CLAIMS

1. A composition comprising a first Enzyme Amplifier Substrate oligonucleotide (EAS1) and a second Enzyme Amplifier Substrate oligonucleotide (EAS2), wherein:

a portion of the EAS1 is complementary to a portion of the EAS2,

the EAS1 and EAS2 form a first Complete Enzyme Signal Amplifier (CESA) complex comprising a recognition and a cleavage sequence for a first nuclease only on assembly with a first Driver Fragment oligonucleotide (DF),

a portion of the first DF is complementary to a portion of the EAS1, and

binding of the first DF to said EAS1 completes a partial nuclease recognition or cleavage sequence.

2. A composition comprising an EAS1, an EAS2, and a first nuclease, wherein:

a portion of the EAS1 is complementary to a portion of the EAS2,

the EAS1 and EAS2 form a first CESA complex comprising a recognition sequence and a cleavage sequence for said first nuclease only on assembly with a first DF,

a portion of the first DF is complementary to a portion of the EAS1, and

binding of the first DF to said EAS1 completes a partial nuclease recognition or cleavage sequence.

3. A composition comprising a multi-component nucleic acid enzyme (MNAzyme), an MNAzyme substrate, an EAS1, an EAS2, and a first nuclease wherein:

the MNAzyme comprises at least a first partzyme and a second partzyme that self-assemble in the presence of an MNAzyme assembly facilitator to form the MNAzyme, wherein each of said at least first and said second partzymes comprise a substrate arm portion, a catalytic core portion, and a sensor arm portion, and wherein the sensor arms interact with said MNAzyme assembly facilitator so as to maintain the first and second partzymes in proximity for association of their respective catalytic core portions to form the catalytic core of the MNAzyme and said catalytic core is capable of modifying said MNAzyme substrate to form a first DF;

and wherein:

a portion of the EAS1 is complementary to a portion of the EAS2 and a portion of the EAS1 is complementary to a portion of the first DF,

the first DF is capable of assembly with the EAS1 and the EAS2 to form a first CESA complex containing a recognition site and a cleavage site for said at least first nuclease, and

binding of the first DF to said EAS1 completes a partial nuclease recognition or cleavage sequence.

4. The composition of claim 3, wherein said MNAzyme substrate is a first strand of an oligonucleotide complex comprising first and second strands, wherein said first strand comprises an internal loop portion and bases within the internal loop portion are not hybridised to bases of the second strand, and wherein the MNAzyme is capable of cleaving the internal loop portion.

5. The composition of claim 4, wherein said second strand comprises the first DF.

6. The composition of claim 4 or claim 5, wherein said first and second strands are linked at one end by a hairpin loop portion.

7. The composition of claim 3, wherein said MNAzyme substrate is a hairpin loop portion of a hairpin oligonucleotide, said MNAzyme is capable of cleaving the hairpin loop portion, and said first driver fragment is located in one strand of a double stranded stem portion in said hairpin oligonucleotide.

8. The composition of any one of claims 3 to 7, wherein the assembly facilitator is a target to be identified.

9. A composition comprising a first Synthetic Initiator Oligonucleotide (SIO), an EAS1, an EAS2, a first nuclease, and a second nuclease wherein:

the SIO is capable of hybridizing with a target to form a duplex substrate wherein said first nuclease is capable of cleaving the duplex substrate to generate a first DF; and wherein:

a portion of the EAS1 is complementary to a portion of the EAS2 and a portion of the EAS1 is complementary to a portion of the first DF,

the EAS1 and the EAS2 form a first CESA complex containing a recognition sequence and a cleavage sequence for said second nuclease only on assembly with the first DF, and

binding of the first DF to said EAS1 completes a partial nuclease recognition or cleavage sequence.

10. The composition of claim 9, wherein said first nuclease is capable of cleaving the SIO to generate said first DF only when the SIO is hybridised with the target.

11. The composition of claim 9, wherein said first nuclease is capable of cleaving the target to generate said first DF only when the target is hybridised with the SIO.

12. The composition of claim 11, wherein said first nuclease is not a restriction enzyme.

13. The composition of any one of claims 9 to 12, wherein said first nuclease is an exonuclease.

14. The composition of any one of claims 9 to 13, wherein the first and second nuclease are the same nuclease.

15. The composition of any one of claims 9 to 13, wherein the first and second nucleases are different nucleases.

16. The composition of any one of claims 1 to 8, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

17. The composition of any one of claims 9 to 15, wherein said second nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

18. The composition of claim 16 or claim 17, wherein said nick is located within the nuclease recognition site, at the nuclease cleavage site, or between the nuclease recognition and cleavage sites.

19. The composition of any one of claims 16 to 18, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

20. The composition of any one of claims 1 to 19, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition sequence.

21. The composition of any one of claims 1 to 19, wherein binding of the first DF to said EAS1 completes a partial nuclease cleavage sequence.

22. The composition of claim 20 or claim 21, wherein the first DF contributes at least one base to said sequence.

23. The composition of any one of claims 20 to 22, wherein the first DF contributes at least two bases to said sequence.

24. The composition of any one of claims 20 to 23, wherein the first DF contributes at least three bases to said sequence.

25. The composition of any one of claims 22 to 24, wherein said bases are immediately 3' of a partial nuclease recognition site formed by the binding of said EAS1 and EAS2.

26. The composition of any one of claims 22 to 24, wherein said bases are immediately 5' of a partial nuclease recognition site formed by the binding of said EAS1 and EAS2.

27. The composition of any one of claims 1 to 26, wherein said EAS1 and EAS2 are components of a hairpin oligonucleotide comprising a double-stranded stem portion

formed by hybridisation of complementary portions of said EAS1 and the EAS2, and a hairpin loop portion linking one end of said EAS1 with one end of said EAS2.

28. The composition of claim 27, wherein said hairpin loop portion is an oligonucleotide linker or a non-oligonucleotide linker.

29. The composition of claim 27 or claim 28, wherein said hairpin oligonucleotide comprises a single stranded 5' or 3' overhang portion extending from either of said EAS1 or EAS2.

30. The composition of any one of claims 1 to 29, wherein a portion of said EAS1 or EAS2 comprises a second DF, and wherein said second DF can be released upon modification of said first CESA complex by the nuclease.

31. The composition of any one of claims 27 to 29, wherein a portion of said hairpin loop portion comprises a second DF, and wherein said second DF can be released upon modification of said first CESA complex by the nuclease.

32. The composition of claim 30 or claim 31, wherein said first DF and said second DF are not identical.

33. The composition of claim 30 or claim 31, wherein said first DF and said second DF are identical.

34. The composition of claim 30 or claim 31, wherein said second DF is a fragment of said first DF, or said first DF is a fragment of said second DF.

35. The composition of claim 33 or claim 34, wherein said second DF, EAS1, and EAS2 are capable of assembly to form said first CESA complex.

36. The composition of any one of claims 30 to 32, further comprising a third Enzyme Amplifier Substrate oligonucleotide (EAS3) and a fourth Enzyme Amplifier Substrate oligonucleotide (EAS4), wherein a portion of the EAS3 is complementary to a portion of the EAS4 and a portion of the EAS3 is complementary to a portion of the second DF, and wherein the EAS3 and the EAS4 form a second CESA complex containing a recognition sequence and a cleavage sequence for an additional nuclease only on assembly with the second DF.

37. The composition of claim 36, wherein the EAS3 or EAS4 comprises a third DF.

38. The composition of claim 37, wherein the third DF is identical to said first DF.

39. The composition of claim 37, wherein the third DF is not identical said first DF.

40. The composition of any one of claims 36 to 39, wherein said additional nuclease is identical to another nuclease in said composition.

41. The composition of any one of claims 36 to 39, wherein said additional nuclease is not identical to another nuclease in said composition, and wherein said composition comprises said additional nuclease.

42. The composition of any one of claims 36 to 41, wherein said additional nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said second CESA complex

43. The composition of any one of claims 36 to 42, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

44. The composition of any one of claims 36 to 43, wherein binding of the second DF to said EAS3 completes a partial nuclease recognition sequence and/or a nuclease cleavage sequence.

45. The composition of any one of claims 36 to 43, wherein the second DF does not contribute any bases to said nuclease recognition sequence or said nuclease cleavage sequence.

46. The composition of any one of claims 36 to 45, wherein said EAS3 and EAS4 are components of a hairpin oligonucleotide comprising a double-stranded stem portion formed by hybridisation of complementary portions of EAS3 and EAS4, and a hairpin loop portion linking one end of said EAS3 with one end of said EAS4.

47. The composition of claim 46, wherein said hairpin loop portion is an oligonucleotide linker or a non-oligonucleotide linker.

48. The composition of claim 46 or claim 47, wherein said hairpin oligonucleotide comprises a single stranded 5' or 3' overhang portion extending from either of said EAS3 or EAS4.

49. A composition comprising a first complex, said first complex comprising a backbone oligonucleotide, an EAS1, an EAS2, an EAS3, and an EAS4, wherein said backbone oligonucleotide comprises:

(i) a first portion comprising said EAS1, wherein:

a portion of said EAS1 is complementary to a portion of EAS2, a portion of the EAS2 is complementary to a portion of a first DF, and a portion of the EAS1 or EAS2 comprises a second DF,

the EAS1 and the EAS2 form a first CESA complex containing a recognition sequence and a cleavage sequence for a first nuclease only on assembly with said first DF, and

binding of said first DF to said EAS2 completes a partial nuclease recognition sequence and/or a partial nuclease cleavage sequence;

(ii) a second portion comprising said EAS3, wherein a portion of said EAS3 is complementary to a portion of said EAS4, a portion of said EAS3 is complementary to a portion of the second DF, and a portion of said EAS3 or EAS4 comprises said first DF, and wherein the EAS3 and the EAS4 form a second CESA complex containing a recognition sequence and a cleavage sequence for a second nuclease only on assembly with said second DF; and

(iii) a third portion connecting the first and second portions.

