

Claims

1. A ribozyme comprising:

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

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

c) one or more target-binding domains, each of which is for the binding of a target RNA molecule;

10 wherein each of the one or more catalytic domains is linked to one of the one or more target-binding domains;

 wherein the catalytic domain is in an inactive state when the target-binding domain linked to said catalytic domain is not bound by the target RNA molecule, and wherein the catalytic domain is in an active state when the target-binding domain
15 linked to said catalytic domain is bound by the target RNA molecule; and

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

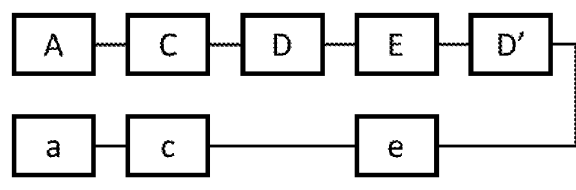
20 2. The ribozyme according to claim 1, further comprising one or more inhibitory domains;

 wherein at least one of the one or more catalytic domains is linked to one of the one or more inhibitory domains, wherein the catalytic domain is in an inactive state due to inhibition from the inhibitory domain, said inhibitory domain being linked to one of the one or more target-binding domains;

25 wherein when one of the one or more target-binding domains is bound to the target RNA molecule, the inhibitory domain linked to said target-binding domain ceases to inhibit the catalytic domain linked to said inhibitory domain, which results in the catalytic domain switching to an active state.

30 3. The ribozyme of claim 1, wherein the ribozyme comprises one catalytic domain and one target binding domain, wherein the ribozyme comprises the following structure:

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wherein [A] to [a] is in the 5' to 3' directionality or the 3' to 5' directionality, and wherein:

5 motifs [A] and [a] constitute the target-binding domain for binding the target RNA molecule;

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

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

10 motif [D'] comprises the second cleavage site capable of being cleaved by the catalytic domain;

motif [E] comprises a releasable RNA segment;

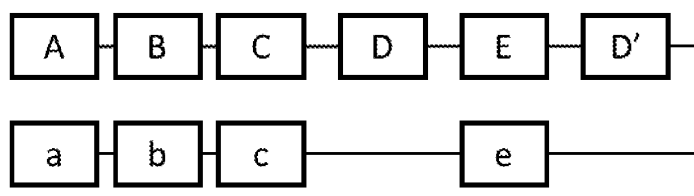
motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E];

each of the horizontal lines connecting the motifs represents an optional linker region;

15 and

wherein the catalytic domain is in an active state when the target-binding domain is bound to the target RNA molecule.

4. The ribozyme of claim 2, wherein the ribozyme comprises one catalytic domain, one inhibitory domain, and one target binding domain, wherein the ribozyme comprises
20 an RNA strand with the following structure:



wherein [A] to [a] is in the 5' to 3' directionality or the 3' to 5' directionality, and wherein:

motifs [A] and [a] constitute the target-binding domain for binding the target RNA molecule;

5 motifs [B] and [b] constitute the inhibitory domain;

motifs [C] and [c] constitute the first catalytic domain;

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

10 motif [D'] comprises the second cleavage site capable of being cleaved by the catalytic domain;

motif [E] comprises a releasable RNA segment;

motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E];

15 each of the horizontal lines connecting the motifs represents an optional linker region; and

wherein the inhibitory domain is characterized by i) or ii) below:

i) motif [b] anneals with [C] when the target-binding domain is not bound by the target RNA molecule, but anneals with [B] when the target-binding domain is bound by the target RNA molecule,

20 ii) motif [B] anneals with motif [c] when the first target-binding domain is not bound by the target RNA molecule, but anneals with [b] when the target-binding domain is bound by the target RNA molecule;

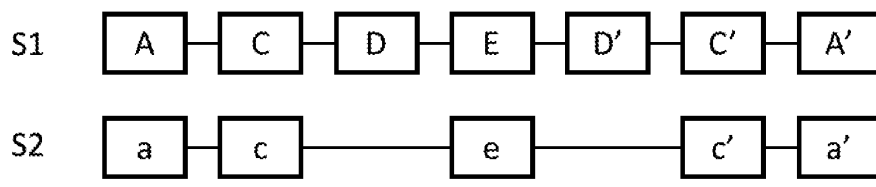
wherein the catalytic domain is in an active state when motif [b] is annealed with motif [B].

