequence LGGDLGTYVINK
(SEQ ID NO: 14);

wherein said method comprises purifying by immunocapture the unprocessed TAT-GG-FXN from said sample prior to MS;

wherein said immunocapture is carried out using a binding protein comprising an antigen binding domain, wherein said binding protein is capable of binding a TAT protein transduction domain.

282. The method of claim 271, wherein said sample is to be obtained from a subject being administered the unprocessed TAT-GG-FXN.

293. The method of claim ~~27~~ or 281 or 2, wherein said method comprises the following steps:

a) purifying by immunocapture the unprocessed TAT-GG-FXN from said sample, thereby obtaining immunocaptured complexes comprising the unprocessed TAT-GG-FXN;

b) subjecting said immunocaptured complexes comprising the unprocessed TAT-GG-FXN to digestion by trypsin, thereby producing the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14; and

c) determining the amount of the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14 by LC/MS-MS.

~~304~~. The method of claim ~~293~~, wherein in part c) the LC comprises reversed-phase chromatography.

~~315~~. The method of claim ~~293~~ or ~~304~~, wherein the MS-MS comprises monitoring the transition 607.3 → 669.3 for the peptide comprising SEQ ID NO: 13 and the transition 625.3 → 794.4 for the peptide comprising SEQ ID NO: 14.

~~326~~. The method of any one of claims ~~27-311-5~~, wherein the sample is a tissue sample.

~~337~~. The method of claim ~~326~~, wherein the tissue sample comprises a buccal swab or a skin biopsy.

~~34~~. ~~The method of claim 32, wherein the tissue sample comprises a skin biopsy.~~

~~358~~. The method of any one of claims ~~27-311-5~~, wherein the sample is derived from the blood of the subject.

~~369~~. The method of claim ~~358~~, wherein the sample comprises platelets or wherein the sample is a plasma sample.

~~37~~. ~~The method of claim 35, wherein the sample is a plasma sample.~~

~~3810~~. The method of any one of claims ~~27-371-9~~, wherein the method has a precision as measured by % coefficient of variation (%CV) of 20% or less; ~~1~~.

~~3911~~. The method of any one of claims ~~27-381-10~~, wherein the method has an accuracy of ~~about~~ 80% to ~~about~~ 120%.

~~4012~~. The method of any one of claims ~~27-391-11~~, wherein the method is linear for concentrations of unprocessed TAT-GG-FXN ranging from ~~about~~ 0.250 ng/mL to ~~about~~ 25.000 ng/mL.

~~4113~~. A method for determining the amount of FXN in a tissue sample, said method comprising determining the amount of at least one peptide derived from said FXN in said sample by using MS,

wherein said at least one peptide is selected from the group consisting of:

a) a peptide comprising the amino acid sequence
SGTLGHPGSLDETTYER (SEQ ID NO: 13); and

b) a peptide comprising the amino acid sequence LGGDLGTYVINK
(SEQ ID NO: 14);

wherein the method is characterized by at least one of the following:

the method has a precision as measured by % coefficient of variation (%CV)
of 20% or less; and

the method has an accuracy of ~~about~~ 80% to ~~about~~ 120%.

~~42~~14. The method of claim ~~41~~13, wherein said sample is to be obtained from a subject.

~~43~~15. The method of claim ~~42~~14, wherein said subject is ~~being to be~~ administered
unprocessed FXN fusion protein.

~~44~~16. The method of claim ~~43~~15, wherein said unprocessed FXN fusion protein comprises,
from N-terminus to C-terminus, a cell penetrating peptide (CPP), a mitochondrial targeting
sequence (MTS) of SEQ ID NO: 3 and FXN.

~~45~~17. The method of claim ~~44~~16, wherein said CPP comprises HIV-TAT (SEQ ID NO: 4)
or HIV-TAT+M (SEQ ID NO: 15).

~~46~~18. The method of claim ~~45~~17, wherein said unprocessed FXN fusion protein is TAT-
GG-FXN (SEQ ID NO: 11).

~~47~~19. The method of any one of claims ~~41-46~~13-18, wherein said method comprises
purifying by immunocapture the FXN from said sample prior to MS.