50. The composition of claim 49, further comprising a second complex, said second complex comprising a backbone oligonucleotide, a fifth Enzyme Amplifier Substrate Oligonucleotide (EAS5), a sixth Enzyme Amplifier Substrate Oligonucleotide (EAS6), a seventh Enzyme Amplifier Substrate Oligonucleotide (EAS7), and an eighth Enzyme Amplifier Substrate Oligonucleotide (EAS8), wherein said backbone oligonucleotide comprises:

(i) a first portion comprising said EAS5, wherein a portion of said EAS5 is complementary to a portion of said EAS6, a portion of said EAS5 is complementary to a portion of said second DF, and a portion of said EAS5 or EAS6 comprises said first DF, and wherein the EAS5 and the EAS6 form a third CESA complex containing a recognition sequence and a cleavage sequence for a third nuclease only on assembly with said second DF; and

(ii) a second portion comprising said EAS7, wherein a portion of said EAS7 is complementary to a portion of EAS8, a portion of said EAS8 is complementary to a portion of said first DF, and a portion of said EAS7 or EAS8 comprises said second DF, and wherein the EAS7 and the EAS8 form a fourth CESA complex containing a recognition sequence and a cleavage sequence for a fourth nuclease only on assembly with said first DF; and

(iii) a third portion connecting the first and second portions, wherein said third portion is complementary to the third portion of the backbone of said first complex.

51. The composition of claim 50, wherein EAS1 is identical to EAS7, EAS2 is identical to EAS8, EAS3 is identical to EAS5, and/or EAS4 is identical to EAS6.

52. The composition of claim 50 or claim 51, wherein the first nuclease is identical to the third nuclease, and/or the second nuclease is identical to the fourth nuclease, and/or the first, second, third and fourth nucleases are identical.

53. The composition of any one of claims 50 to 52, wherein first and second complexes are hybridised via their respective complementary third portions forming a first double complex.

54. The composition of claim 53, wherein said first double complex is linked to a second double complex.

55. The composition of claim 54, wherein said first double complex is linked to said second double complex by linking any one or more of EAS1-EAS8 of said first double complex with any one or more of EAS1-EAS8 of said second double complex.

56. The composition of claim 54 or claim 55, wherein said first double complex is linked to said second double complex by linking EAS2 and/or EAS8 of said first double complex with EAS2 and/or EAS8 of said second double complex.

57. The composition of claim 55 or claim 56, wherein said linking is achieved using any one or more of chemical hybridisation, antibodies, oligonucleotide linkers, non-oligonucleotide linkers, covalent bonding and peptide linkers.

58. The composition of claim 55 or claim 56, wherein said linking is achieved via biotinylation of any one or more of said Enzyme Amplifier Substrate Oligonucleotides and the complexing of multiple biotinylated Enzyme Amplifier Substrate Oligonucleotides using avidin.

59. The composition of any one of claims 49 to 58, wherein said first and/or said second nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said second CESA complex

60. The composition of any one of claims 49 to 59, wherein said first and/or said second nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

61. The composition of any one of claims 50 to 60, wherein binding of said first DF to said EAS2 or EAS8 and/or the binding of said second DF to said EAS3 or EAS5 completes a partial nuclease recognition sequence and/or a partial nuclease cleavage sequence.

62. The composition of any one of claims 50 to 61, wherein a pair of Enzyme Amplifier Substrates selected from EAS1 and EAS2; EAS3 and EAS4; EAS5 and EAS6; and EAS7 and EAS8, is a component of a hairpin oligonucleotide comprising a double-stranded stem portion formed by hybridisation of complementary portions of each member of said pair, and a hairpin loop portion linked to one end of the stem portion.

63. The composition of claim 62, wherein said hairpin loop portion is an oligonucleotide linker or a non-oligonucleotide linker.

64. The composition of claim 62 or claim 63, wherein said hairpin oligonucleotide comprises a single stranded 5' or 3' overhang portion.

65. A composition comprising:

(i) a SIO, said SIO comprising a first portion complementary to a target strand and a second portion that is not complementary to said target strand, wherein said first and second portions are separated by a phosphorothioate, and said second portion comprises a first DF; and,

(ii) an EAS1 and an EAS2, wherein

a portion of the EAS1 is complementary to a portion of the EAS2 and hybridization of the EAS1 and the EAS2 provides a duplex structure with a 3' overhang at either end,

a portion of the EAS1 is complementary to a portion of the first DF, and

the EAS1 and the EAS2 are capable of forming a first CESA complex comprising a recessed 3' end capable of digestion by a first nuclease, only on assembly with said first DF.

66. The composition of claim 65, wherein said SIO is a hairpin oligonucleotide comprising a double-stranded stem formed by hybridisation of two complementary portions, a single stranded hairpin loop, and a 3'overhang.

67. The composition of claim 65 or claim 66, wherein the nuclease is an exonuclease.

68. The composition of claim 67, wherein the exonuclease cannot digest single stranded oligonucleotides, double stranded oligonucleotides comprising a 3' overhang of 5 or more bases, or phosphorothioate internucleotide linkages.

69. The composition of any one of claims 1 to 68, wherein any said first DF is generated using an endonuclease or an exonuclease.

70. The composition of claim 69, wherein the exonuclease is selected from the group consisting of Nuclease BAL-31, Exonuclease I, Exonuclease III, T7 Exonuclease, T7 Exonuclease I and Exonuclease T.

71. The composition of claim 69 or claim 70, wherein the exonuclease is Exonuclease III.

72. The composition of claim 69, wherein the endonuclease is T7 Endonuclease I, RNase H, Flap Nuclease, or Mung Bean Nuclease.

73. The composition of any one of claims 1 to 72, wherein any said EAS comprises one or more detectable labels.

74. The composition of any one of claims 1 to 73, wherein any said EAS comprises a fluorophore portion and/or a quencher portion.

75. The composition of any one of claims 1 to 74, wherein any said partzyme, assembly facilitator, MNAzyme substrate, DF, EAS1, EAS2, EAS3, EAS4, EAS5, EAS6, EAS7, EAS8, or SIO comprises at least one nucleotide substitution or addition selected from the group consisting of phosphorothioate, 4-acetylcytidine, 5-(carboxyhydroxylmethyl)uridine, 2'-O-methylcytidine, 5-carboxymethylaminomethyl thiouridine, dihydrouridine, 2'-O-methylpseudouridine, beta D-galactosylqueosine, 2'-O-methylguanosine, inosine, N6-isopentenyladenosine, 1-methyladenosine, 1-methylpseudouridine, 1-methylguanosine, 1-methylinosine, 2,2-dimethylguanosine, 2-methyladenosine, 2-methylguanosine, 3-methylcytidine, 5-methylcytidine, N6-methyladenosine, 7-methylguanosine, 5-methylaminomethyluridine, 5-methoxyaminomethyl-2-thiouridine, beta D-mannosylmethyluridine, 5-methoxycarbonylmethyluridine, 5-methoxyuridine, 2-methylthio-N6-isopentenyladenosine, N-((9-beta-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine, N-((9-beta-ribofuranosylpurine-6-yl)N-methyl-carbamoyl)threonine, uridine-5-oxyacetic acid methylester, uridine-5-oxyacetic acid (v), wybutoxosine, pseudouridine, queosine, 2-thiocytidine, 5-methyl-2-thiouridine, 2-thiouridine, 4-thiouridine, 5-methyluridine, N-((9-beta-D-ribofuranosylpurine-6-yl)carbamoyl)threonine, 2'-O-methyl-5-methyluridine, 2'-O-methyluridine, wybutosine, 3-(3-amino-3-carboxypropyl)uridine, beta D-arabinosyl uridine and beta D-arabinosyl thymidine.

76. The composition of any one of claims 3 to 8, wherein at least one of the MNAzyme partzymes, MNAzyme substrate or a combination thereof further comprises an aptamer or portion thereof.

77. The composition claim 76, wherein the aptamer or portion thereof comprises at least one of: a nucleic acid, peptide, polypeptide, protein, a derivative thereof, or a combination thereof.

78. The composition of any one of claims 1 to 77, wherein any said MNAzyme partzyme, MNAzyme substrate, EAS1, EAS2, EAS3, EAS4, EAS5, EAS6, EAS7, EAS8, SIO, DF or nuclease is attached to a solid support.

79. The composition of any one of claims 1, 2, or 49, wherein said first DF is produced only in the presence of a target.

80. The composition of any one of claims 3, 9 or 49, wherein said first DF is distinct from said target.

81. The composition of claim 1 or claim 2, wherein said composition is for detecting a target, and first DF is distinct from said target.

82. The composition of any one of claims 1 to 81, wherein any said EAS can hybridise with another EAS to from a Partial Enzyme Signal Amplifier (PESA) complex capable of hybridizing with more than one DF.

83. The composition of any one of claims 3 to 8, wherein said composition comprises first and second MNAzymes specific for different portions of a target molecule.

84. The composition of claim 83, wherein said MNAzymes specific for different portions of said target molecule recognize and cleave the same substrate upon assembly in the presence of said target.

85. The composition of any one of claims 3 to 8, wherein said composition comprises at least two different MNAzymes having specificity for distinct targets.

86. The composition of claim 9 or claim 65, wherein said composition comprises at least two different SIO with complementarity for distinct targets.

87. The composition of any one of claims 1 to 3, 9, 49 or 65, wherein said composition comprises at least two distinct first CESA complexes assembled with a different first DF.

88. A method for detecting a target comprising:

(a) providing two or more partzymes and at least one multi-component nucleic acid (MNAzyme) substrate, wherein the partzymes self-assemble in the presence of the target to form at least one MNAzyme;

(b) contacting the partzymes with a sample putatively containing the target under conditions permitting self-assembly and catalytic activity of the MNAzyme, and wherein catalytic activity of said MNAzyme produces a first Driver Fragment oligonucleotide (DF) from said at least one MNAzyme substrate;

(c) providing a first Enzyme Amplifier Substrate Oligonucleotide (EAS1) and a second Enzyme Amplifier Substrate Oligonucleotide (EAS2) wherein a portion of the EAS1 is complementary to a portion of the EAS2, and wherein a portion of the EAS1 is complementary to a portion of the first DF and;

(d) contacting the EAS1 and the EAS2 with the first DF under conditions permitting;

(1) assembly of the first DF with the EAS1 and the EAS2 to form a first Complete Enzyme Signal Amplifier (CESA) complex, and

(2) formation of a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(e) providing the first nuclease; and

(f) contacting the first nuclease with the first CESA complex under conditions permitting interaction of the nuclease with the recognition site and cleavage at the cleavage site wherein the cleavage by said first nuclease produces a detectable effect indicative of the presence of the target.

89. The method of claim 88, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said recognition site and/or said cleavage site for said first nuclease.

90. The method of claim 88 or claim 89, wherein the first DF is produced by cleavage of the MNAzyme substrate.

91. The method of claim 88 or claim 89, wherein the first DF is produced by ligation of two or more MNAzyme substrates.

92. The method of any one of claims 88 to 90, wherein said MNAzyme substrate is a first strand of an oligonucleotide complex comprising first and second strands, wherein said first strand comprises an internal loop portion and bases within the internal loop portion are not hybridised to bases of the second strand, and wherein the MNAzyme is capable of cleaving the internal loop portion.

