

TREM COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/255,420, filed on October 13, 2021; U.S. Provisional Application No. 63/255,426, filed on October 13, 2021; U.S. Provisional Application No. 63/284,934, filed on December 1, 2021; and U.S. Provisional Application No. 63/284,946, filed on December 1, 2021; the entire contents of each of the foregoing applications is hereby incorporated by reference.

BACKGROUND

Transfer RNAs (tRNAs) are complex, naturally occurring RNA molecules that possess a number of functions including initiation and elongation of proteins.

SUMMARY

The present disclosure features modified tRNA-based effector molecules (TREMs, e.g., a TREM or TREM fragment), as well as related compositions and uses thereof. As provided herein, TREMs are complex molecules which can mediate a variety of cellular processes. The TREMs disclosed herein comprise at least one modification (e.g., a non-naturally occurring modification), e.g., on a component nucleotide (e.g., a nucleobase or sugar) or within an internucleotide region, e.g., the TREM backbone. In one example, provided herein is a TREM comprising a sequence of Formula A: [L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2], wherein independently, [L1] and [VL Domain], are optional; and one of [L1], [ASt Domain1], [L2]-[DH Domain], [L3], [ACH Domain], [VL Domain], [TH Domain], [L4], and [ASt Domain2] comprises a nucleotide comprising a non-naturally occurring modification.

In one aspect, the present disclosure refers to a tRNA effector molecule (TREM) comprising a sequence of Formula (I):

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2] (I),

wherein:

independently, [L1] and [VL Domain], are optional; and

5 a nucleotide within [L1]-[ASt Domain1]-[L2] comprises a nucleotide having a non-naturally occurring modification.

In one aspect, the present disclosure refers to a tRNA effector molecule (TREM) comprising a sequence of Formula (I):

10 [L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2] (I),

wherein:

independently, [L1] and [VL Domain], are optional; and

a nucleotide within [DH Domain], or [L3] comprises a nucleotide having a non-naturally occurring modification.

15 In one aspect, the present disclosure refers to a tRNA effector molecule (TREM) comprising a sequence of Formula (I):

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2] (I),

wherein:

20 independently, [L1] and [VL Domain], are optional; and

a nucleotide within [ACH Domain] comprises a nucleotide having a non-naturally occurring modification.

In one aspect, the present disclosure refers to a tRNA effector molecule (TREM) comprising a sequence of Formula (I):

25 [L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2] (I),

wherein:

independently, [L1] and [VL Domain], are optional; and

30 a nucleotide within [VL Domain] comprises a nucleotide having a non-naturally occurring modification.

In one aspect, the present disclosure refers to a tRNA effector molecule (TREM) comprising a sequence of Formula (I):

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2] (I),

5 wherein:

independently, [L1] and [VL Domain], are optional; and
a nucleotide within [TH Domain] comprises a nucleotide having a non-naturally occurring modification.

In one aspect, the present disclosure refers to a tRNA effector molecule (TREM) comprising a sequence of Formula (I):

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2] (I),

wherein:

independently, [L1] and [VL Domain], are optional; and
15 a nucleotide within [L4] or [ASt Domain 2] comprises a nucleotide having a non-naturally occurring modification.

In one aspect, the present disclosure refers to a TREM, according to Design Guidance 1, Design Guidance 2, Design Guidance 3, Design Guidance 4, Design Guidance 5, or Design Guidance 6.

20 In one aspect, the present disclosure refers to a TREM, according to any two, three, four, five, or all of Design Guidances 1, 2, 3, 4, 5, and 6.

