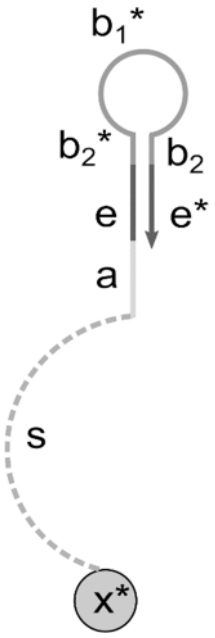
CLAIMS

1. A composition for detecting the presence of one or more target(s) of interest, wherein the composition comprises the following components:

- a first hairpin initiator molecule according to structure I:



(Structure I)

wherein the structure comprises domains a, b_1*, b_2, b_2*, e, e*, s and x*;

wherein neighbouring domains are connected directly to each other or via a linker;

wherein domain x* binds, or is complementary, to the one or more target(s) of interest;

wherein domain b_1* forms a hairpin loop and is at least 3 nucleotides long;

wherein domains b_2* and b_2 are of the same length and are complementary to each other;

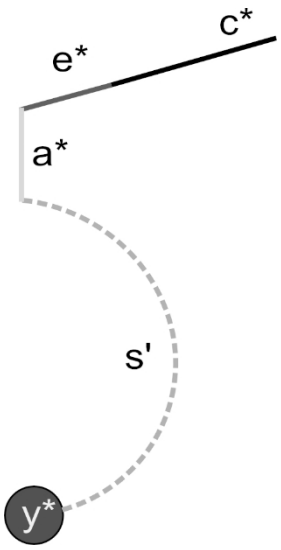
wherein domains b_2* and b_2 are between 2 to 6 nucleotides in length;

wherein domains e and e* are of the same length, are between 2 and 8 nucleotides in length, and are complementary to each other;

wherein domain a is at least 3 nucleotides in length;

wherein domain s is a spacer; and

- a second initiator molecule according to structure II:



(Structure II)

wherein the structure comprises domains a*, c*, e*, s' and y*;

wherein domain y* is complementary, or binds to, the one or more target(s) of interest;

wherein domains a and a* are complementary to each other;

wherein domain a* is between 3 to 10 nucleotides in length;

wherein domain e* of structure II is at least 2 nucleotides in length and binds to domain e of structure I upon binding the target of interest,

wherein domain c* is capable of binding to a signal generating molecule/signal generating complex; and

wherein domain s' is a spacer,

wherein the length of domains s and s' are selected to allow domains b_1*, b_2* and c* to be adjacent to each other upon binding,

and wherein upon binding to the one or more target(s) of interest by domains x* and y*, structures I and II are brought into close proximity with domain a binding to domain a*; domain e* of structure II displaces domain e* of structure I, with domain e* of structure II then binding to domain e on structure I; and domain b2* dissociates from b2 and hairpin loop b1* opens so that b1* and b2* are exposed in single stranded form.

2. The composition of claim 1, wherein all domains, except domains x*, y*, s, and s', are nucleic acid sequences.

3. The composition of claim 1, wherein domains x* and y* are selected from the group consisting of nucleic acid sequence, protein sequence, including post-translational modified versions thereof, antibody, antigen, and small molecule.

4. The composition of any of the preceding claims, wherein domain c* is at least the length of domain b_1*.

5. The composition of any of the preceding claims, wherein domain s is between 1 to 50 nm long.

6. The composition of any of the preceding claims, wherein domain c* is at least the length of domains b_1* and wherein domain d* is between 6 to 12 nucleotides long.

7. The composition of any of the preceding claims, wherein domain c* is at least the length of domains b_1*; wherein domain d* is between 6 to 12 nucleotides long; and wherein domain s is between 1 – 50 nm long.

8. The composition according to any one of the preceding claims, wherein domain b_2* is 2 or 3 nucleotides in length.

9. The composition according to any one of the preceding claims, wherein the one or more target(s) of interest are selected from the group consisting of DNA, RNA, single nucleotide polymorphism (SNP), microRNA (miRNA), genomic DNA, viral DNA, protein, post-translational modified proteins, cell surface receptors, metabolites, lipids, carbohydrates and small molecules.

10. The composition according to any one of the preceding claims, wherein the composition further comprises one or more signal generating molecules/signal generating complexes.