93. The method of claim 92, wherein said second strand comprises the first DF.

94. The method of claim 92 or claim 93, wherein said first and second strands are linked at one end by a hairpin loop portion.

95. The method of any one of claims 88 to 90, wherein said MNAzyme substrate is a hairpin loop portion of a hairpin oligonucleotide, said MNAzyme is capable of cleaving the hairpin loop portion, and said first driver fragment is located in one strand of a double stranded stem portion in said hairpin oligonucleotide.

96. A method of detecting a target comprising:

(a) providing at least a first Synthetic Initiator Oligonucleotide (SIO);

(b) contacting the SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target thus creating a duplex substrate for a first nuclease;

(c) providing a first nuclease capable of cleaving the duplex substrate formed by hybridization of the SIO and the target wherein cleavage of the duplex substrate by the first nuclease generates a first DF;

(d) providing an EAS1 and an EAS2 wherein a portion of the EAS1 is complementary to a portion of the EAS2, and wherein a portion of the EAS1 is complementary to a portion of the first DF and;

(e) contacting the EAS1 and the EAS2 with the first DF under conditions permitting:

(1) assembly of the first DF with the EAS1 and the EAS2 to form a first CESA, and

(2) formation of a recognition site and a cleavage site for a second nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(f) providing a second nuclease; and

(g) contacting the second nuclease with the first CESA under conditions permitting interaction of the second nuclease with the recognition site and cleavage at the cleavage site wherein cleavage by the second nuclease produces a detectable effect indicative of the presence of the target.

97. The method of claim 96, wherein the first nuclease cleaves said SIO to generate said first DF only when said SIO is hybridised with the target.

98. The method of claim 96, wherein the first nuclease cleaves said target to generate said first DF only when said target is hybridised with the SIO.

99. The method of claim 98, wherein said first nuclease is not a restriction enzyme.

100. The method of any one of claims 96 to 99, wherein said first nuclease is an exonuclease.

101. The method of any one of claims 88 to 100, wherein cleavage of the first CESA complex allows release of a further DF, and the further DF assembles with further Enzyme Amplifier Substrate Oligonucleotides to form a further CESA complex, and at least one nuclease is used to cleave the further CESA complex to produce further detectable effect and release further DF, thereby facilitating a further increase in the detectable effect.

102. A method of detecting a target using a cascade comprising:

(a) producing a first DF, wherein said first DF is produced only in the presence of said target,

(b) providing:

(i) an EAS1 and an EAS2 wherein:

a portion of the EAS1 is complementary to a portion of the EAS2:

a portion of the EAS1 is complementary to a portion of the first DF; and

a portion of the EAS1 or the EAS2 comprises a second DF;

and,

(ii) a third Enzyme Amplifier Substrate Oligonucleotide (EAS3) and a fourth Enzyme Amplifier Substrate Oligonucleotide (EAS4) wherein:

a portion of the EAS3 is complementary to a portion of the EAS4;

a portion of the EAS3 is complementary to a portion of the second DF;

(c) contacting:

(i) the EAS1 and the EAS2 with the first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA complex comprising a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA complex with the first nuclease under conditions permitting interaction of the first nuclease with the recognition site and cleavage site of the first CESA complex, wherein cleavage at the cleavage site by the first nuclease releases the second DF;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA complex comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA complex with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA complex, wherein the second nuclease cleaves said second CESA complex at said cleavage site;

and wherein cleavage of said first CESA complex and/or said second CESA complex produces a detectable effect.

103. The method of claim 102, wherein cleavage of said first CESA complex and said second CESA complex each produces a detectable effect.

104. The method of claim 102 and claim 103, wherein said first nuclease and said second nuclease are the same nuclease.

105. The method of claim 102 and claim 103, wherein said first nuclease and said second nuclease are different nucleases.

106. The method of any one of claims 102 to 105, wherein a portion of said EAS3 or said EAS4 comprises an additional DF and cleavage at said cleavage site of the second CESA complex by the second nuclease releases said additional DF.

107. The method of claim 106, wherein a portion of said additional DF is complementary to a first portion of a fifth Enzyme Amplifier Substrate Oligonucleotide (EAS5), wherein a second portion of said EAS5 is complementary to a portion of a sixth Enzyme Amplifier Substrate Oligonucleotide (EAS6), and wherein said EAS5 and EAS6 assemble with said additional DF to form third CESA complex.

108. The method of claim 106, wherein:

(i) a portion of the additional DF is identical to said first DF;

(ii) the additional DF is identical to said first DF; or

(iii) the additional DF is a fragment of said first DF;

and wherein said additional DF can assemble with said EAS1 and EAS2 to form said first CESA complex.

109. A method of detecting a plurality of distinct targets using a cascade comprising:

(a) producing at least a first DF and a second DF, wherein said first DF is produced only in the presence of a first target, and said second DF is produced only in the presence of a second target;

(b) providing:

(i) an EAS1 and an EAS2 wherein:

a portion of the EAS1 is complementary to a portion of the EAS2:

a portion of the EAS1 is complementary to a portion of the first DF; and

(ii) an EAS3 and an EAS4 wherein:

a portion of the EAS3 is complementary to a portion of the EAS4:

a portion of the EAS3 is complementary to a portion of the second DF;

(c) contacting:

(i) the EAS1 and the EAS2 with the first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA complex comprising a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA complex with the first nuclease under conditions permitting interaction of the first nuclease with said recognition site and cleavage site of the first CESA complex, wherein said first nuclease cleaves said first CESA complex at said cleavage site producing a first detectable effect;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA complex comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA complex with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA complex, wherein said second nuclease cleaves said second CESA complex at said cleavage site producing a second detectable effect;

and wherein said first detectable effect is distinct from said second detectable effect.

110. The method of claim 109, wherein said first nuclease and said second nuclease are the same nuclease.

111. The method of claim 109 and claim 110, wherein said first nuclease and said second nuclease are different nucleases.

112. The method of any one of claims 109 to 111, wherein

(i) a portion of said EAS1 or said EAS2 comprises an additional DF and cleavage at said cleavage site of the first CESA complex by the first nuclease releases said additional DF; and

(ii) said additional DF assembles with at least two additional EAS oligonucleotides to form an additional CESA complex and cleavage of said additional CESA complex by a nuclease increases said first detectable effect.

113. The method of any one of claims 109 to 112, wherein

(i) a portion of said EAS3 or said EAS4 comprises an additional DF and cleavage at said cleavage site of the second CESA complex by the second nuclease releases said additional DF; and

(ii) said additional DF assembles with at least two additional EAS oligonucleotides to form an additional CESA complex and cleavage of said additional CESA complex by a nuclease increases said second detectable effect.

114. A method of detecting a target using a cascade comprising:

(a) producing a first driver fragment, wherein said first driver fragment is provided only in the presence of said target;

(b) providing:

(i) an EAS1 and an EAS2 wherein:

a portion of the EAS1 is complementary to a portion of the EAS2;

a portion of the EAS1 is complementary to a portion of the first DF;

a portion of the EAS1 or EAS2 comprises a second DF; and

the EAS1 or the EAS2 is tethered to a support;

(ii) an EAS3 and an EAS4 wherein:

a portion of the EAS3 is complementary to a portion of the EAS4:

a portion of the EAS3 is complementary to a portion of the second DF;

a portion of the EAS3 or EAS4 comprises a third DF; and

the EAS3 or the EAS4 is tethered to a support;

(c) contacting:

(i) the EAS1 and the EAS2 with said first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA comprising a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA with the first nuclease under conditions permitting interaction of the first nuclease with the recognition site and cleavage site of the first CESA, wherein cleavage at said cleavage site by the first releases the second DF;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA, wherein cleavage at said cleavage site by the second nuclease releases the third DF which can assemble with said EAS1 and EAS2 to form said first CESA;

and wherein cleavage of said first CESA complex and/or said second CESA complex produces a detectable effect.

116. The method of any one of claims 102 to 114, wherein said first DF of (a) is produced by

contacting a SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target to form a duplex structure amenable to modification by an initiator nuclease, and

contacting the duplex structure with said initiator nuclease,

wherein modification of paired or unpaired regions in the duplex structure by the initiator nuclease releases said first DF.

116. The method of any one of claims 109 to 113, wherein said second DF of (a) is produced by

contacting a SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target to form a duplex structure amenable to modification by an initiator nuclease, and

contacting the duplex structure with said initiator nuclease,

wherein modification of paired or unpaired regions in the duplex structure by the initiator nuclease releases said second DF.

117. The method of claim 115 or claim 116, wherein said initiator nuclease cleaves the SIO to generate said DF only when the SIO is hybridised with the target.

118. The method of claim 115 or claim 116, wherein said initiator nuclease cleaves the target to generate said DF only when the target is hybridised with the SIO.

119. The method of claim 118, wherein said initiator nuclease is not a restriction enzyme.

120. The method of any one of claims 115 to 119, wherein said initiator nuclease is an exonuclease.

121. The method of any one of claims 115 to 120, wherein said SIO is attached to a support.

122. The method of any one of claims 102 to 114, wherein said first DF of (a) is produced by providing two or more partzymes and at least one MNAzyme substrate, and, contacting the partzymes with a sample putatively containing the target under conditions permitting self-assembly and catalytic activity of the MNAzyme in the presence of said target, wherein said catalytic activity modifies said substrate thereby providing said first DF.

123. The method of any one of claims 109 to 113, wherein said second DF of (a) is produced by providing two or more partzymes and at least one MNAzyme substrate, and, contacting the partzymes with a sample putatively containing the target under conditions permitting self-assembly and catalytic activity of the MNAzyme in the presence of said

target, wherein said catalytic activity modifies said substrate thereby providing said second DF.

124. The method of claim 122 or claim 123, wherein said MNAzyme substrate is a first strand of an oligonucleotide complex comprising first and second strands, wherein said first strand comprises an internal loop portion and bases within the internal loop portion are not hybridised to bases of the second strand, and wherein the MNAzyme is capable of cleaving the internal loop portion.

125. The method of claim 124, wherein said second strand comprises said DF.

126. The method of claim 124 or claim 125, wherein said first and second strands are linked at one end by a hairpin loop portion.

127. The method of claim 126, wherein said hairpin loop portion is an oligonucleotide linker or a non-oligonucleotide linker.