~~48~~20. The method of any one of claims ~~41-47~~13-19, wherein said method comprises the
following steps:

a) purifying by immunocapture the FXN from said sample, thereby obtaining
immunocaptured complexes comprising FXN;

b) subjecting said immunocaptured complexes comprising FXN to digestion by trypsin, thereby producing the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14; and

c) determining the amount of at least one peptide selected from the group consisting of the peptide comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14 by LC/MS-MS.

[4921](#). The method of claim [4820](#), wherein in part c) the LC comprises reversed-phase chromatography.

[5022](#). The method of claim [48-20](#) or [4921](#), wherein the tandem mass spectrometry (MS-MS) comprises monitoring the transition 607.3 → 669.3 for the peptide comprising SEQ ID NO: 13 and the transition 625.3 → 794.4 for the peptide comprising SEQ ID NO: 14.

[5123](#). The method of any one of claims [47-5019-22](#), wherein the immunocapture is carried out using a binding protein comprising an antigen binding domain that specifically binds FXN.

[5224](#). The method of any one of claims [47-5019-22](#), wherein said FXN is a part of an FXN fusion protein also comprising HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15) and wherein said binding protein specifically binds the HIV-TAT.

[5325](#). The method of any one of claims [41-5213-24](#), wherein the tissue sample comprises a buccal swab.

[5426](#). The method of any one of claims [41-5213-24](#), wherein the tissue sample comprises a skin biopsy.

[5527](#). A method for determining the amount of FXN in a skin sample, said method comprising determining the amount of at least one peptide derived from said FXN in said skin sample by using MS,

wherein said at least one peptide is selected from the group consisting of:

a) ~~a peptide comprising the amino acid sequence GGMWTLGR (SEQ ID NO: 12);~~

~~ba)~~ a peptide comprising the amino acid sequence
SGTLGHPGSLDETTYER (SEQ ID NO: 13); and

~~eb)~~ a peptide comprising the amino acid sequence LGGDLGTYVINK
(SEQ ID NO: 14).

~~5628~~. The method of claim ~~5527~~, wherein said skin sample is to be obtained from a subject.

~~5729~~. The method of claim ~~5628~~, wherein said subject is being to be administered
unprocessed FXN fusion protein.

~~5830~~. The method of claim ~~5729~~, wherein said unprocessed FXN fusion protein comprises,
from N-terminus to C-terminus, a cell penetrating peptide (CPP), a mitochondrial targeting
sequence (MTS) of SEQ ID NO: 3 and FXN.

~~5931~~. The method of claim ~~5830~~, wherein said CPP comprises HIV-TAT (SEQ ID NO: 4)
or HIV-TAT+M (SEQ ID NO: 15).

~~6032~~. The method of claim ~~5931~~, wherein said unprocessed FXN fusion protein is TAT-
GG-FXN (SEQ ID NO: 11).

~~6133~~. The method of any one of claims ~~55-6027-32~~, wherein said method comprises
purifying by immunocapture the FXN from said skin sample prior to MS.

~~6234~~. The method of any one of claims ~~55-6127-33~~, wherein said method comprises the
following steps:

a) purifying by immunocapture FXN from said skin sample, thereby obtaining
immunocaptured complexes comprising FXN;

b) subjecting said immunocaptured complexes comprising FXN to digestion by
trypsin, thereby producing the peptide comprising SEQ ID NO: 12 and/or the peptide
comprising SEQ ID NO: 13 and/or the peptide comprising SEQ ID NO: 14; and

c) determining the amount of at least one peptide selected from the group
consisting of ~~the peptide comprising SEQ ID NO: 12 and/or~~ the peptide comprising SEQ ID
NO: 13 and/or the peptide comprising SEQ ID NO: 14 by LC/MS-MS.

~~63~~35. The method of any one of claims ~~61-62~~33-34, wherein said immunocapture is carried out using a binding protein comprising an antigen binding domain that specifically binds FXN.

~~64~~36. The method of any one of claims ~~61-63~~33-35, wherein said FXN is a part of an FXN fusion protein also comprising HIV-TAT (SEQ ID NO: 4) or HIV-TAT+M (SEQ ID NO: 15) and wherein said binding protein specifically binds the HIV-TAT.