

CLAIMS

~~What is claimed is:~~

1. A library of RNA oligonucleotides comprising a plurality of non-identical oligonucleotides, wherein the individual oligonucleotides comprise a structural scaffold comprising:

- a) a first sequence comprising a helix domain;
- b) a second sequence comprising a first hairpin domain; ~~and~~
- c) a third sequence comprising a second hairpin domain; and
- d) an oligonucleotide junction;

wherein the oligonucleotide junction comprises (i) sequence joining the helix domain, first hairpin domain and second hairpin domain ~~form an oligonucleotide junction containing~~ and (ii) a ligand-binding domain, and

wherein the library comprises a plurality of non-identical ligand-binding domains.

~~2. The library of oligonucleotides of claim 1, wherein each helix domain independently is a fully complementary helix optionally comprising one or more destabilizing nucleotides selected from the group consisting of a mismatched base pair, a G•U wobble base pair and a bulge~~

~~3. The library of oligonucleotides of claim 1, wherein each helix domain is a fully complementary helix.~~

~~4. The library of oligonucleotides of claim 1, wherein each first hairpin domain independently comprises one or more destabilizing nucleotides selected from the group consisting of a mismatched base pair, a G•U wobble base pair and a bulge.~~

~~5. The library of oligonucleotides of claim 1, wherein each second hairpin domain independently comprises one or more destabilizing nucleotides selected from the group consisting of a mismatched base pair, a G•U wobble base pair and a bulge.~~

~~6. The library of oligonucleotides of claim 1, wherein the helix domain is at least 4 to 10 base pairs in length.~~

7. — The library of oligonucleotides of claim 1, wherein the helix domain is at least 10 base-pairs in length.

8. — The library of oligonucleotides of claim 1, wherein the oligonucleotides are oligoribonucleotides.

9. — The library of oligonucleotides of claim 1, wherein the oligonucleotides individually comprise a sequence having a series of linked sequences according to Formula I:

$$P1-J1/2-P2-L2-P2'-J2/3-P3-L3-P3'-J3/1-P1'$$

5 (I)

— wherein

— represents a bond;

— P1 and P1' form the helix;

— P2, L2 and P2' form the first hairpin;

10 — P3, L3 and P3' form the second hairpin; and

— J1/2, J2/3 and J3/1 together form the oligonucleotide junction.

10. — The library of oligonucleotides of claim 9, wherein J2/3 comprises a T-loop motif.

11. — The library of oligonucleotides of claim 10, wherein the T-loop motif comprises the sequence UUGAA.

12. — The library of oligonucleotides of claim 11, wherein the guanosine of the T-loop forms a Watson-Crick base pair with a cytidine in J3/1.

13. — The library of oligonucleotides of claim 1, wherein the helix domain has a first end and a second end, and the first end is proximal to the oligonucleotide junction, and the second end is linked to an oligonucleotide-based readout module.

14. — The library of oligonucleotides of claim 13, wherein the oligonucleotide-based readout module is a fluorogenic or switch-based readout module.

~~15. The library of oligonucleotides of claim 14, wherein the fluorogenic module is a Broccoli fluorophore binding aptamer.~~

~~16. The library of oligonucleotides of claim 14, wherein the switch-based module is a *pbuE* switch.~~

~~17. The library of oligonucleotides of claim 13, wherein the oligonucleotide-based readout module is an oligoribonucleotide-based readout module.~~

~~18. The library of oligonucleotides of claim 1, wherein individual oligonucleotides have sequence correspondence to a *Bacillus subtilis* xpt-pbuX guanine riboswitch sequence, comprising about 23 variable nucleotide residues within the oligonucleotide junction.~~

~~19. The library of oligonucleotides of claim 1, wherein individual oligonucleotides have sequence correspondence to a *Vibrio cholera* Vc2 cyclic di-GMP riboswitch sequence, comprising about 21 variable nucleotide residues within the oligonucleotide junction.~~

~~20. The library of oligonucleotides of claim 1, wherein individual oligonucleotides have sequence correspondence to a *Schistosoma mansoni* hammerhead ribozyme sequence, comprising about 21 variable nucleotide residues within the oligonucleotide junction.~~

~~21. The library of oligonucleotides of claim 1, wherein the oligonucleotide junction is an N-way junction, wherein N is two, three, four or five.~~

~~22. The library of oligonucleotides of claim 1, wherein the oligonucleotide junction is an N-way junction, wherein N is two.~~

~~23. The library of oligonucleotides of claim 1, wherein the oligonucleotide junction is an N-way junction, wherein N is three.~~

~~24. The library of oligonucleotides of claim 1, wherein the oligonucleotide junction is an N-way junction, wherein N is four.~~

~~25. The library of oligonucleotides of claim 1, wherein the oligonucleotide junction is an N-way junction, wherein N is five.~~