128. The method of claim 122 or claim 123, wherein said MNAzyme substrate is a hairpin loop portion of a hairpin oligonucleotide, said MNAzyme is capable of cleaving the hairpin loop portion, and said driver fragment is located in one strand of a double stranded stem portion in said hairpin oligonucleotide.

129. The method of any one of claims 102 to 128, wherein said EAS3 and EAS4 are components of a hairpin oligonucleotide complex comprising a double-stranded portion formed between complementary portions of said EAS3 and EAS4, and a hairpin loop portion linking one end of said EAS3 with one end of said EAS4.

130. The method of any one of claims 88 to 128, wherein said EAS1 and EAS2 are components of a hairpin oligonucleotide complex comprising a double-stranded portion formed between complementary portions of said EAS1 and EAS2, and a hairpin loop portion linking one end of said EAS1 with one end of said EAS2.

131. The method of claim 129 or claim 130, wherein said hairpin loop portion is an oligonucleotide linker or a non-oligonucleotide linker.

132. The method of any one of claims 129 to 131, wherein said hairpin oligonucleotide complex further comprises a 5' or a 3' overhanging single stranded portion extending from one EAS oligonucleotide.

133. The method of any one of claims 129 to 132, wherein said hairpin loop portion comprises a detectable portion and/or a quencher portion.

134. The method of any one of claims 102 to 128, wherein the EAS3 and/or EAS4 comprises a detectable portion and/or a quencher portion, and said detectable portion and

quencher portion separate upon cleavage of the second CESA by the second nuclease providing a detectable effect.

135. The method of claim 134, wherein the EAS3 comprises a detectable portion and a quencher portion, the EAS4 comprises a further quencher portion, and said detectable portion and further quencher portion separate upon cleavage of the second CESA by the second nuclease providing a detectable effect.

136. The method of any one of claims 88 to 128, wherein the EAS1 and/or EAS2 comprises a detectable portion and/or a quencher portion, and said detectable portion and quencher portion separate upon cleavage of the first CESA by the first nuclease providing a detectable effect.

137. The method of claim 136, wherein the EAS1 comprises a detectable portion and a quencher portion, the EAS2 comprises a further quencher portion, and said detectable portion and further quencher portion separate upon cleavage of the first CESA by the first nuclease providing a detectable effect.

138. The method of any one of claims 133 to 137, wherein said detectable portion is a fluorophore.

139. The method of any one of claims 88 to 95, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

140. The method of any one of claims 96 to 101, wherein said second nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

141. The method of any one of claims 102 to 135, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex, and/or said second nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said second CESA complex.

142. The method of any one of claims 139 to 141, wherein said nick is located within the nuclease recognition site, at the nuclease cleavage site, or between the nuclease recognition and cleavage sites.

143. The method of any one of claims 139 to 142, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

144. The method of any one of claims 88 to 143, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site.

145. The method of any one of claims 102 to 135, wherein binding of the second DF to said EAS2 completes a partial nuclease recognition site.

146. The method of claim 144 or claim 145, wherein binding of the first DF to said EAS1 completes a partial nuclease cleavage site.

147. The method of any one of claims 144 to 146, wherein binding of the second DF to said EAS2 completes a partial nuclease cleavage site.

148. The method of any one of claims 144 to 147, wherein the DF contributes at least one, two, or three bases to said partial nuclease recognition site and/or a partial nuclease cleavage site

149. The method of any one of claims 146 to 148, wherein said bases are immediately 3' of a partial nuclease recognition site formed by the binding of said Enzyme Amplifier Substrate oligonucleotides.

150. The method of any one of claims 146 to 148, wherein said bases are immediately 5' of a partial nuclease recognition site formed by the binding of said Enzyme Amplifier Substrate oligonucleotides.

151. The method of any one of claims 102 to 135, wherein the second DF does not contribute any bases to said nuclease recognition site or said nuclease cleavage site.

152. A method of detecting a target using a cascade comprising:

(a) producing a first driver fragment, wherein said first driver fragment is produced only in the presence of said target;

(b) providing a first complex comprising a first backbone oligonucleotide, said first backbone oligonucleotide comprising:

(i) a first portion comprising an EAS1, wherein

a portion of said EAS1 is complementary to a portion of an EAS2;

a portion of the EAS2 is complementary to a portion of the first driver fragment; and

a portion of the EAS1 or EAS2 comprises a second driver fragment;

(ii) a second portion comprising an EAS3, wherein

a portion of said EAS3 is complementary to an EAS4;

a portion of said EAS3 is complementary to a portion of the second driver fragment; and

a portion of the EAS3 or EAS4 comprises said first driver fragment; and,

(iii) a third portion connecting the first and second portions;

and,

(c) contacting:

(i) the EAS1 and the EAS2 with said first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA comprising a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA with the first nuclease under conditions permitting interaction of the first nuclease with the recognition site and cleavage site of the first CESA, wherein cleavage at said cleavage site by the first nuclease releases the second DF;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA, wherein cleavage at said cleavage site by the second nuclease releases the first DF which can assemble with said EAS1 and EAS2 to form said first CESA;

and wherein cleavage of said first CESA complex and/or said second CESA complex produces a detectable effect.

153. The method of claim 152, wherein cleavage of said first CESA complex and cleavage of said second CESA complex each produces a detectable effect.

154. The method of claim 152 or claim 153, further comprising:

(a) providing a second complex, said second complex comprising a backbone oligonucleotide comprising:

(i) a first portion comprising a fifth Enzyme Amplifier Substrate Oligonucleotide (EAS5), wherein

a portion of said EAS5 is complementary to a portion of a sixth Enzyme Amplifier Substrate Oligonucleotide (EAS6); and

a portion of the EAS5 is complementary to a portion of the second driver fragment; and

a portion of the EAS5 or EAS6 comprises the first driver fragment; and

(ii) a second portion comprising a seventh Enzyme Amplifier Substrate Oligonucleotide (EAS7) , wherein

a portion of said EAS7 is complementary to a portion of an eighth Enzyme Amplifier Substrate Oligonucleotide (EAS8);

a portion of said EAS8 is complementary to a portion of the first driver fragment;

a portion of the EAS7 or EAS8 comprises said second driver fragment; and,

(iii) a third portion connecting the first and second portions, wherein said third portion is complementary to the third portion of the backbone of said first complex; and,

(b) contacting said first and second complexes under conditions permitting hybridisation of the third portion of said first complex with the third portion of said second complex, thereby forming a first double complex;

(c) contacting:

(i) the EAS5 and the EAS6 with said second DF of (a) under conditions permitting assembly of the second DF with the EAS5 and the EAS6 to form a third CESA comprising a recognition site and a cleavage site for a third nuclease;

(ii) the third CESA with the third nuclease under conditions permitting interaction of the third nuclease with the recognition site and cleavage site of the third CESA, wherein cleavage at said cleavage site by the third nuclease releases the first DF which can assemble with said EAS1 and EAS2 to form said first CESA, and assemble with said EAS7 and EAS8 to form said fourth CESA; and,

(d) contacting:

(i) the EAS7 and the EAS8 with the first DF under conditions permitting assembly of the first DF with the EAS7 and the EAS8 to form a fourth CESA comprising a recognition site and a cleavage site for a fourth nuclease;

(ii) the fourth CESA with the fourth nuclease under conditions permitting interaction of the fourth nuclease with the recognition site and cleavage site of the fourth CESA, wherein cleavage at said cleavage site by the fourth nuclease releases the second DF which can assemble with said EAS3 and EAS4 to form said second CESA, and assemble with said EAS5 and EAS6 to form said third CESA;

and wherein cleavage of said third CESA complex and/or said fourth CESA complex produces a detectable effect.

155. The method of claim 154, wherein cleavage of said third CESA complex and cleavage of said fourth CESA complex each produces a detectable effect.

156. The method of claim 154 or claim 155, wherein EAS1 is identical to EAS7, EAS2 is identical to EAS8, EAS3 is identical to EAS5, and/or EAS4 is identical to EAS6.

157. The method of any one of claims 154 to 156, wherein said first double complex is linked to a second double complex.

158. The method of claim 157, wherein said first double complex is linked to said second double complex by linking any one or more of EAS1-EAS8 of said first double complex with any one or more of EAS1-EAS8 of said second double complex.

159. The method of claim 157 or claim 158, wherein said first double complex is linked to said second double complex by linking EAS2 and/or EAS8 of said first double complex with EAS2 and/or EAS8 of said second double complex.

160. The method of claim 158 or claim 159, wherein said linking is achieved using any one or more of chemical hybridisation, antibodies, oligonucleotide linkers, non-oligonucleotide linkers, and peptide linkers.

161. The method of claim 158 or claim 159, wherein said linking is achieved via biotinylation of any one or more of said Enzyme Amplifier Substrate Oligonucleotides and the complexing of multiple biotinylated Enzyme Amplifier Substrate Oligonucleotides using avidin.

162. The method of any one of claims 152 to 161, wherein said first DF of (a) is produced by

contacting a SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target to form a duplex structure amenable to modification by an initiator nuclease, and

contacting the duplex structure with said initiator nuclease,

wherein modification of paired or unpaired regions in the duplex structure by the initiator nuclease releases said second DF.

163. The method of claim 162, wherein said initiator nuclease cleaves the SIO to generate said DF only when the SIO is hybridised with the target.

164. The method of claim 162, wherein said initiator nuclease cleaves the target to generate said DF only when the target is hybridised with the SIO.

165. The method of claim 164, wherein said initiator nuclease is not a restriction enzyme.

166. The method of any one of claims 152 to 161, wherein said first DF of (a) is produced by providing two or more partzymes and at least one MNAzyme substrate, and, contacting the partzymes with a sample putatively containing the target under conditions permitting self-assembly and catalytic activity of the MNAzyme in the presence of said target, wherein said catalytic activity modifies said substrate thereby providing said first DF.

167. The method of any one of claims 154 to 166, wherein any one or more of said EAS1, EAS2, EAS3, EAS4, EAS5, EAS6, EAS7 and EAS8 comprises a detectable portion and a quencher portion, wherein said detectable portion and quencher portion separate upon cleavage of the first, second, third, and/or fourth CESA providing a detectable effect.

168. The method of any one of claims 154 to 166, wherein:

(i) the EAS1 comprises a detectable portion and said EAS2 comprises a quencher portion or visa versa; and/or

(ii) the EAS3 comprises a detectable portion and said EAS4 comprises a quencher portion or visa versa; and/or

(iii) the EAS5 comprises a detectable portion and said EAS6 comprises a quencher portion or visa versa;

(iv) the EAS7 comprises a detectable portion and said EAS8 comprises a quencher portion or visa versa; and,

wherein said detectable portion and quencher portion separate upon cleavage of the first, second, third, and/or fourth CESA providing a detectable effect.