In an example, the TREM: (a) has the ability to: (i) support protein synthesis, (ii) be charged by a synthetase, (iii) be bound by an elongation factor, (iv) introduce an amino acid into a peptide chain, (v) support elongation, or (vi) support initiation; (b) comprises at least X
25 contiguous nucleotides without a non-naturally occurring modification, wherein X is greater than 3, 4, 5, 6, 7, 8, 9, or 10; (c) comprises at least 3, but less than all of the nucleotides of a type (e.g., A, T, C, G or U) comprise the same non-naturally occurring modification; (d) comprises at least

X nucleotides of a type (e.g., A, T, C, G or U) that do not comprise a non-naturally occurring modification, wherein X= than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, or 80; (e) comprises no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, or 80 nucleotides of a type (e.g., A, T, C, G or U) that comprise a non-naturally occurring modification; and/or (f) comprises no more than than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, or 80 nucleotides of a type (e.g., A, T, C, G or U) that do not comprise a non-naturally occurring modification.

In an example, the TREM comprises feature (a)(i). In an example, the TREM comprises feature (a)(ii). In an example, the TREM comprises feature (a)(iii). In an example, the TREM comprises feature (a)(iv). In an example, the TREM comprises feature (a)(v). In an example, the TREM comprises feature (a)(vi). In an example, the TREM comprises feature (b). In an example, the TREM comprises feature (c). In an example, the TREM comprises feature (d). In an example, the TREM comprises feature (e). In an example, the TREM comprises feature (f). In an example, the TREM comprises all of features (a)-(f) or a combination thereof.

In an example, the TREM Domain comprising the non-naturally occurring modification has a function, e.g., a domain function described herein.

In an example, provided herein is a TREM core fragment comprising a sequence of Formula B:

$[L1]_y-[ASt\ Domain1]_x-[L2]_y-[DH\ Domain]_y-[L3]_y-[ACH\ Domain]_x-[VL\ Domain]_y-[TH\ Domain]_y-[L4]_y-[ASt\ Domain2]_x,$

wherein $x=1$ and $y=0$ or 1 ; and one of [ASt Domain1], [ACH Domain], and [ASt Domain2] comprises a nucleotide having a non-naturally occurring modification.

In an example, the TREM has the ability to support protein synthesis. In an example, the TREM has the ability to be able to be charged by a synthetase. In an example, the TREM has the ability to be bound by an elongation factor. In an example, the TREM has the ability to introduce an amino acid into a peptide chain. In an example, the

TREM has the ability to support elongation. In an example, the TREM has the ability to support initiation.

In an example, the [ASt Domain 1] and/or [ASt Domain 2] comprising the non-naturally occurring modification has the ability to initiate or elongate a polypeptide chain.

5 In an example, the [ACH Domain] comprising the non-naturally occurring modification has the ability to mediate pairing with a codon.

In an example, $y=1$ for any one, two, three, four, five, six, all or a combination of [L1], [L2], [DH Domain], [L3], [VL Domain], [TH Domain], [L4].

10 In an example, $y=0$ for any one, two, three, four, five, six, all or a combination of [L1], [L2], [DH Domain], [L3], [VL Domain], [TH Domain], [L4].

In an example, $y=1$ for linker [L1], and L1 comprises a nucleotide having a non-naturally occurring modification.

In an example, $y=1$ for linker [L2], and L2 comprises a nucleotide having a non-naturally occurring modification.

15 In an example, $y=1$ for [DH Domain (DHD)], and DHD comprises a nucleotide having a non-naturally occurring modification. In an example, the DHD comprising the non-naturally occurring modification has the ability to mediate recognition of aminoacyl-tRNA synthetase.

In an example, $y=1$ for linker [L3], and L3 comprises a nucleotide having a non-naturally occurring modification.

20 In an example, $y=1$ for [VL Domain (VLD)], and VLD comprises a nucleotide having a non-naturally occurring modification.

In an example, $y=1$ for [TH Domain (THD)], and THD comprises a nucleotide having a non-naturally occurring modification. In an example, the THD comprising the non-naturally occurring modification has the ability to mediate recognition of the ribosome.

25 In an example, $y=1$ for linker [L4], and L4 comprises a nucleotide having a non-naturally occurring modification.

In another example, the disclosure provides a TREM fragment comprising a portion of a TREM, wherein the TREM comprises a sequence of Formula A:

[L1]-[ASt Domain1]-[L2]-[DH Domain]-[L3]-[ACH Domain] -[VL Domain]-[TH Domain]-[L4]-[ASt Domain2], and wherein the TREM fragment comprises a non-naturally occurring modification.

