

Summary

[0013] The disclosure described herein alleviates at least some of the problems in the prior art associated with signal amplification assays utilising reaction components tethered to fixed/immobile supports. The present inventors have devised a system capable of maintaining spatial separation of nucleic acid enzymes and their components (e.g. partzyme oligonucleotide components of MNazymes) from their substrates without the inherent drawbacks of assays that rely on fixed/immobilised supports. The assays described herein thus achieve the enhanced reaction efficiency and/or specificity that has been sought by signal amplification assays utilising reaction components tethered to fixed/immobile supports. In general, the assays of the present disclosure utilise a selectively permeable membrane to separate nucleic acid enzymes and/or their components from their substrates. The components remain mobile in the assay mix which provides various advantages over assays relying on fixed/immobile supports. Upon undergoing a modification (e.g. cleavage of a mobile support component, alteration in charge and/or size, or other), which is triggered only when target molecule is present, the enzymes/enzyme components become capable of permeating the membrane and thereby coming into contact with their substrates to modify them and provide a signal.

[0014] More specifically, the present inventors have devised a rapid detection assay utilising subZyme oligonucleotides each comprising a catalytic nucleic acid substrate component, a catalytic nucleic acid component (either a full catalytic nucleic acid or a portion thereof such as a partzyme oligonucleotide), and a component and/or property preventing the subZyme oligonucleotide from moving through an otherwise permeable membrane utilised in the assay. The subZyme oligonucleotides are mobile in solution rather than, for example, being tethered to a planar surface. This provides a number of advantages including, but not limited to, improved accessibility of catalytic nucleic acids to their substrates and increased rates of diffusion to opposite sides of the membrane leading to faster detection. The catalytic nucleic acid component of the subZyme oligonucleotide exists in a catalytically active form without, for example, any conformational constraint/s (e.g. a hybridised inhibitory molecule/oligonucleotide) that would reduce or prevent its catalytic activity in the presence of its target substrate. Providing the catalytic nucleic acid component in an uninhibited form avoids the reduction in catalytic activity associated with utilising enzymes subjected to conformational constraints. In the assays described herein, the presence of a target molecule triggers cleavage of the subZyme oligonucleotide releasing the catalytic nucleic acid component from the subZyme oligonucleotide and allowing the released catalytic nucleic acid component to permeate the membrane. Once through the membrane the released catalytic nucleic acid component can cleave

other subZyme oligonucleotides comprising its substrate and thereby release other catalytic nucleic acid components. These can in turn permeate to the other side of the membrane to cleave further subZyme oligonucleotides, and so on. The assays of the present disclosure thus rely on the transfer of catalytically active nucleic acid enzymes, and/or components thereof such as partzyme oligonucleotides, across the membrane following an initial target recognition event. The remainder of the cleaved subZyme which includes the substrate component remains on one side of the membrane and is still unable to permeate through it. Accordingly, the assays described herein do not rely on or require the transfer of nucleic acid enzyme substrates across the membrane. Rather, they rely on the provision of catalytically active nucleic acids, and/or components thereof, as components of subZyme oligonucleotides, and their subsequent release and transfer across the membrane upon a target recognition event.

[0015] The target recognition event of the assays described herein utilises an ‘initiating’ catalytic nucleic enzyme which specifically detects the target and upon doing so effects cleavage of a subZyme oligonucleotide. The present inventors have observed that the use of initiating enzymes in the manner disclosed herein can increase the sensitivity and/or specificity of the detection assay in comparison to other methods such as, for example, those relying on target molecules to remove inhibitory component/s from catalytic nucleic acids to facilitate their activity.

[0016] The assays of the present disclosure may incorporate feedback loops making them capable of signal amplification, independent of the number of target molecules present. A singletarget molecule can initiate a cascade in which an initial subZyme is cleaved in a target-dependent fashion by an initiating enzyme. This releases the catalytic nucleic acid component of the subZyme which is free to permeate across the membrane. Once across the membrane, the released catalytically active nucleic acid, and/or components thereof, can access and cleave its substrate, which exists as a component of another subZyme. This event in turn releases another catalytically active nucleic acid, or components thereof (e.g. partzyme oligonucleotides), from the cleaved subZyme which can move across the permeable membrane and cleave its substrate, which again is a component of another subZyme. SubZyme cleavage events can thus be perpetuated in this manner and a feedback amplification loop is established that is independent of additional target molecules being present. This provides a significant advantage over, for example, linear cascades in which the amount of signal generated is more restricted by the number of target molecules present.