169. The method of any one of claims 152 to 168, wherein any said nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said recognition sequence for said first, second or third nuclease.

170. The method of claim 169, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, *and TspR*I.

171. The method of any one of claims 152 to 168, wherein binding of any said DF to an Enzyme Amplifier Substrate oligonucleotide completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

172. The method of any one of claims 154 to 165, wherein a pair of Enzyme Amplifier Substrates selected from EAS1 and EAS2; EAS3 and EAS4; EAS5 and EAS6; and EAS7 and EAS8, is a component of a hairpin oligonucleotide comprising a double-stranded

stem portion formed by hybridisation of complementary portions of each member of said pair, and a hairpin loop portion linked to one end of the stem portion.

173. The method of any one of claims 88 to 172, wherein any said first DF is generated using a nuclease selected from an endonuclease and an exonuclease.

174. The method of claim 173, wherein the exonuclease is selected from the group consisting of Nuclease BAL-31, Exonuclease I, Exonuclease III, T7 Exonuclease, T7 Exonuclease I and Exonuclease T.

175. The method of claim 173, wherein the endonuclease is T7 Endonuclease I, RNase H, Flap Nuclease, or Mung Bean Nuclease.

176. A method of detecting a target using a cascade comprising:

(a) providing:

(i) an SIO, said SIO comprising a first portion complementary to a target strand and a second portion that is not complementary to said target strand, wherein said first and second portions are separated by a phosphorothioate, and said second portion comprises a first DF;

(ii) an EAS1 and an EAS2, wherein a portion of the EAS1 is complementary to a portion of the EAS2 and hybridization of the EAS1 and the EAS2 provides a duplex structure with a 3' overhang at either end, and a portion of the EAS1 is complementary to a portion of the first DF; and

(iii) a first exonuclease; and

(b) contacting:

(i) said SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target thus creating a duplex structure for a first exonuclease, wherein modification of the duplex structure by the first exonuclease releases said first DF from said duplex substrate;

(ii) the EAS1 and EAS2 with the first DF of (b) under conditions permitting assembly of the first DF and said EAS1 and EAS2 to form a first CESA complex comprising a substrate for a second exonuclease;

(iii) the first CESA with the second exonuclease under conditions permitting interaction of the second exonuclease with the first CESA complex, wherein modification of the first CESA complex by the second exonuclease releases said first DF from said first CESA complex which can assemble with additional EAS1 and EAS2 to form an additional first CESA complex;

and wherein said modification of the duplex structure and/or said modification of the first CESA complex provides a detectable effect.

177. The method of claim 176, wherein said modification of the duplex structure and said modification of the first CESA complex provides a detectable effect.

178. The method of claim 176 or claim 177, wherein said SIO is a hairpin oligonucleotide comprising a double-stranded stem formed by hybridisation of two complementary portions, a single stranded hairpin loop, and a 3'overhang.

179. The method of any one of claims 176 to 178, wherein the first and/or second exonuclease is Exonuclease III.

180. The method of any one of claims 176 to 179, wherein said SIO and/or said EAS1 comprises a detectable portion and a quencher portion, and wherein said detectable portion and quencher portion can separate upon modification by said exonuclease to provide a detectable effect.

181. The method according to claim 180, wherein said detectable portion is a fluorophore.

182. The method of any one of claims 90 to 184, wherein said detectable effect is detected by fluorescence spectroscopy, surface plasmon resonance, mass spectroscopy, NMR, electron spin resonance, polarization fluorescence spectroscopy, circular dichroism, immunoassay, chromatography, radiometry, photometry, scintigraphy, electronic methods, UV, visible light or infra red spectroscopy, enzymatic methods or any combination thereof.

183. The method of any one of claims 88 to 182, wherein the detectable effect is measured and wherein the magnitude of said measurement and/or rate of accumulation of the detectable effect is indicative of the quantity of a target.

184. The method of any one of claims 88 to 183, wherein the target is selected from the group consisting of nucleic acids, proteins, glycoproteins, lipids, lipoproteins, cells, viruses, bacteria, archaea, fungi, antibodies, metabolites, pathogens, toxins, contaminants, poisons, small molecules, polymers, metal ions, metal salts, prions, nucleic acids or any derivatives, portions or combinations thereof.

185. The method of claim 184, wherein the nucleic acid is selected from the group consisting of DNA, methylated DNA, alkylated DNA, RNA, methylated RNA, microRNA, siRNA, shRNA, mRNA, tRNA, snoRNA, stRNA, smRNA, pre- and pri-microRNA, other non-coding RNAs, ribosomal RNA, derivatives thereof, amplicons thereof and any combination thereof.

186. The method of claim 184 or claim 185, wherein the source of the nucleic acid is selected from the group consisting of synthetic, mammalian, human, animal, plant, fungal, bacterial, viral, archael and any combination thereof.

187. The method of any one of claims 184 to 186, wherein the nucleic acid is amplified.

188. The method of any one of claims 96, 102, 109, 114 or 152, wherein said first DF is distinct from said target.

189. The method of any one of claims 88 to 188, wherein any said EAS can hybridise with another EAS to from a Partial Enzyme Signal Amplifier (PESA) complex capable of hybridizing with more than one DF.

190. The method of any one of claims 88 to 95, 122, 123 or 166, wherein said method comprises using first and second MNAzymes specific for different portions of a target molecule.

191. The method of claim 190, wherein said MNAzymes specific for different portions of said target molecule recognize and cleave the same substrate upon assembly in the presence of said target.

192. The method of any one of claims 88 to 95, 122, 123 or 166, wherein said method comprises using at least two different MNAzymes having specificity for distinct targets.

193. The method of any one of claims 96, 115, 116, and 162, wherein said method comprises using at least two different SIO with complementarity for distinct targets.

194. The method of any one of claims 88, 96, 102, 114 or 152, wherein said method comprises using at least two distinct first CESA complexes assembled with a different first DF.

195. A kit for amplifying a signal comprising;

a nuclease; and

an EAS1 and an EAS2 wherein:

a portion of the EAS1 and EAS2 are complementary,

the EAS1 and EAS2 form a complex comprising a recognition sequence and a cleavage sequence for said nuclease only on assembly with a Driver Fragment oligonucleotide, and

binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

196. A kit for detecting a target comprising;

a nuclease;

an EAS1 and an EAS2 wherein a portion of the EAS1 and EAS2 are complementary

a plurality of partzymes designed to assemble an MNAzyme corresponding to the target and

an MNAzyme substrate wherein a portion of said substrate is complementary to a portion of the EAS1; and

wherein the EAS1 and EAS2 form a complex comprising a recognition sequence and a cleavage sequence for said nuclease only on assembly with a Driver Fragment oligonucleotide, and binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

197. A kit for detecting a target comprising

a nuclease;

an EAS1 and an EAS2 wherein a portion of the EAS1 and EAS2 are complementary;

a plurality of SIOs designed to hybridize to the target to form a nuclease substrate;

wherein a portion of said nuclease substrate is complementary to a portion of the EAS1

wherein the EAS1 and EAS2 form a complex comprising a recognition sequence and a cleavage sequence for said nuclease only on assembly with a Driver Fragment Oligonucleotide (DF), and binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

198. A kit comprising a first Enzyme Amplifier Substrate oligonucleotide (EAS1) and a second Enzyme Amplifier Substrate oligonucleotide (EAS2), wherein:

a portion of the EAS1 is complementary to a portion of the EAS2, and wherein the EAS1 and EAS2 form a first Complete Enzyme Signal Amplifier (CESA) complex comprising a recognition and a cleavage sequence for a first nuclease only on assembly with a first Driver Fragment oligonucleotide (DF),

a portion of the first DF is complementary to a portion of the EAS1, and

binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

199. A kit comprising an EAS1, an EAS2, and a first nuclease, wherein:

a portion of the EAS1 is complementary to a portion of the EAS2,

the EAS1 and EAS2 form a first CESA complex comprising a recognition sequence and a cleavage sequence for said first nuclease only on assembly with a first DF,

a portion of the first DF is complementary to a portion of the EAS1, and

binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

200. A kit comprising a multi-component nucleic acid enzyme (MNAzyme), an MNAzyme substrate, an EAS1, an EAS2, and a first nuclease wherein:

the MNAzyme comprises at least a first partzyme and a second partzyme that self-assemble in the presence of an MNAzyme assembly facilitator to form the MNAzyme, wherein each of said at least first and said second partzymes comprise a substrate arm portion, a catalytic core portion, and a sensor arm portion, and wherein the sensor arms interact with said MNAzyme assembly facilitator so as to maintain the first and second partzymes in proximity for association of their respective catalytic core portions to form the catalytic core of the MNAzyme and said catalytic core is capable of modifying said MNAzyme substrate to form a first DF;

and wherein a portion of the EAS1 is complementary to a portion of the EAS2 and a portion of the EAS1 is complementary to a portion of the first DF, the first DF is capable of assembly with the EAS1 and the EAS2 to form a first CESA complex containing a recognition site and a cleavage site for said at least first nuclease, and binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

201. The kit of claim 200, wherein said MNAzyme substrate is a first strand of an oligonucleotide complex comprising first and second strands, wherein said first strand comprises an internal loop portion and bases within the internal loop portion are not hybridised to bases of the second strand, and wherein the MNAzyme is capable of cleaving the internal loop portion.

202. The kit of claim 201, wherein said second strand comprises the first DF.

203. The kit of claim 201 or claim 202, wherein said first and second strands are linked at one end by a hairpin loop portion.

204. The kit of claim 200, wherein said MNAzyme substrate is a hairpin loop portion of a hairpin oligonucleotide, said MNAzyme is capable of cleaving the hairpin loop portion, and said first driver fragment is located in one strand of a double stranded stem portion in said hairpin oligonucleotide.

205. The kit of any one of claims 200 to 204, wherein the assembly facilitator is a target to be identified.

206. A kit comprising a first Synthetic Initiator Oligonucleotide (SIO), an EAS1, an EAS2, a first nuclease, and a second nuclease wherein:

the SIO is capable of hybridizing with a target to form a duplex substrate wherein said first nuclease is capable of cleaving the duplex substrate to generate a first DF; and wherein;

a portion of the EAS1 is complementary to a portion of the EAS2 and a portion of the EAS1 is complementary to a portion of the first DF,

the EAS1 and the EAS2 form a first CESA complex containing a recognition sequence and a cleavage sequence for said second nuclease only on assembly with the first DF, and

binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site.