~~26. The library of oligonucleotides of claim 1, wherein the library comprises from about 4²⁴ to about 4²³ non-identical members.~~

~~272. A library of RNA oligonucleotides comprising a plurality of non-identical oligonucleotides, wherein the individual oligonucleotides comprise a structural scaffold comprising:~~

- ~~a) a first sequence comprising a helix domain;~~
- ~~b) a second sequence comprising a first hairpin domain; and~~
- ~~c) a third sequence comprising a second hairpin domain; and~~
- ~~d) an oligonucleotide junction;~~

~~wherein the oligonucleotide junction comprises (i) sequence joining the helix domain, the first hairpin domain and the second hairpin domain form an oligonucleotide junction containing and (ii) a pre-selected ligand-binding domain, and~~

~~wherein the library comprises a plurality of non-identical ligand-binding domains.~~

~~283. The library of RNA oligonucleotides of claim 1 or 27, wherein each helix domain independently is a fully complementary helix optionally comprising one or more destabilizing nucleotides selected from the group consisting of a mismatched base pair, a G•U wobble base pair, and a bulge.~~

~~294. The library of RNA oligonucleotides of claim 1 or 27, wherein each helix domain is a fully complementary helix.~~

~~530. The library of RNA oligonucleotides of claim 1 or 27, wherein each first hairpin domain independently comprises one or more destabilizing nucleotides selected from the group consisting of a mismatched base pair, a G•U wobble base pair, and a bulge.~~

~~634. The library of RNA oligonucleotides of claim 1 or 27, wherein each second hairpin domain independently comprises one or more destabilizing nucleotides selected from the group consisting of a mismatched base pair, a G•U wobble base pair, and a bulge.~~

~~732.~~ The library of RNA oligonucleotides of claim 1 or 27, wherein the helix domain is at least 4 to 10 base-pairs in length.

~~338.~~ The library of RNA oligonucleotides of claim 27, wherein the helix domain is at least 10 base-pairs in length.

~~349.~~ ~~The library of oligonucleotides of claim 27, wherein the oligonucleotides are oligoribonucleotides.~~

~~359.~~ The library of RNA oligonucleotides of claim 1 or 27, wherein the individual oligonucleotides comprise a sequence having a series of linked sequences according to Formula I:

(I) P1-J1/2-P2-L2-P2'-J2/3-P3-L3-P3'-J3/1-P1'

$\oplus$

wherein

- represents a bond;

P1 and P1' form the helix;

P2, L2 and P2' form the first hairpin;

P3, L3 and P3' form the second hairpin; and

J1/2, J2/3 and J3/1 together form the oligonucleotide junction.

~~36-10.~~ The library of RNA oligonucleotides of claim 935, wherein J2/3 comprises a T-loop motif.

~~3711.~~ The library of RNA oligonucleotides of claim 1036, wherein the T-loop motif comprises the sequence UUGAA.

~~3812.~~ The library of RNA oligonucleotides of claim 1137, wherein the guanosine of the T-loop forms a Watson-Crick base-pair with a cytidine in J3/1.

139. The library of RNA oligonucleotides of claim 1 or 27, wherein the helix domain has a first end and a second end, and the first end is proximal to the oligonucleotide junction, and the second end of is linked to an oligonucleotide-based readout module.

~~140.~~ The library of RNA oligonucleotides of claim ~~139~~, wherein the oligonucleotide-based readout module is a fluorogenic or switch-based readout module.

~~4115.~~ The library of RNA oligonucleotides of claim ~~140~~, wherein the fluorogenic module is a Broccoli fluorophore binding aptamer.

~~4216.~~ The library of RNA oligonucleotides of claim ~~140~~, wherein the switch-based module is a *pbuE* switch.

~~4317.~~ The library of RNA oligonucleotides of claim ~~139~~, wherein the oligonucleotide-based readout module is an oligoribonucleotide-based readout module.

~~4418.~~ The library of RNA oligonucleotides of claim ~~1 or 27~~, wherein the individual oligonucleotides comprise sequences having sequence correspondence to a *Bacillus subtilis* xpt-pbuX guanine riboswitch sequence, comprising ~~about~~ 23 variable nucleotide residues within the oligonucleotide junction.

~~4519.~~ The library of RNA oligonucleotides of claim ~~1 or 27~~, wherein the individual oligonucleotides comprise sequences having sequence correspondence to a *Vibrio cholera* Vc2 cyclic di-GMP riboswitch sequence, comprising ~~about~~ 21 variable nucleotide residues within the oligonucleotide junction.

~~4620.~~ The library of RNA oligonucleotides of claim ~~1 or 27~~, wherein the individual oligonucleotides comprise sequences having sequence correspondence to a *Schistosoma mansoni* hammerhead ribozyme sequence, comprising ~~about~~ 21 variable nucleotide residues within the oligonucleotide junction.