25 5. The ribozyme of claim 1 or 2, wherein the ribozyme comprises two catalytic domains.

6. The ribozyme claim 5, wherein the ribozyme comprises two target-binding domains.

7. The ribozyme of claim 5 or 6, wherein the ribozyme comprises a first RNA strand and a second RNA strand.

8. The ribozyme of claim 7, wherein the ribozyme comprises the following structure:



5 wherein:

S1 is the first RNA strand and S2 is the second RNA strand, wherein [A] to [A'] and [a] to [a'] represent opposite directionalities; and

motifs [A] and [a] constitute a first target-binding domain for binding a first target RNA molecule;

10 motifs [C] and [c] constitute a first catalytic domain;

motif [D] comprises a first cleavage site capable of being cleaved by the first catalytic domain;

motifs [A'] and [a'] constitute a second target-binding domain for binding a second target RNA molecule;

15 motifs [C'] and [c'] constitute a second catalytic domain;

motif [D'] comprises a second cleavage site capable of being cleaved by the second catalytic domain;

motif [E] comprises a releasable RNA segment;

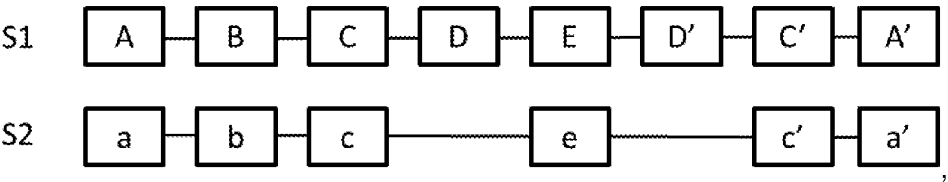
20 motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E];

each of the horizontal lines connecting the motifs represents an optional linker region; and

wherein the first catalytic domain is in an active state when the first target-binding domain is bound to first target RNA molecule; and wherein the second catalytic

domain is in an active state when the second target-binding domain is bound to the second target RNA molecule.

9. The ribozyme of claim 2, wherein the ribozyme comprises the following structure:



5 wherein:

S1 is the first RNA strand and S2 is the second RNA strand, wherein [A] to [A'] and [a] to [a'] represent opposite directionalities; wherein

motifs [A] and [a] constitute a first target-binding domain for binding a first target RNA molecule;

10 motifs [B] and [b] constitute a inhibitory domain;

motifs [C] and [c] constitute a first catalytic domain;

motif [D] comprises a first cleavage site for being cleaved by the first catalytic domain;

motifs [A'] and [a'] constitute a second target-binding domain for binding a second target RNA molecule;

15 motifs [C'] and [c'] constitute a second catalytic domain;

motif [D'] comprises a second cleavage site capable of being cleaved by the second catalytic domain;

motif [E] comprises a releasable RNA segment;

20 motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E];

each of the horizontal lines connecting the motifs represents an optional linker region;

wherein the inhibitory domain is characterized by i) or ii) below:

i) motif [b] anneals with [C] when the first target-binding domain is not bound by the first target RNA molecule, but anneals with [B] when the first target-binding domain is bound by the first target RNA molecule,

5 ii) motif [B] anneals with motif [c] when the first target-binding domain is not bound by the first target RNA molecule, but anneals with [b] when the first target-binding domain is bound by the first target RNA molecule;

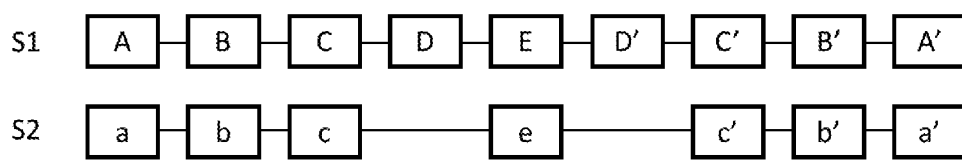
wherein the first catalytic domain is in an active state when motif [b] is annealed with motif [B].