207. The kit of claim 206, wherein said first nuclease is capable of cleaving the SIO to generate said first DF only when the SIO is hybridised with the target.

208. The kit of claim 206, wherein said first nuclease is capable of cleaving the target to generate said first DF only when the target is hybridised with the SIO.

209. The kit of claim 208, wherein said first nuclease is not a restriction enzyme.

210. The kit of any one of claims 206 to 209, wherein said first nuclease is an exonuclease.

211. The kit of any one of claims 206 to 210, wherein the first and second nuclease are the same nuclease.

212. The kit of any one of claims 206 to 210, wherein the first and second nucleases are different nucleases.

213. The kit of any one of claims 198 to 206, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

214. The kit of any one of claims 206 to 212, wherein said second nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

215. The kit of claim 213 or claim 214, wherein said nick is located within the nuclease recognition site, at the nuclease cleavage site, or between the nuclease recognition and cleavage sites.

216. The kit of any one of claims 213 to 215, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

217. The kit of any one of claims 195 to 216, wherein said EAS1 and EAS2 are components of a hairpin oligonucleotide comprising a double-stranded stem portion formed by hybridisation of complementary portions of said EAS1 and the EAS2, and a hairpin loop portion linking one end of said EAS1 with one end of said EAS2.

218. The kit of claim 217, wherein said hairpin oligonucleotide comprises a single stranded 5' or 3' overhang portion extending from either of said EAS1 or EAS2.

219. The kit of any one of claims 195 to 218, wherein a portion of said EAS1 or EAS2 comprises a second DF, and wherein said second DF can be released upon modification of said first CESA complex by the nuclease.

220. The kit of any one of claims 217 to 218, wherein a portion of said hairpin loop portion comprises a second DF, and wherein said second DF can be released upon modification of said first CESA complex by the nuclease.

221. The kit of claim 219 or claim 220, wherein said first DF and said second DF are not identical.

222. The kit of claim 219 or claim 220, wherein said first DF and said second DF are identical.

223. The kit of claim 219 or claim 220, wherein said second DF is a fragment of said first DF, or said first DF is a fragment of said second DF.

224. The kit of claim 222 or claim 223, wherein said second DF, EAS1, and EAS2 are capable of assembly to form said first CESA complex.

225. The kit of any one of claims 219 to 221, further comprising a third Enzyme Amplifier Substrate oligonucleotide (EAS3) and a fourth Enzyme Amplifier Substrate oligonucleotide (EAS4), wherein a portion of the EAS3 is complementary to a portion of the EAS4 and a portion of the EAS3 is complementary to a portion of the second DF, and wherein the EAS3 and the EAS4 form a second CESA complex containing a recognition sequence and a cleavage sequence for an additional nuclease only on assembly with the second DF.

226. The kit of claim 225, wherein the EAS3 or EAS4 comprises a third DF.

227. The kit of claim 226, wherein the third DF is identical to said first DF.

228. The kit of claim 226, wherein the third DF is not identical said first DF.

229. The kit of any one of claims 225 to 228, wherein said additional nuclease is identical to another nuclease in said kit.

230. The kit of any one of claims 225 to 228, wherein said additional nuclease is not identical to another nuclease in said kit, and wherein said kit comprises said additional nuclease.

231. The kit of any one of claims 225 to 230, wherein said additional nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said second CESA complex

232. The kit of any one of claims 225 to 231, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

233. The kit of any one of claims 225 to 232, wherein said EAS3 and EAS4 are components of a hairpin oligonucleotide comprising a double-stranded stem portion formed by hybridisation of complementary portions of EAS3 and EAS4, and a hairpin loop portion linking one end of said EAS3 with one end of said EAS4.

234. The kit of 233, wherein said hairpin oligonucleotide comprises a single stranded 5' or 3' overhang portion extending from either of said EAS3 or EAS4.

235. A kit comprising a first complex, said first complex comprising a backbone oligonucleotide, an EAS1, an EAS2, an EAS3, and an EAS4, wherein said backbone oligonucleotide comprises:

(i) a first portion comprising said EAS1, wherein:

a portion of said EAS1 is complementary to a portion of EAS2, a portion of the EAS2 is complementary to a portion of a first DF, and a portion of the EAS1 or EAS2 comprises a second DF,

the EAS1 and the EAS2 form a first CESA complex containing a recognition sequence and a cleavage sequence for a first nuclease only on assembly with said first DF,

and binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) a second portion comprising said EAS3, wherein a portion of said EAS3 is complementary to a portion of said EAS4, a portion of said EAS3 is complementary to a portion of the second DF, and a portion of said EAS3 or EAS4 comprises said first DF, and wherein the EAS3 and the EAS4 form a second CESA complex containing a recognition sequence and a cleavage sequence for a second nuclease only on assembly with said second DF; and

(iii) a third portion connecting the first and second portions.

236. The kit of claim 235, further comprising a second complex, said second complex comprising a backbone oligonucleotide, a fifth Enzyme Amplifier Substrate Oligonucleotide (EAS5), a sixth Enzyme Amplifier Substrate Oligonucleotide (EAS6), a seventh Enzyme Amplifier Substrate Oligonucleotide (EAS7), and an eighth Enzyme Amplifier Substrate Oligonucleotide (EAS8), wherein said backbone oligonucleotide comprises:

(i) a first portion comprising said EAS5, wherein a portion of said EAS5 is complementary to a portion of said EAS6, a portion of said EAS5 is complementary to a portion of said second DF, and a portion of said EAS5 or EAS6 comprises said first DF, and wherein the EAS5 and the EAS6 form a third CESA complex containing a recognition sequence and a cleavage sequence for a third nuclease only on assembly with said second DF; and

(ii) a second portion comprising said EAS7, wherein a portion of said EAS7 is complementary to a portion of EAS8, a portion of said EAS8 is complementary to a portion of said first DF, and a portion of said EAS7 or EAS8 comprises said second DF, and wherein the EAS7 and the EAS8 form a fourth CESA complex containing a recognition sequence and a cleavage sequence for a fourth nuclease only on assembly with said first DF; and

(iii) a third portion connecting the first and second portions, wherein said third portion is complementary to the third portion of the backbone of said first complex.

237. The kit of claim 236, wherein EAS1 is identical to EAS7, EAS2 is identical to EAS8, EAS3 is identical to EAS5, and/or EAS4 is identical to EAS6.

238. The kit of claim 236 or claim 237, wherein the first nuclease is identical to the third nuclease, and/or the second nuclease is identical to the fourth nuclease, and/or the first, second, third and fourth nucleases are identical.

239. The kit of any one of claims 236 to 238, wherein first and second complexes are hybridised via their respective complementary third portions forming a first double complex.

240. The kit of claim 239, wherein said first double complex is linked to a second double complex.

241. The kit of claim 240, wherein said first double complex is linked to said second double complex by linking any one or more of EAS1-EAS8 of said first double complex with any one or more of EAS1-EAS8 of said second double complex.

242. The kit of claim 240 or claim 241, wherein said first double complex is linked to said second double complex by linking EAS2 and/or EAS8 of said first double complex with EAS2 and/or EAS8 of said second double complex.

243. The kit of claim 241 or claim 242, wherein said linking is achieved using any one or more of chemical hybridisation, antibodies, oligonucleotide linkers, non-oligonucleotide linkers, and peptide linkers.

244. The kit of claim 241 or claim 242, wherein said linking is achieved via biotinylation of any one or more of said Enzyme Amplifier Substrate Oligonucleotides and the complexing of multiple biotinylated Enzyme Amplifier Substrate Oligonucleotides using avidin.

245. The kit of any one of claims 236 to 244, wherein said first and/or said second nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said second CESA complex

246. The kit of any one of claims 236 to 245, wherein said first and/or said second nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

247. The kit of any one of claims 236 to 246, wherein a pair of Enzyme Amplifier Substrates selected from EAS1 and EAS2; EAS3 and EAS4; EAS5 and EAS6; and EAS7 and EAS8, is a component of a hairpin oligonucleotide comprising a double-stranded stem portion formed by hybridisation of complementary portions of each member of said pair, and a hairpin loop portion linked to one end of the stem portion.

248. A kit comprising:

(i) a SIO, said SIO comprising a first portion complementary to a target strand and a second portion that is not complementary to said target strand, wherein said first and second portions are separated by a phosphorothioate, and said second portion comprises a first DF; and,

(ii) an EAS1 and an EAS2, wherein

a portion of the EAS1 is complementary to a portion of the EAS2 and hybridization of the EAS1 and the EAS2 provides a duplex structure with a 3' overhang at either end,

a portion of the EAS1 is complementary to a portion of the first DF, and

the EAS1 and the EAS2 are capable of forming a first CESA complex comprising a recessed 3' end capable of digestion by a first nuclease, only on assembly with said first DF.

249. The kit of claim 248, wherein said SIO is a hairpin oligonucleotide comprising a double-stranded stem formed by hybridisation of two complementary portions, a single stranded hairpin loop, and a 3'overhang.

250. The kit of claim 248 or 249, wherein the nuclease is an exonuclease.

251. The kit of any one of claims 195 to 250, wherein any said EAS comprises one or more detectable labels.

252. The kit of any one of claims 195 to 251, wherein any said EAS comprises a fluorophore portion and a quencher portion.

253. The kit of any one of claims 195 to 252, wherein any said MNAzyme partzyme, MNAzyme substrate, EAS1, EAS2, EAS3, EAS4, EAS5, EAS6, EAS7, EAS8, SIO, DF or nuclease is attached to a solid support.

254. The kit of any one of claims 196, 197, 205, or 206, wherein said first DF is distinct from said target.

255. The kit of claim 196 or claim 200, wherein said kit comprises first and second MNAzymes specific for different portions of a target molecule.

256. The kit of claim 255, wherein said MNAzymes specific for different portions of said target molecule recognize and cleave the same substrate upon assembly in the presence of said target.

257. The kit claim 196 or claim 200, wherein said kit comprises at least two different MNAzymes having specificity for distinct targets.

258. The kit of 197, 206 or 248, wherein said kit comprises at least two different SIO with complementarity for distinct targets.