In an example, the TREM fragment comprises one, two, three or all or any combination of the following: (a) a TREM half (*e.g.*, from a cleavage in the ACH Domain, *e.g.*, in the anticodon sequence, *e.g.*, a 5' half or a 3' half); (b) a 5' fragment (*e.g.*, a fragment comprising the 5' end, *e.g.*, from a cleavage in a DH Domain or the ACH Domain); (c) a 3' fragment (*e.g.*, a fragment comprising the 3' end, *e.g.*, from a cleavage in the TH Domain); or (d) an internal fragment (*e.g.*, from a cleavage in any one of the ACH Domain, DH Domain or TH Domain).

In an example, the TREM fragment comprise (a) a TREM half which comprises a nucleotide having a non-naturally occurring modification.

In an example, the TREM fragment comprise (b) a 5' fragment which comprises a nucleotide having a non-naturally occurring modification.

In an example, the TREM fragment comprise (c) a 3' fragment which comprises a nucleotide having a non-naturally occurring modification.

In an example, the TREM fragment comprise (d) an internal fragment which comprises a nucleotide having a non-naturally occurring modification.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM Domain comprises a plurality of nucleotides each having a non-naturally occurring modification. In an example, the non-naturally occurring modification comprises a nucleobase modification, a sugar (*e.g.*, ribose) modification, or a backbone modification. In an example, the non-naturally occurring modification is a sugar (*e.g.*, ribose) modification. In an example, the non-naturally occurring modification is 2'-ribose modification, *e.g.*, a 2'-OMe, 2'-halo (*e.g.*, 2'-F), 2'-MOE, or 2'-deoxy modification. In an example, the non-naturally occurring modification is a backbone modification, *e.g.*, a phosphorothioate modification.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM sequence comprises a CCA sequence on a terminus, *e.g.*, the 3' terminus. In an example, the TREM sequence does not comprise a CCA sequence on a terminus, *e.g.*, the 3' terminus.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a modification in a base or a backbone of a nucleotide, e.g., a modification chosen from any one of Tables 5, 6, 7, 8 or or 9.

5 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a base modification chosen from a modification listed in Table 5.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a base modification chosen from a modification listed in Table 6.

10 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a base modification chosen from a modification listed in Table 7.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a backbone modification chosen from a modification listed in Table 8.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the non-naturally occurring modification is a backbone modification chosen from a modification listed in Table 9.

20 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in Table 1, e.g., any one of SEQ ID NOs 1-451.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM, TREM core fragment, or TREM fragment is encoded by a consensus sequence chosen from any one of SEQ ID NOs: 562-621.

25 In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM, TREM core fragment, or TREM fragment is encoded by a sequence provided in FIG. 2, e.g., any one of SEQ ID NOs: 622-9757. In an example, the TREM, TREM core fragment, or TREM fragment comprises a TREM having at least 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity with a TREM provided in FIG. 2, e.g., any one of SEQ ID NOs: 622-9757. In an example, the TREM, TREM core fragment, or TREM fragment comprises
30 a sequence that differs by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10

nucleotides from a TREM provided in FIG. 2, e.g., any one of SEQ ID NOs: 622-3284. In an example, the TREM, TREM core fragment, or TREM fragment comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 additional non-naturally occurring modifications compared with a TREM, TREM core fragment, or TREM fragment provided in FIG. 2 (e.g., 2'-ribose modifications or an internucleotide modification, e.g., 2'OMe, 2'-halo, 2'-MOE, 2'-deoxy, or phosphorothiorate modifications), e.g., any one of SEQ ID NOs: 622-9757.

