affected. They are also CRISPR-specific and are not generalisable for transducing RNA signals to other functional non-coding RNA pathways, e.g. shRNA, anti-sense or splice-switching oligonucleotides and RNA aptamers.

Therefore, there is a need for a molecular system that can transduce binding of an RNA or other nucleic acid signal, into release of a functional RNA. The present disclosure provides a solution in the form of a ribozyme engineered to be capable of releasing functional RNA upon binding to a sequence-complementary trigger.

SUMMARY

In one aspect, the present disclosure relates to a ribozyme comprising:

a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and

wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with

motifs [A] and [a], wherein motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b], wherein motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently 1 nucleotide or, 2, 3 or 4 nucleotides in length;

motifs [C] and [c], wherein motifs [C] and [c] constitute the catalytic domain

motif [D], wherein motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'], wherein motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [E], wherein motif [E] comprises the releasable RNA segment;

motif [e], wherein motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

and wherein the motifs are connected by one or more optional linker region.

In one example, there is provided a ribozyme comprising:

a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and

wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with

motifs [A] and [a], wherein motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b], wherein motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently at least 1 nucleotide in length;

motifs [C] and [c], wherein motifs [C] and [c] constitute the catalytic domain;

CLAIMS

1. A ribozyme comprising:

a) one or more catalytic domains capable of switching between an active state and an inactive state;

b) one or more releasable RNA segments, wherein each of said releasable RNA segments is flanked by two ribozyme cleavage sites, wherein cleavage at each cleavage site is catalysed by at least one of the one or more catalytic domains in an active state;

c) one or more trigger-binding domains, each of which is for the binding of a trigger nucleic acid molecule; wherein each of the one or more catalytic domains is linked to one of the one or more trigger-binding domains;

wherein the catalytic domain is in an inactive state when the trigger-binding domain linked to said catalytic domain is not bound by the trigger nucleic acid molecule, and wherein the catalytic domain is in an active state when the trigger-binding domain linked to said catalytic domain is bound by the trigger nucleic acid molecule; and

wherein when both cleavage sites flanking a releasable RNA segment are cleaved, the one or more releasable RNA segment is released from the ribozyme,

wherein the ribozyme comprises an RNA strand with

motifs [A] and [a], wherein motifs [A] and [a] constitute the trigger-binding domain for binding the trigger nucleic acid molecule;

motifs [B] and [b], wherein motifs [B] and [b] constitute a linker that functions as a communication module to stabilise the catalytic domain when the trigger nucleic acid is bound, wherein motif [B] and [b] are independently 1 nucleotide or, 2, 3 or 4 nucleotides in length;

motifs [C] and [c], wherein motifs [C] and [c] constitute the catalytic domain;

motif [D], wherein motif [D] comprises the first cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [D'], wherein motif [D'] comprises the second cleavage site capable of being cleaved when catalysed by the catalytic domain;

motif [E], wherein motif [E] comprises the releasable RNA segment;

motif [e], wherein motif [e] comprises a sequence that is partially or fully complementary to the sequence of motif [E];

and wherein the motifs are connected by one or more optional linker region.

2. The ribozyme of claim 1, wherein the linker between motifs [C] and [D] is selected from the group consisting of two-way junction, three-way junction, four-way junction, a stem, single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges and combinations thereof.

3. The ribozyme of claim 1 or 2, wherein the linker between motifs [C] and [D] comprises a three-way junction and a stem.

4. The ribozyme of claim 3, wherein the stem sequence connecting the junction to motif [D] is 4 to 12 nucleotides in length.

5. The ribozyme of claim 3, wherein the stem sequence connecting motif [C] and [c] to the junction is 4 to 12 nucleotides in length.

6. The ribozyme of any one of the preceding claims, wherein the trigger nucleic acid molecule comprises a region that is complementary to the trigger-binding domain, wherein said region is more than 10 nucleotides in length, optionally the one or more trigger -binding domains are for binding the same trigger nucleic acid molecule.

7. The ribozyme of any one of the preceding claims, wherein the releasable RNA segment is 6 to 150 nucleotides in length.