259. A kit comprising the composition of any one of claims 1 to 87.

260. The kit of any one of claims 195 to 259, further comprising instructions for use of said kit.

261. A composition comprising a first Enzyme Amplifier Substrate oligonucleotide (EAS1) and a second Enzyme Amplifier Substrate oligonucleotide (EAS2), wherein:

a portion of the EAS1 is complementary to a portion of the EAS2,

the EAS1 and EAS2 form a first Complete Enzyme Signal Amplifier (CESA) complex comprising a recognition and a cleavage sequence for a first nuclease only on assembly with a first Driver Fragment oligonucleotide (DF),

a portion of the first DF is complementary to a portion of the EAS1, and

the first DF is capable of binding to said EAS1 immediately adjacent to the first nuclease cleavage sequence.

262. A method for detecting a target comprising:

(a) providing two or more partzymes and at least one multi-component nucleic acid (MNAzyme) substrate, wherein the partzymes self-assemble in the presence of the target to form at least one MNAzyme;

(b) contacting the partzymes with a sample putatively containing the target under conditions permitting self-assembly and catalytic activity of the MNAzyme, and wherein catalytic activity of said MNAzyme produces a first Driver Fragment oligonucleotide (DF) from said at least one MNAzyme substrate;

(c) providing a first Enzyme Amplifier Substrate Oligonucleotide (EAS1) and a second Enzyme Amplifier Substrate Oligonucleotide (EAS2) wherein a portion of the EAS1 is complementary to a portion of the EAS2, and wherein a portion of the EAS1 is complementary to a portion of the first DF and;

(d) contacting the EAS1 and the EAS2 with the first DF under conditions permitting;

(1) assembly of the first DF with the EAS1 and the EAS2 to form a first Complete Enzyme Signal Amplifier (CESA) complex, and

(2) formation of a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 is immediately adjacent to the first nuclease cleavage site;

(e) providing the first nuclease; and

(f) contacting the first nuclease with the first CESA complex under conditions permitting interaction of the nuclease with the recognition site and cleavage at the cleavage site wherein the cleavage by said first nuclease produces a detectable effect indicative of the presence of the target.

263. A method of detecting a target comprising:

(a) providing at least a first Synthetic Initiator Oligonucleotide (SIO);

(b) contacting the SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target thus creating a duplex substrate for a first nuclease;

(c) providing a first nuclease capable of cleaving the duplex substrate formed by hybridization of the SIO and the target wherein cleavage of the duplex substrate by the first nuclease generates a first DF;

(d) providing an EAS1 and an EAS2 wherein a portion of the EAS1 is complementary to a portion of the EAS2, and wherein a portion of the EAS1 is complementary to a portion of the first DF and;

(e) contacting the EAS1 and the EAS2 with the first DF under conditions permitting:

(1) assembly of the first DF with the EAS1 and the EAS2 to form a first CESA, and

(2) formation of a recognition site and a cleavage site for a second nuclease, wherein binding of the first DF to said EAS1 is immediately adjacent to the second nuclease cleavage site;

(f) providing a second nuclease; and

(g) contacting the second nuclease with the first CESA under conditions permitting interaction of the second nuclease with the recognition site and cleavage at the cleavage site wherein cleavage by the second nuclease produces a detectable effect indicative of the presence of the target.

264. A kit for amplifying a signal comprising;

a nuclease; and

an EAS1 and an EAS2 wherein:

a portion of the EAS1 and EAS2 are complementary,

the EAS1 and EAS2 form a complex comprising a recognition sequence and a cleavage sequence for said nuclease only on assembly with a Driver Fragment oligonucleotide, and

the first DF is capable of binding to said EAS1 immediately adjacent to the nuclease cleavage site.

CLAIMS

1. A composition comprising an EAS1, an EAS2, and a first nuclease, wherein:
a portion of the EAS1 is complementary to a portion of the EAS2,
the EAS1 and EAS2 form a first CESA complex comprising a recognition sequence and a cleavage sequence for said first nuclease only on assembly with a first DF,
a portion of the first DF is complementary to a portion of the EAS1, and
binding of the first DF to said EAS1 completes a partial nuclease recognition or cleavage sequence.

2. A composition comprising a multi-component nucleic acid enzyme (MNAzyme), an MNAzyme substrate, an EAS1, an EAS2, and a first nuclease wherein:

the MNAzyme comprises at least a first partzyme and a second partzyme that self-assemble in the presence of an MNAzyme assembly facilitator to form the MNAzyme, wherein each of said at least first and said second partzymes comprise a substrate arm portion, a catalytic core portion, and a sensor arm portion, and wherein the sensor arms interact with said MNAzyme assembly facilitator so as to maintain the first and second partzymes in proximity for association of their respective catalytic core portions to form the catalytic core of the MNAzyme and said catalytic core is capable of modifying said MNAzyme substrate to form a first DF;

and wherein:

a portion of the EAS1 is complementary to a portion of the EAS2 and a portion of the EAS1 is complementary to a portion of the first DF,

the first DF is capable of assembly with the EAS1 and the EAS2 to form a first CESA complex containing a recognition site and a cleavage site for said at least first nuclease, and

binding of the first DF to said EAS1 completes a partial nuclease recognition or cleavage sequence.

3. A composition comprising a first Synthetic Initiator Oligonucleotide (SIO), an EAS1, an EAS2, a first nuclease, and a second nuclease wherein:

the SIO is capable of hybridizing with a target to form a duplex substrate wherein said first nuclease is capable of cleaving the duplex substrate to generate a first DF; and wherein:

a portion of the EAS1 is complementary to a portion of the EAS2 and a portion of the EAS1 is complementary to a portion of the first DF,

the EAS1 and the EAS2 form a first CESA complex containing a recognition sequence and a cleavage sequence for said second nuclease only on assembly with the first DF, and

binding of the first DF to said EAS1 completes a partial nuclease recognition or cleavage sequence.

4. The composition of claim 1 or claim 2, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

5. The composition of claim 3, wherein said second nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

6. The composition of claim 4 or claim 5, wherein said nick is located within the nuclease recognition site, at the nuclease cleavage site, or between the nuclease recognition and cleavage sites.

7. The composition of any one of claims 4 to 6, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

8. The composition of claim 1 to 7, wherein said EAS1 and EAS2 are components of a hairpin oligonucleotide comprising a double-stranded stem portion formed by hybridisation of complementary portions of said EAS1 and the EAS2, and a hairpin loop portion linking one end of said EAS1 with one end of said EAS2.

9. The composition of claim 8, wherein said hairpin loop portion is an oligonucleotide linker or a non-oligonucleotide linker.

10. The composition of any one of claims 1 to 9, wherein a portion of said EAS1 or EAS2 comprises a second DF, and wherein said second DF can be released upon modification of said first CESA complex by the nuclease.

11. The composition of any one of claims 8 to 10, wherein a portion of said hairpin loop portion comprises a second DF, and wherein said second DF can be released upon modification of said first CESA complex by the nuclease.

12. The composition of claim 10 or claim 11, wherein said first DF and said second DF are not identical.

13. The composition of claim 10 or claim 11, wherein said first DF and said second DF are identical.

14. The composition of claim 10 or claim 11, wherein said second DF is a fragment of said first DF, or said first DF is a fragment of said second DF.

15. The composition of any one of claims 10 to 12, further comprising a third Enzyme Amplifier Substrate oligonucleotide (EAS3) and a fourth Enzyme Amplifier Substrate oligonucleotide (EAS4), wherein a portion of the EAS3 is complementary to a portion of the EAS4 and a portion of the EAS3 is complementary to a portion of the second DF, and wherein the EAS3 and the EAS4 form a second CESA complex containing a recognition sequence and a cleavage sequence for an additional nuclease only on assembly with the second DF.

16. The composition of claim 15, wherein the EAS3 or EAS4 comprises a third DF.

17. The composition of claim 16, wherein the third DF is identical to said first DF.

18. The composition of claim 16, wherein the third DF is not identical said first DF.

19. The composition of any one of claims 15 to 18, wherein said additional nuclease is identical to another nuclease in said composition.

20. The composition of any one of claims 15 to 18, wherein said additional nuclease is not identical to another nuclease in said composition, and wherein said composition comprises said additional nuclease.

21. The composition of claim 2, wherein at least one of the MNAzyme partzymes, MNAzyme substrate or a combination thereof further comprises an aptamer or portion thereof.

22. The composition claim 21, wherein the aptamer or portion thereof comprises at least one of: a nucleic acid, peptide, polypeptide, protein, a derivative thereof, or a combination thereof.

23. The composition of any one of claims 1 to 22, wherein any said MNAzyme partzyme, MNAzyme substrate, EAS1, EAS2, EAS3, EAS4, EAS5, EAS6, SIO, DF or nuclease is attached to a solid support.

24. The composition of claim 1, wherein said first DF is produced only in the presence of a target.

25. The composition of claim 2 or claim 3, wherein said first DF is distinct from said target.

26. The composition of any one of claims 1 to 3, wherein said composition comprises at least two distinct first CESA complexes assembled with a different first DF.

27. A method for detecting a target comprising:

(a) providing two or more partzymes and at least one multi-component nucleic acid (MNAzyme) substrate, wherein the partzymes self-assemble in the presence of the target to form at least one MNAzyme;

(b) contacting the partzymes with a sample putatively containing the target under conditions permitting self-assembly and catalytic activity of the MNAzyme, and wherein catalytic activity of said MNAzyme produces a first Driver Fragment oligonucleotide (DF) from said at least one MNAzyme substrate;

(c) providing a first Enzyme Amplifier Substrate Oligonucleotide (EAS1) and a second Enzyme Amplifier Substrate Oligonucleotide (EAS2) wherein a portion of the EAS1 is complementary to a portion of the EAS2, and wherein a portion of the EAS1 is complementary to a portion of the first DF and;

(d) contacting the EAS1 and the EAS2 with the first DF under conditions permitting;

(1) assembly of the first DF with the EAS1 and the EAS2 to form a first Complete Enzyme Signal Amplifier (CESA) complex, and

(2) formation of a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(e) providing the first nuclease; and

(f) contacting the first nuclease with the first CESA complex under conditions permitting interaction of the nuclease with the recognition site and cleavage at the cleavage site wherein the cleavage by said first nuclease produces a detectable effect indicative of the presence of the target.

28. The method of claim 27, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said recognition site and/or said cleavage site for said first nuclease.

29. The method of claim 27 or claim 28, wherein the first DF is produced by cleavage of the MNAzyme substrate.

30. The method of any one of claims 27 to 29, wherein said MNAzyme substrate is a first strand of an oligonucleotide complex comprising first and second strands, wherein said first strand comprises an internal loop portion and bases within the internal loop portion are not hybridised to bases of the second strand, and wherein the MNAzyme is capable of cleaving the internal loop portion.