In an example of any of the TREMs, TREM core fragments, or TREM fragments disclosed herein, the TREM, TREM core fragment, or TREM fragment is a TREM provided in FIG. 2, e.g., any one of TREM NOs: 1-9757. In an example, the TREM, TREM core fragment, or TREM fragment comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 additional non-naturally occurring modifications compared with a TREM provided in FIG. 2 (e.g., 2'-ribose modifications or an internucleotide modification, e.g., 2'OMe, 2'-halo, 2'-MOE, 2'-deoxy, or phosphorothiorate modifications), e.g., any one of TREM NOs: 1-2663.

In another example, the disclosure provides a pharmaceutical composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein. In one aspect, the present disclosure refers to a pharmaceutical composition comprising a TREM disclosed herein.

In one aspect, the present disclosure refers to a lipid nanoparticle formulation comprising a TREM disclosed herein or the pharmaceutical composition disclosed herein.

In another example, a TREM or a related composition thereof can be used, *inter alia*, to modulate a production parameter (e.g., an expression parameter and/or a signaling parameter) of an RNA corresponding to, or a polypeptide encoded by, a nucleic acid sequence comprising an endogenous open reading frame (ORF) having a premature termination codon (PTC).

In another example, provided herein is a method of modulating a production parameter of an mRNA corresponding to, or polypeptide encoded by, an endogenous open reading frame (ORF) in a subject, which ORF comprises a premature termination codon (PTC), contacting the subject with a TREM composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein in an amount and/or for a time sufficient to modulate the production parameter of the mRNA or polypeptide, wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with the codon having the first sequence, thereby modulating the production parameter in the subject. In an example, the production parameter comprises a signaling parameter and/or an expression parameter, e.g., as described herein.

In another example, disclosed herein is a method of modulating expression of a protein in a cell, wherein the protein is encoded by a nucleic acid comprising an endogenous open reading frame (ORF), which ORF comprises a premature termination codon (PTC), comprising

contacting the cell with a TREM composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein in an amount and/or for a time sufficient to modulate expression of the encoded protein, wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with the PTC, thereby modulating expression of the protein in the cell. In an example, the PTC comprises UAA, UGA or UAG.

In another example, provided herein is a method of increasing expression of a protein in a subject wherein the protein is encoded by a nucleic acid comprising an endogenous open reading frame (ORF), which ORF comprises a premature termination codon (PTC), comprising contacting the subject, in an amount and/or for a time sufficient to increase expression of the protein, with a TREM composition that (i) has an anticodon that pairs with the PTC, (ii) recognizes an aminoacyl-tRNA synthetase specific for Trp, Tyr, Cys, Glu, Lys, Gln, Ser, Leu, Arg, or Gly, (iii) comprises a sequence of Formula A, or (iv) comprises a non-naturally occurring modification. In an example, the PTC comprises UAA, UGA or UAG. In an example, the TREM composition comprises (i). In an example, the TREM composition comprises (ii). In an example, the TREM composition comprises (iii). In an example, the TREM composition comprises (iv). In an example, the TREM composition comprises two of (i)-(iv). In an example, the TREM composition comprises three of (i)-(iv). In an example, the TREM composition comprises each of (i)-(iv).

In one aspect, the present disclosure refers to use of a TREM, TREM core fragment, or TREM fragment described herein, in the manufacture of a medicament for treating a subject having a disease or disorder associated with a PTC.

In another example, the disclosure provides a method of treating a subject having an endogenous open reading frame (ORF) which comprises a premature termination codon (PTC), comprising providing a TREM composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein, wherein the TREM comprises an anticodon that pairs with the PTC in the ORF; contacting the subject with the composition comprising a TREM, TREM core fragment or TREM fragment in an amount and/or for a time sufficient to treat the subject, thereby treating the subject. In an example, the PTC comprises UAA, UGA or UAG.

In another example, the disclosure provides a method of treating a subject having a disease or disorder associated with a premature termination codon (PTC), comprising providing a TREM composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein; contacting the subject with the composition comprising a TREM, TREM core fragment or TREM fragment in an amount and/or for a time sufficient to treat the subject, thereby treating the subject. In an example, the PTC comprises UAA, UGA or UAG. In an example, the

disease or disorder associated with a PTC is a disease or disorder described herein, e.g., a cancer or a monogenic disease.