10 10. The ribozyme of claim 4 or 9, wherein the inhibitory domain is further characterized by i) or ii) below:

i) motif [b] is at least 50% complementary to motif [C], and at least 20% complementary to motif [B], and

ii) motif [B] is at least 50% complementary to motif [c], and at least 20% complementary to motif [b].

15 11. The ribozyme of claim 2, wherein the ribozyme comprises the following structure:



wherein:

S1 is the first RNA strand and S2 is the second RNA strand, wherein [A] to [A'] and [a] to [a'] represent opposite directionalities; wherein

20 motifs [A] and [a] constitute a first target-binding domain for binding a first target RNA molecule;

motifs [B] and [b] constitute a first inhibitory domain;

motifs [C] and [c] constitute a first catalytic domain;

25 motif [D] comprises a first cleavage site capable of being cleaved by the first catalytic domain;

motifs [A'] and [a'] constitute a second target-binding domain for binding a second target RNA molecule;

motifs [B'] and [b'] constitute a second inhibitory domain;

motifs [C'] and [c'] constitute a second catalytic domain;

5 motif [D'] comprises a second cleavage site capable of being cleaved by the second catalytic domain;

motif [E] comprises a releasable RNA segment;

motif [e] comprises a sequence which is optionally complementary to the sequence of motif [E];

10 each of the horizontal lines connecting the motifs represents an optional linker region;

wherein the first inhibitory domain is characterized by i) or ii) below:

i) motif [b] anneals with [C] when the first target-binding domain is not bound by the first target RNA molecule, but anneals with [B] when the first target-binding domain is bound by the first target RNA molecule,

15 ii) motif [B] anneals with motif [c] when the first target-binding domain is not bound by the first target RNA molecule, but anneals with [b] when the first target-binding domain is bound by the first target RNA molecule,

wherein the second inhibitory domain is characterized by i) or ii) below:

20 i) motif [b'] anneals with motif [C'] when the second target-binding domain is not bound by the first target RNA molecule, but anneals with [B'] when the second target-binding domain is bound by the second target RNA molecule,

ii) motif [B'] anneals with motif [c'] when the second target-binding domain is not bound by the second target RNA molecule, but anneals with [b'] when the second target-binding domain is bound by the second target RNA molecule,

25 wherein the first catalytic domain is in an active state when motif [b] is annealed with motif [B], and the second catalytic domain is in an active state when motif [b'] is annealed with motif [B'].

12. The ribozyme of claim 11, wherein the first inhibitory domain is further characterized by i) or ii) below:

i) motif [b] is at least 50% complementary to motif [C], and at least 20% complementary to motif [B], and

5 ii) motif [B] is at least 50% complementary to motif [c], and at least 20% complementary to motif [b]; and

the second inhibitory domain is further characterized by i) or ii) below:

i) motif [b'] is at least 50% complementary to motif [C'], and at least 20% complementary to motif [B'],

10 ii) motif [B'] is at least 50% complementary to motif [c'], and at least 20% complementary to motif [b'].

13. The ribozyme of any one of claims 3-12, wherein the optional linker regions individually or collectively form one or more secondary structures.

14. The ribozyme of claim 13, wherein the one or more secondary structures are
15 selected from the group consisting of: single-nucleotide bulges, three-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.

20 15. The ribozyme complex of any one of claims 3-14, wherein motifs [E] and [e] are complementary to each other with a complementarity of less than 70%.

16. The ribozyme of any one of claims 11-15, wherein the first RNA strand (S1) and second RNA strand (S2) comprise the sequences of SEQ ID NO.: 26 and SEQ ID NO.: 29 respectively.

25 17. The ribozyme of any one of claims 1-16, wherein the target RNA molecule is more than 16 nucleotides in length.

18. The ribozyme of any one of claims 1-17, wherein the target RNA molecule comprises a region which is complementary to the target-binding domain, wherein said region is more than 16 nucleotides in length.