8. The ribozyme of any one of the preceding claims, wherein the releasable RNA segment comprises a sequence that is identical to at least one of the one or more trigger RNA molecules.

9. The ribozyme of any one of the preceding claims, wherein the releasable RNA segment is a functional RNA selected from the group consisting of single-guide RNA (sgRNA), guide RNA (gRNA), short hairpin RNA (shRNA), and RNA aptamer.

10. The ribozyme of any one of the preceding claims, wherein motifs [B] and [b] are independently 3 nucleotides in length.

11. The ribozyme of any one of the preceding claims, wherein motifs [B] and [b] has a sequence selected from the group consisting of SEQ ID NO: 1 (5'-ACG/CGU-3'), SEQ ID NO: 2 (5'-ACG/CGA-3'), SEQ ID NO: 449 (5'- ACG/UGA -3'), SEQ ID NO: 450 (5'- AUG/CGA -3'), SEQ ID NO: 451 (5'- AUG/UGA -3'), SEQ ID NO: 452 (5'- CG/CG -3'), SEQ ID NO: 453 (5'- UUG/UGG -3'), SEQ ID NO: 454 (5'- UAU/AUA -3'), SEQ ID NO: 455 (5'- ACU/AGA -3'), SEQ ID NO: 456 (5'- AUG/CAA -3'), SEQ ID NO: 457 (5'- CU/AG -3'), and SEQ ID NO: 458 (5'- UG/CA -3').

12. The ribozyme of any one of the preceding claims, wherein if motifs [e] and [E] are partially complementary to each other, the complementarity between motif [e] and [E] is characterised by alternating regions of complementarity and regions of non-complementarity.

13. The ribozyme of any one of the preceding claims, wherein motif [D] comprises a mutation of nucleotide N_7 to pair with nucleotide N_{+3}.

14. The ribozyme of any one of the preceding claims, wherein the optional linker regions individually or collectively form one or more secondary structures, optionally the one or more secondary structures are selected from the group consisting of: single-nucleotide bulges, two-nucleotide bulges, three-nucleotide bulges, multi-nucleotide bulges, stems, stem loops, t-RNA type structures, cloverleaves, tetraloops, pseudoknots, symmetrical internal loops, asymmetrical internal loops, three stem junctions (3-way junctions), four stem junctions (4-way junctions), two-stem junctions (2-way junctions) or coaxial stacks or combinations thereof.

15. The ribozyme of any one of the preceding claims, wherein the ribozyme complex comprises the sequences of any one or more of SEQ ID NOs: 3 to SEQ ID NOs: 448.

16. The ribozyme of any one of the preceding claims, wherein the ribozyme further comprises one or more modification.

17. The ribozyme of any one of the preceding claims, wherein the trigger nucleic acid comprises one or more modified nucleotide.

18. A method of detecting presence of a target/trigger nucleic acid molecule in a sample, wherein the method comprises:

incubating the sample with a ribozyme according to any of the claims 1 to 17 at temperature T1 which allows the binding of the target/trigger nucleic acid molecule with one or more target/trigger -binding domains comprised in the ribozyme;

incubating the sample at temperature T2 which allows the nucleic acid molecule and the RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme.

19. A method of detecting presence of a sequence or mutation of interest on a nucleic acid of interest in a sample, wherein the method comprises:

incubating the sample with a ribozyme according to any of the claims 1 to 17, thereby allowing binding of the nucleic acid molecule of interest with one or more target/trigger-binding domains comprised in the ribozyme;

incubating the sample which allows the nucleic acid molecule and a releasable RNA segment to be released from the ribozyme;

detecting the release of the releasable RNA segment from the ribozyme;

wherein the releasable RNA segment is an sgRNA or shRNA; wherein detection of the sequence or mutation of interest in the sample results in a signal being generated.

20. The ribozyme of any one of claims 1 to 17 or the method of any one of claims 18 to 19, wherein the trigger nucleic acid molecule is a genome of a virus, or a fragment thereof.

21. A kit comprising the ribozyme of any one of claims 1 to 17 or 20.