11. The composition according to any of the preceding claims, further comprising a pair of nucleic acid hairpins comprising a first hairpin reporter (HP1) and a second hairpin reporter (HP2) according to structures III and IV, respectively:



(S(Structure III) (Structure IV)

- wherein the first hairpin reporter comprises domains b, c, d and c*,
- wherein domain b is a 5' nucleic acid overhang with a length between 6 to 12 nucleotides,
- wherein domain d is a hairpin loop with a length between 6 to 12 nucleotides,
- wherein domain c* is as defined in any one of claims 1 to 11.
- wherein the second hairpin reporter (HP2) comprises domains d*, c', b_3* and c*',
- wherein domain b_3* is a hairpin loop of the same length as domain b,
- wherein domain d* is a nucleic acid overhang of the same length as domain d,
- wherein domains c' and c*' are of the same length, and are capable of binding to each other, and
- wherein domains b_3* and d each form a hairpin loop.

12. The composition of claim 11, wherein domain d and d* are complementary to each other.

13. The composition of claim 11 or 12, wherein the domains c' and c*' are complementary to each other.

14. The composition of any of claims 11 to 13, wherein the first hairpin reporter (HP1) comprises a first detection moiety.

15. The composition of any of claims 11 to 14, wherein the second hairpin comprises a second detection moiety.

16. The composition of claim 14 or 15, wherein the detection moiety is selected from the group consisting of fluorophores, small molecules, proteins and inorganic nanomaterials.

17. The composition of any of claims 11 to 16, wherein domain d* is between 6 to 12 nucleotides long.

18. A method for detecting one or more target(s) of interest in a sample, the method comprising providing a sample and detecting the one or more target(s) of interest using the composition according to any one of claims 1 to 17, whereby each composition is specific for one target of interest.

19. A method for detecting the presence of one or more target(s) of interest in a sample, the method comprising:

- adding one or more compositions according to any one of claims 1 to 17 to the sample;
- allowing binding of the one or more compositions to the one or more target(s) of interest;
- measuring one or more signals resulting from the binding of the composition(s) to the target(s) of interest;

wherein the generation of one or more signals detects the presence of one or more of the target(s) of interest in the sample.

20. The method according to claim 19, wherein each component of the one or more compositions, as defined in any of claims 1 to 17, is added simultaneously or sequentially.

21. The method of any one of claims 18 to 20, wherein the first hairpin initiator molecule and the second initiator molecule, as defined in claim 1, bind to at least one target(s) of interest.

22. The method of any once of claims 18 to 21, wherein the binding of the composition of claim 1 to the one or more target(s) of interest does not require enzymes.

23. The method of any one of claims 18 to 22, wherein the method is performed at a single temperature, at which the composition(s) bound to the target(s) of interest are stable.

24. The method of any one of claims 18 to 23, wherein the method is performed in a single reaction vessel.

25. The method according to any one of claims 18 to 24, wherein the one or more target(s) of interest are selected from the group consisting of DNA, RNA, single nucleotide polymorphism (SNP), microRNA (miRNA), genomic DNA, viral DNA, protein, protein interactions, post-translationally modified proteins, cell surface receptors metabolites, lipids, carbohydrates and small molecules.

26. The composition according to any one of claims 1 to 17 wherein presence of the one or more target(s) of interest indicates the presence of a disease.

27. A method of identifying a disease, the method comprising
    - adding one or more compositions according to any one of claims 1 to 17 or 26 to a sample obtained from a subject suspected to have the disease;
    - allowing binding of the one or more compositions to the one or more target(s) of interest;
    - measuring one or more signals resulting from the binding of the composition(s) to the target(s) of interest; and
    - identifying the disease, wherein the presence of one or more signals indicates the presence of one or more of the target(s) of interest in the sample; and wherein the one or more of the target(s) of interest are disease-specific.

28. A kit comprising the composition as defined in any one of claims 1 to 17 or 26.

29. The kit of claim 28, wherein each component of the composition is provided separately, in the form of a lyophilised powder or as a concentrated stock solution.

30. The kit of claim 28 or 29, optionally comprising i) reagents for performing affinity conjugation, or ii) reagents for performing covalent conjugation with a molecule selected from the group consisting of DNA, antibody and proteins.

31. Use of a method according to claim 22 or 23 in drug discovery.