In an example of any of the methods disclosed herein, the codon having the first sequence comprises a mutation (e.g., a point mutation, e.g., a nonsense mutation), resulting in a premature termination codon (PTC) chosen from UAA, UGA or UAG. In an example, the codon having the first sequence or the PTC comprises a UAA mutation. In an example, the codon having the first sequence or the PTC comprises a UGA mutation. In an example, the codon having the first sequence or the PTC comprises a UAG mutation

In another example, the disclosure provides a method of making a TREM, a TREM core fragment, or a TREM fragment disclosed herein, comprising linking a first nucleotide to a second nucleotide to form the TREM.

In an example, the TREM, TREM core fragment or TREM fragment is non-naturally occurring (e.g., synthetic).

In an example, the TREM, TREM core fragment or TREM fragment is made by cell-free solid phase synthesis.

In another example, the disclosure provides a method of modulating a tRNA pool in a cell comprising: providing a TREM, a TREM core fragment, or a TREM fragment disclosed herein, and contacting the cell with the TREM, TREM core fragment or TREM fragment, thereby modulating the tRNA pool in the cell.

In an example, the disclosure provides a method of contacting a cell, tissue, or subject with a TREM, a TREM core fragment, or a TREM fragment disclosed herein, comprising: contacting the cell, tissue or subject with the TREM, TREM core fragment or TREM fragment, thereby contacting the cell, tissue, or subject with the TREM, TREM core fragment or TREM fragment.

In another example, the disclosure provides a method of delivering a TREM, TREM core fragment or TREM fragment to a cell, tissue, or subject, comprising: providing a cell, tissue, or subject, and contacting the cell, tissue, or subject, a TREM, a TREM core fragment, or a TREM fragment disclosed herein.

In an example, the disclosure provides a method of modulating a tRNA pool in a cell comprising an endogenous open reading frame (ORF), which ORF comprises a codon having a first sequence, comprising:

optionally, acquiring knowledge of the abundance of one or both of (i) and (ii), e.g.,
acquiring knowledge of the relative amounts of: (i) and (ii) in the cell, wherein (i) is a tRNA
moiety having an anticodon that pairs with the codon of the ORF having a first sequence (the
first tRNA moiety) and (ii) is an isoacceptor tRNA moiety having an anticodon that pairs with a
5 codon other than the codon having the first sequence (the second tRNA moiety) in the cell;

contacting the cell with a TREM, a TREM core fragment, or a TREM fragment disclosed
herein, wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs
with: the codon having the first sequence; or the codon other than the codon having the first
sequence, in an amount and/or for a time sufficient to modulate the relative amounts of the first
10 tRNA moiety and the second tRNA moiety in the cell,

thereby modulating the tRNA pool in the cell.

In another example, the disclosure provides a method of modulating a tRNA pool in a
subject having an ORF, which ORF comprises a codon having a first sequence, comprising:

optionally, acquiring knowledge of the abundance of one or both of (i) and (ii), e.g.,
15 acquiring knowledge of the relative amounts of: (i) and (ii) in the subject, wherein (i) is a tRNA
moiety having an anticodon that pairs with the codon of the ORF having a first sequence (the
first tRNA moiety) and (ii) is an isoacceptor tRNA moiety having an anticodon that pairs with a
codon other than the codon having the first sequence (the second tRNA moiety) in the subject;

contacting the subject with a TREM, a TREM core fragment, or a TREM fragment
20 disclosed herein, wherein the TREM, TREM core fragment or TREM fragment has an anticodon
that pairs with: the codon having the first sequence; or the codon other than the codon having the
first sequence, in an amount and/or for a time sufficient to modulate the relative amounts of the
first tRNA moiety and the second tRNA moiety in the subject,

thereby modulating the tRNA pool in the subject.