19. The ribozyme of any one of claims 1-18, wherein the releasable RNA segment is 6 to 150 nucleotides in length.
20. The ribozyme of any one of claims 1-19, wherein the one or more target-binding domains are for binding the same target RNA molecule.
- 5 21. The ribozyme of any one of claims 1-20, wherein the releasable RNA segment comprises a sequence that is identical to at least one of the one or more target RNA molecules.
22. A method of detecting presence of a target RNA molecule in a sample, wherein the method comprises:
- 10 a) incubating the sample with an ribozyme according to any of the claims 1-21 at temperature T1 which allows the binding of the target RNA molecule with one or more target-binding domains comprised in the ribozyme;
- b) incubating the sample at temperature T2 which allows the RNA molecule and the RNA segment to be released from the ribozyme;
- 15 c) detecting the release of the releasable RNA segment from the ribozyme.
23. The method of claim 22, wherein steps a) to b) are repeated for one or more times before step c) is carried out, wherein the RNA molecules released from step b) are capable of binding to another copy of the ribozyme.
24. A method of amplifying a target RNA molecule, wherein the method comprises:
- 20 a) incubating the target RNA molecule with an ribozyme according to any of the claims 1-21 at temperature T1 which allows the binding of the target RNA molecule with one or more target-binding domains comprised in the ribozyme, and wherein the releasable RNA segment comprises a sequence identical to the target RNA molecule;
- 25 b) incubating the ribozyme bound to the target RNA molecule at temperature T2 which allows the target RNA molecule and the RNA segment to be released from the ribozyme.

25. The method of claim 24, wherein steps a) to b) are repeated for one or more times, wherein the target RNA molecules and the releasable RNA segment released from step b) are capable of binding to another copy of the ribozyme.
26. The method of any of claims 22-25, wherein T1 is a temperature not more than 50°C.
- 5 27. The method of any of claims 22-26, wherein T2 is a temperature between 20°C to 100°C.
28. The ribozyme of any one of claims 1-21 or the method of any one of claims 22-27, wherein the target RNA molecule is an RNA molecule obtained from animals, viruses, bacteria, yeast or plants.
- 10 29. The ribozyme of any one of claims 1-21 or the method of any one of claims 22-28, wherein the target RNA molecule is a microRNA, or a precursor thereof, or a fragment thereof.
30. The ribozyme of any one of claims 1-21 or the method of any one of claims 22-28, wherein the target RNA molecule is a genome of an RNA virus, or a fragment thereof.
- 15 31. The ribozyme or method of claim 30, wherein the RNA virus is selected from the group consisting of: a Retroviridae virus, a Lentiviridae virus, a Coronaviridae virus, a Picornaviridae virus, a Caliciviridae virus, a Flaviviridae virus, a Togaviridae virus, a Bornaviridae, a Filoviridae, a Paramyxoviridae, a Pneumoviridae, a Rhabdoviridae, an Arenaviridae, a Bunyaviridae, an Orthomyxoviridae, and a Deltavirus.
- 20 32. The ribozyme or method of claim 31, wherein the RNA virus is selected from the group consisting of: Lymphocytic choriomeningitis virus, Coronavirus, human immunodeficiency virus (HIV), Severe acute respiratory syndrome virus (SARS), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Poliovirus, Rhinovirus, Hepatitis A, Norwalk virus, Yellow fever virus, West Nile virus, Hepatitis C virus, Dengue fever virus, Zika virus, Rubella virus, Ross River virus, Sindbis virus, Chikungunya virus, Borna disease virus, Ebola virus, Marburg virus, Measles virus, Mumps virus, Nipah virus, Hendra virus, Newcastle disease virus, Human respiratory syncytial virus, Rabies virus, Lassa virus, Hantavirus, Crimean-Congo hemorrhagic fever virus, Influenza virus, and Hepatitis D virus.
- 25 30 33. A polynucleotide encoding the ribozyme of claims 1-21.

- 34. A kit comprising the ribozyme of claims 1-21.
- 35. The kit of claim 34, further comprising an RNA detection system.
- 36. The kit of claim 35, wherein the RNA detection system comprises an RNA-activated fluorescent sensor.
- 5 37. The kit of claim 35, wherein the RNA detection system is a CRISPR-Cas based nucleic acid detection system.