31. The method of claim 30, wherein said second strand comprises the first DF.

32. The method of claim 30 or claim 31, wherein said first and second strands are linked at one end by a hairpin loop portion.

33. The method of any one of claims 27 to 29, wherein said MNAzyme substrate is a hairpin loop portion of a hairpin oligonucleotide, said MNAzyme is capable of cleaving

the hairpin loop portion, and said first driver fragment is located in one strand of a double stranded stem portion in said hairpin oligonucleotide.

34. A method of detecting a target comprising:

(a) providing at least a first Synthetic Initiator Oligonucleotide (SIO);

(b) contacting the SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target thus creating a duplex substrate for a first nuclease;

(c) providing a first nuclease capable of cleaving the duplex substrate formed by hybridization of the SIO and the target wherein cleavage of the duplex substrate by the first nuclease generates a first DF;

(d) providing an EAS1 and an EAS2 wherein a portion of the EAS1 is complementary to a portion of the EAS2, and wherein a portion of the EAS1 is complementary to a portion of the first DF and;

(e) contacting the EAS1 and the EAS2 with the first DF under conditions permitting:

(1) assembly of the first DF with the EAS1 and the EAS2 to form a first CESA, and

(2) formation of a recognition site and a cleavage site for a second nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(f) providing a second nuclease; and

(g) contacting the second nuclease with the first CESA under conditions permitting interaction of the second nuclease with the recognition site and cleavage at the cleavage site wherein cleavage by the second nuclease produces a detectable effect indicative of the presence of the target.

35. The method of any one of claims 27 to 34, wherein cleavage of the first CESA complex allows release of a further DF, and the further DF assembles with further Enzyme Amplifier Substrate Oligonucleotides to form a further CESA complex, and at least one nuclease is used to cleave the further CESA complex to produce further detectable effect and release further DF, thereby facilitating a further increase in the detectable effect.

36. A method of detecting a target using a cascade comprising:

(a) producing a first DF, wherein said first DF is produced only in the presence of said target,

(b) providing:

(i) an EAS1 and an EAS2 wherein:

a portion of the EAS1 is complementary to a portion of the EAS2:

a portion of the EAS1 is complementary to a portion of the first DF; and

a portion of the EAS1 or the EAS2 comprises a second DF;

and,

(ii) a third Enzyme Amplifier Substrate Oligonucleotide (EAS3) and a fourth Enzyme Amplifier Substrate Oligonucleotide (EAS4) wherein:

a portion of the EAS3 is complementary to a portion of the EAS4;

a portion of the EAS3 is complementary to a portion of the second DF;

(c) contacting:

(i) the EAS1 and the EAS2 with the first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA complex comprising a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA complex with the first nuclease under conditions permitting interaction of the first nuclease with the recognition site and cleavage site of the first CESA complex, wherein cleavage at the cleavage site by the first nuclease releases the second DF;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA complex comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA complex with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA complex, wherein the second nuclease cleaves said second CESA complex at said cleavage site;

and wherein cleavage of said first CESA complex and/or said second CESA complex produces a detectable effect.

37. The method of claim 36, wherein said first nuclease and said second nuclease are the same nuclease.

38. The method of claim 36, wherein said first nuclease and said second nuclease are different nucleases.

39. The method of any one of claims 36 to 38, wherein a portion of said EAS3 or said EAS4 comprises an additional DF and cleavage at said cleavage site of the second CESA complex by the second nuclease releases said additional DF.

40. The method of claim 39, wherein:

(i) a portion of the additional DF is identical to said first DF;

(ii) the additional DF is identical to said first DF; or

(iii) the additional DF is a fragment of said first DF;

and wherein said additional DF can assemble with said EAS1 and EAS2 to form said first CESA complex.

41. A method of detecting a plurality of distinct targets using a cascade comprising:

(a) producing at least a first DF and a second DF, wherein said first DF is produced only in the presence of a first target, and said second DF is produced only in the presence of a second target;

(b) providing:

(i) an EAS1 and an EAS2 wherein:

a portion of the EAS1 is complementary to a portion of the EAS2:

a portion of the EAS1 is complementary to a portion of the first DF; and

(ii) an EAS3 and an EAS4 wherein:

a portion of the EAS3 is complementary to a portion of the EAS4:

a portion of the EAS3 is complementary to a portion of the second DF;

(c) contacting:

(i) the EAS1 and the EAS2 with the first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA complex comprising a recognition site and a cleavage site for a first nuclease, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA complex with the first nuclease under conditions permitting interaction of the first nuclease with said recognition site and cleavage site of the first CESA complex, wherein said first nuclease cleaves said first CESA complex at said cleavage site producing a first detectable effect;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA complex comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA complex with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA complex, wherein said second nuclease cleaves said second CESA complex at said cleavage site producing a second detectable effect;

and wherein said first detectable effect is distinct from said second detectable effect.

42. A method of detecting a target using a cascade comprising:

(a) producing a first driver fragment, wherein said first driver fragment is provided only in the presence of said target;

(b) providing:

(i) an EAS1 and an EAS2 wherein:

a portion of the EAS1 is complementary to a portion of the EAS2;

a portion of the EAS1 is complementary to a portion of the first DF;

a portion of the EAS1 or EAS2 comprises a second DF; and

the EAS1 or the EAS2 is tethered to a support;

(ii) an EAS3 and an EAS4 wherein:

a portion of the EAS3 is complementary to a portion of the EAS4:

a portion of the EAS3 is complementary to a portion of the second DF;

a portion of the EAS3 or EAS4 comprises a third DF; and

the EAS3 or the EAS4 is tethered to a support;

(c) contacting:

(i) the EAS1 and the EAS2 with said first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA comprising a recognition site and a cleavage site for a first nuclease, wherein binding of

the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA with the first nuclease under conditions permitting interaction of the first nuclease with the recognition site and cleavage site of the first CESA, wherein cleavage at said cleavage site by the first releases the second DF;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA, wherein cleavage at said cleavage site by the second nuclease releases the third DF which can assemble with said EAS1 and EAS2 to form said first CESA;

and wherein cleavage of said first CESA complex and/or said second CESA complex produces a detectable effect.

43. The method of any one of claims 36 to 42, wherein said first DF of (a) is produced by

contacting a SIO with a sample putatively containing the target under conditions permitting hybridizing of the SIO with the target to form a duplex structure amenable to modification by an initiator nuclease, and

contacting the duplex structure with said initiator nuclease,

wherein modification of paired or unpaired regions in the duplex structure by the initiator nuclease releases said first DF.

44. The method of claim 43, wherein said SIO is attached to a support.

45 The method of any one of claims 27 to 33, wherein said first nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said first CESA complex.

46. The method of claim 45, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, and *TspR* I.

47. The method of any one of claims 27 to 46, wherein binding of the first DF to said EAS1 completes a partial nuclease recognition site.

48. The method of any one of claims 36 to 45, wherein binding of the second DF to said EAS2 completes a partial nuclease recognition site.

49. The method of claim 47, wherein binding of the first DF to said EAS1 completes a partial nuclease cleavage site.

50. The method of claim 48, wherein binding of the second DF to said EAS2 completes a partial nuclease cleavage site.

51. A method of detecting a target using a cascade comprising:

(a) producing a first driver fragment, wherein said first driver fragment is produced only in the presence of said target;

(b) providing a first complex comprising a first backbone oligonucleotide, said first backbone oligonucleotide comprising:

(i) a first portion comprising an EAS1, wherein

a portion of said EAS1 is complementary to a portion of an EAS2;

a portion of the EAS2 is complementary to a portion of the first driver fragment; and

a portion of the EAS1 or EAS2 comprises a second driver fragment;

(ii) a second portion comprising an EAS3, wherein

a portion of said EAS3 is complementary to an EAS4;

a portion of said EAS3 is complementary to a portion of the second driver fragment; and

a portion of the EAS3 or EAS4 comprises said first driver fragment; and,

(iii) a third portion connecting the first and second portions;

and,

(c) contacting:

(i) the EAS1 and the EAS2 with said first DF of (a) under conditions permitting assembly of the first DF with the EAS1 and the EAS2 to form a first CESA comprising a recognition site and a cleavage site for a first nuclease, wherein binding of

the first DF to said EAS1 completes a partial nuclease recognition site and/or a partial nuclease cleavage site;

(ii) the first CESA with the first nuclease under conditions permitting interaction of the first nuclease with the recognition site and cleavage site of the first CESA, wherein cleavage at said cleavage site by the first nuclease releases the second DF;

(d) contacting:

(i) the EAS3 and the EAS4 with the second DF under conditions permitting assembly of the second DF with the EAS3 and the EAS4 to form a second CESA comprising a recognition site and a cleavage site for a second nuclease;

(ii) the second CESA with the second nuclease under conditions permitting interaction of the second nuclease with the recognition site and cleavage site of the second CESA, wherein cleavage at said cleavage site by the second nuclease releases the first DF which can assemble with said EAS1 and EAS2 to form said first CESA;

and wherein cleavage of said first CESA complex and/or said second CESA complex produces a detectable effect.

52. The method of claim 51, wherein any said nuclease is capable of cleaving a double stranded oligonucleotide comprising a nick in at least one of two strands forming said recognition sequence for said first, second or third nuclease.

53. The method of claim 52, wherein said nuclease is selected from the group consisting of *Mnl* I, *Rsa* I, *Pme* I, *Hpy* 8I, *Msp* I, *Ear* I, *and TspR*I.

54. The method of any one of claims 27 to 53, wherein any said first DF is generated using a nuclease selected from an endonuclease and an exonuclease.

55. The method of claim 54 wherein the exonuclease is selected from the group consisting of Nuclease BAL-31, Exonuclease I, Exonuclease III, T7 Exonuclease, T7 Exonuclease I and Exonuclease T.

56. The method of claim 55, wherein the endonuclease is T7 Endonuclease I, RNase H, Flap Nuclease, or Mung Bean Nuclease.

57. The method of any one of claims 27 to 56, wherein the target is selected from the group consisting of nucleic acids, proteins, glycoproteins, lipids, lipoproteins, cells, viruses, bacteria, archaea, fungi, antibodies, metabolites, pathogens, toxins, contaminants, poisons, small molecules, polymers, metal ions, metal salts, prions, nucleic acids or any derivatives, portions or combinations thereof.

58. The method of any one of claims 34, 36, 41, 42 or 51, wherein said first DF is distinct from said target.

59. The method of any one of claims 27 to 58, wherein any said EAS can hybridise with another EAS to from a Partial Enzyme Signal Amplifier (PESA) complex capable of hybridizing with more than one DF.