25 In an example, the disclosure provides a method of modulating a tRNA pool in a subject
having an endogenous ORF comprising a codon comprising a synonymous mutation (a
synonymous mutation codon or SMC), comprising:

providing a composition comprising a TREM, a TREM core fragment, or a TREM
fragment disclosed herein, wherein the TREM, TREM core fragment or TREM fragment
30 comprises an isoacceptor tRNA moiety comprising an anticodon sequence that pairs with the
SMC (the TREM);

contacting the subject with the composition in an amount and/or for a time sufficient to modulate the tRNA pool in the subject,

thereby modulating the tRNA pool in the subject.

In another example, the disclosure provides a method of modulating a tRNA pool in a cell comprising an endogenous ORF comprising a codon comprising a SMC, comprising:

providing a composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein, wherein the TREM, TREM core fragment or TREM fragment comprises an isoacceptor tRNA moiety comprising an anticodon sequence that pairs with the SMC (the TREM);

contacting the cell with the composition comprising a TREM in an amount and/or for a time sufficient to modulate the tRNA pool in the cell,

thereby modulating the tRNA pool in the cell.

In an example, the disclosure provides a method of modulating expression of a protein in a cell, wherein the protein is encoded by a nucleic acid comprising an ORF, which ORF

comprises a codon having a mutation, comprising:

contacting the cell with a composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein in an amount and/or for a time sufficient to modulate expression of the encoded protein,

wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with the codon having the mutation,

thereby modulating expression of the protein in the cell.

In another example, the disclosure provides a method of modulating expression of a protein in a subject, wherein the protein is encoded by a nucleic acid comprising an endogenous ORF, which ORF comprises a codon having a mutation, comprising:

contacting the subject with a composition comprising a TREM, a TREM core fragment, or a TREM fragment disclosed herein, in an amount and/or for a time sufficient to modulate expression of the encoded protein,

wherein the TREM, TREM core fragment or TREM fragment has an anticodon that pairs with the codon having the mutation,

thereby modulating expression of the protein in the subject.

In an example of any of the methods disclosed herein, the mutation in the ORF is a nonsense mutation, e.g., resulting in a premature stop codon chosen from UAA, UGA or UAG. In an example, the stop codon is UAA. In an example, the stop codon is UGA. In an example, the stop codon is UAG.

5 In an example of any of the methods disclosed herein, the TREM comprises an anticodon that pairs with a stop codon.

TREMs of the disclosure include TREMs, TREM core fragments and TREM fragments. TREMs, TREM core fragments or TREM fragments can be modified with non-naturally occurring modifications to, e.g., increase the level and/or activity (e.g., stability) of the TREM.

10 Pharmaceutical TREM compositions, e.g., comprising TREMs having a non-naturally occurring modification, can be administered to cells, tissues or subjects to modulate these functions, *e.g., in vitro* or *in vivo*. Disclosed herein are TREMs, TREM core fragments or TREM fragments comprising non-naturally occurring modifications, TREM compositions, preparations, methods of making TREM compositions and preparations, and methods of using the same.

15 In an example, the TREM, TREM core fragment, and TREM fragments comprise a non-naturally occurring modification that improves stability or enhances activity of the TREM, TREM core fragment, or TREM fragment.

Additional features of any of the aforesaid TREMs, TREM core fragments, TREM fragments, TREM compositions, preparations, methods of making TREM compositions and preparations, and methods of using TREM compositions and preparations include one or more of the features in the Enumerated Examples, Figures, Description, Examples, or Claims.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific examples of the disclosure described herein. Such equivalents are intended to be encompassed by the following Enumerated Examples, Drawings, Description, Examples, or Claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a depiction of a representative TREM with a summary of a design guidances (e.g., Design Guidance 1-6) described herein.

30 **FIG. 2** is a table summarizing exemplary TREMs, TREM core fragments, and TREM fragments described herein. The sequence of each TREM, TREM core fragment, and TREM