

CLAIMS

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;
- 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 and (ii) a ligand-binding domain,

wherein the individual oligonucleotides comprise a sequence having a series of linked sequences according to Formula I:

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

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; and

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

2. 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;
- 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 and (ii) a pre-selected ligand-binding domain,

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'

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; and

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

3. The library of RNA oligonucleotides of claim 1 or 2, 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.

4. The library of RNA oligonucleotides of claim 1 or 2, wherein each helix domain is a fully complementary helix.

5. The library of RNA oligonucleotides of claim 1 or 2, 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.

6. The library of RNA oligonucleotides of claim 1 or 2, 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.

7. The library of RNA oligonucleotides of claim 1 or 2, wherein the helix domain is at least 4 to 10 base-pairs in length.

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

9. The library of RNA oligonucleotides of claim 1 or 2, wherein J2/3 comprises a T-loop motif.
10. The library of RNA oligonucleotides of claim 9, wherein the T-loop motif comprises the sequence UUGAA.
11. The library of RNA oligonucleotides of claim 10, wherein the guanosine of the T-loop forms a Watson-Crick base-pair with a cytidine in J3/1.
12. The library of RNA oligonucleotides of claim 1 or 2, 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.
13. The library of RNA oligonucleotides of claim 12, wherein the oligonucleotide-based readout module is a fluorogenic or switch-based readout module.
14. The library of RNA oligonucleotides of claim 13, wherein the fluorogenic module is a Broccoli fluorophore binding aptamer.
15. The library of RNA oligonucleotides of claim 13, wherein the switch-based module is a *pbuE* switch.
16. The library of RNA oligonucleotides of claim 12, wherein the oligonucleotide-based readout module is an oligoribonucleotide-based readout module.
17. The library of RNA oligonucleotides of claim 1 or 2, wherein the individual oligonucleotides comprise sequences having sequence correspondence to a *Bacillus subtilis* xpt-pbuX guanine riboswitch sequence, comprising 23 variable nucleotide residues within the oligonucleotide junction.
18. The library of RNA oligonucleotides of claim 1 or 2, wherein the individual oligonucleotides comprise sequences having sequence correspondence to a *Vibrio cholera* Vc2 cyclic di-GMP riboswitch sequence, comprising 21 variable nucleotide residues within the oligonucleotide junction.

19. The library of RNA oligonucleotides of claim 1 or 2, wherein the individual oligonucleotides comprise sequences having sequence correspondence to a *Schistosoma mansoni* hammerhead ribozyme sequence, comprising 21 variable nucleotide residues within the oligonucleotide junction.
20. The library of RNA oligonucleotides of claim 1 or 2, wherein the oligonucleotide junction is an N-way junction, wherein N is three, four or five.
21. The library of RNA oligonucleotides of claim 1 or 2, wherein the oligonucleotide junction is an N-way junction, wherein N is three.
22. The library of RNA oligonucleotides of claim 1 or 2, wherein the oligonucleotide junction is an N-way junction, wherein N is four.
23. The library of RNA oligonucleotides of claim 1 or 2, wherein the oligonucleotide junction is an N-way junction, wherein N is five.
24. The library of RNA oligonucleotides of claim 1 or 2, wherein the library comprises from 4^{21} to 4^{23} non-identical members.
25. The library of RNA oligonucleotides of claim 1, wherein the ligand-binding 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, and an active pharmaceutical ingredient.
26. The library of RNA oligonucleotides of claim 2, wherein the pre-selected ligand-binding 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, and an active pharmaceutical ingredient.
27. The library of RNA oligonucleotides of claim 25, wherein the 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, and a hormone.

28. The library of RNA oligonucleotides of claim 26, 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, and a hormone.
29. The library of RNA oligonucleotides of claim 27, wherein the ligand-binding domain comprises a binding site for a ligand selected from the group consisting of a nucleotide, a neurotransmitter, and a hormone.
30. The library of RNA oligonucleotides of claim 28, wherein the pre-selected ligand-binding domain comprises a binding site for a ligand selected from the group consisting of a nucleotide, a neurotransmitter, and a hormone.
31. 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.
32. The library of RNA oligonucleotides of claim 2, wherein the pre-selected 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.
33. The library of RNA oligonucleotides of claim 31 or 32, wherein the ligand is at least one of 5-hydroxy-L-tryptophan or serotonin.
34. 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;
 - 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;

wherein the individual oligonucleotides comprise a sequence having a series of linked sequences according to Formula I:

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

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; 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 oligonucleotide junction further comprises a ligand-binding domain, and wherein the ligand-binding domains of the library of RNA oligonucleotides comprise variable nucleotide residues.

35. The method of claim 34, 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.