~~4721.~~ The library of RNA oligonucleotides of claim ~~1 or 27~~, wherein the oligonucleotide junction is an N-way junction, wherein N is ~~two~~, three, four or five.

~~482.~~ ~~The library of oligonucleotides of claim 27, wherein the oligonucleotide junction is an N-way junction, wherein N is two.~~

~~49~~22. The library of RNA oligonucleotides of claim 1 or 27, wherein the oligonucleotide junction is an N-way junction, wherein N is three.

~~50~~23. The library of RNA oligonucleotides of claim 1 or 27, wherein the oligonucleotide junction is an N-way junction, wherein N is four.

~~51~~24. The library of RNA oligonucleotides of claim 1 or 27, wherein the oligonucleotide junction is an N-way junction, wherein N is five.

25. The library of RNA oligonucleotides of claim 1 or 2, wherein the library comprises from 4²¹ to 4²³ non-identical members.

~~52~~26. The library of RNA oligonucleotides of claim 127, wherein the ~~preselected~~ ligand-binding ~~site~~domain comprises a binding site for a compound selected from the group consisting of an amino acid, a peptide, a nucleobase, a nucleoside, a nucleotide, a metal ion, a neurotransmitter, a hormone, an active pharmaceutical ingredient, and derivatives thereof.

~~53~~27. The library of RNA oligonucleotides of claim ~~52~~, wherein the pre-selected ligand-binding ~~site~~domain comprises a binding site for a ~~ligand~~compound selected from the group consisting of an amino acid, a peptide, a nucleobase, a nucleoside, a nucleotide, a metal ion, a neurotransmitter, a hormone, an active pharmaceutical ingredient, and derivatives thereof.

~~54~~28. The library of RNA oligonucleotides of claim ~~26~~53, wherein the ~~preselected~~ ligand-binding ~~site~~domain comprises a binding site for a ligand selected from the group consisting of an amino acid, a nucleobase, a nucleoside, a nucleotide, a neurotransmitter, a hormone, and derivatives thereof.

29. The library of RNA oligonucleotides of claim 27, wherein the pre-selected ligand-binding domain comprises a binding site for a ligand selected from the group consisting of an amino acid, a nucleobase, a nucleoside, a nucleotide, a neurotransmitter, a hormone, and derivatives thereof.

30. The library of RNA oligonucleotides of claim 28, wherein the ligand-binding domain comprises a binding site for a ligand selected from the group consisting of a nucleotide, a neurotransmitter, a hormone, and derivatives thereof.

31. The library of RNA oligonucleotides of claim 29, wherein the pre-selected ligand-binding domain comprises a binding site for a ligand selected from the group consisting of a nucleotide, a neurotransmitter, a hormone, and derivatives thereof.

32. The library of RNA oligonucleotides of claim 1, wherein the ligand-binding domain comprises a binding site for at least one ligand selected from the group consisting of 5-hydroxy-L-tryptophan, L-tryptophan, serotonin, and 5-hydroxy-L-tryptophan-methylamide.

~~5533~~. The library of RNA oligonucleotides of claim 27, wherein the pre-selected ligand-binding ~~site~~domain comprises a binding site for at least one ligand selected from the group consisting of 5-hydroxy-L-tryptophan, L-tryptophan, serotonin, and 5-hydroxy-L-tryptophan-methylamide.

~~5634~~. The library of RNA oligonucleotides of claim ~~5532~~ or 33, wherein the ligand is at least one of 5-hydroxy-L-tryptophan or serotonin.

~~5735~~. A method of selecting a plurality of non-identical ligand-binding RNA oligonucleotides, comprising the steps of:

- 1) contacting a library of RNA oligonucleotides comprising a plurality of RNA oligonucleotides with a ligand under conditions suitable for ligand binding, wherein the individual RNA oligonucleotides comprises a structural scaffold comprising:

- a) a first sequence comprising a helix domain;
- b) a second sequence comprising a first hairpin domain; ~~and~~
- c) a third sequence comprising a second hairpin domain; and
- d) an oligonucleotide junction;

wherein the oligonucleotide junction comprises sequence joining the helix domain, first hairpin domain and second hairpin domain ~~form an oligonucleotide junction;~~ and

- 2) partitioning the library of RNA oligonucleotides in a spatially addressable such that the plurality of non-identical ligand-binding RNA oligonucleotides is selected, wherein ~~the oligonucleotides having~~ the oligonucleotide junction further comprises a

ligand-binding domain, and wherein the ligand-binding domains of the library of RNA oligonucleotides comprise variable nucleotide residues, ~~is selected~~.

~~58~~36. The method of claim 357, wherein the method further comprises a step 1a) between step 1) and step 2), step 1a) comprising competitively partitioning the library of RNA oligonucleotides with a solution of free ligand.