[0016a] In one aspect, the present disclosure refers to a method for determining a presence of a target in a sample, the method comprising:

- providing a reaction mix comprising:
 - a population of polynucleotide A (PA) and a population of polynucleotide B (PB),
 - wherein
 - each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,
 - each PB polynucleotide comprises a catalytic nucleic acid B and a catalytic nucleic acid substrate B,
 - the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify catalytic nucleic acid substrate A, and
 - the catalytic nucleic acid B is capable of catalytically modifying the catalytic nucleic acid substrate A and is unable to catalytically modify the catalytic nucleic acid substrate B;
 - a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, and the PA and/or PB is/are mobile in the reaction mix; and
 - (i) two oligonucleotides each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide, at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide; or
 - (ii) a catalytic enzyme D capable of cleaving one or more strands of a nucleic acid duplex formed by hybridisation of the target to the PA;
- contacting:
 - (i) the PA, the PB, and the oligonucleotide(s) with the sample, and using the selectively permeable barrier to separate the PA from the PB, wherein:
 - if the target is present in the sample, each of the two oligonucleotides hybridises to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one further hybridises to the target to thereby form the catalytic nucleic acid C which cleaves the PA polynucleotide to thereby release the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier; or
 - (ii) the PA, the PB, and the catalytic enzyme D with the sample, and using the selectively permeable barrier to separate the PA from the PB, wherein:

if the target is present in the sample, the target hybridises with a PA polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site releasing the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

- wherein:

the released catalytic nucleic acid A moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate B of a PB polynucleotide, and cleaves the PB polynucleotide to thereby release the catalytic nucleic acid B of the PB polynucleotide which is consequently capable of movement through the permeable barrier;

the released catalytic nucleic acid B moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate A of a second PA polynucleotide, and cleaves the second PA polynucleotide to thereby release the catalytic nucleic acid A of the second PA polynucleotide which is consequently capable of movement through the permeable barrier;

detecting the cleavage of any said PA and/or PB polynucleotide indicates the presence of the target in the sample;

and

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.

[0016b] In another aspect, the present disclosure refers to a method for determining a presence of a target in a sample, the method comprising:

(a) providing a reaction mix comprising:

(i) a population of polynucleotide A (PA), a population of polynucleotide B (PB) and a population of polynucleotide C (PC), wherein

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,

each PB polynucleotide comprises a partzyme oligonucleotide B1 and a catalytic nucleic acid substrate B,

each PC polynucleotide comprises a partzyme oligonucleotide B2,

wherein:

a substrate arm of the partzyme oligonucleotide B1 is capable of hybridising to the catalytic nucleic acid substrate A,

a substrate arm of the partzyme oligonucleotide B2 is capable of hybridising to the catalytic nucleic acid substrate A, and

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify the catalytic nucleic acid substrate A;

(ii) an assembly facilitator oligonucleotide capable of hybridising to a sensor arm of partzyme oligonucleotide B1 and to a sensor arm of partzyme oligonucleotide B2;

(iii) a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, and the PA, PB and PC and are mobile in the reaction mix; and either one of:

(iv) two partzyme oligonucleotides C1 and C2 each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide;

or

(v) a catalytic enzyme D capable of cleaving one or more strands of a nucleic acid duplex formed by hybridisation of the target to the PA;

and

(b) contacting:

(i) the partzyme oligonucleotides C1 and C2 with the sample, and using the selectively permeable barrier to separate the PB from the PA, wherein:

if the target is present in the sample, the substrate arm of each of the partzyme oligonucleotides C1 and C2 hybridises to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one of partzyme oligonucleotides C1 and C2 hybridises to the target forming the catalytic nucleic acid C which cleaves the PA polynucleotide to thereby release the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

or

(ii) the catalytic enzyme D with the sample, and using the selectively permeable barrier to separate the PB from the PA, wherein:

if the target is present in the sample, the target hybridises with a PA polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site releasing the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

wherein:

the released catalytic nucleic acid A moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate B of a PB polynucleotide, and cleaves the PB polynucleotide to thereby release the partzyme oligonucleotide B1 of the PB polynucleotide which is consequently capable of movement through the permeable barrier;

the released partzyme oligonucleotide B1 moves through the permeable barrier, the sensor arm of each of partzyme oligonucleotides B1 and B2 hybridises to the assembly facilitator oligonucleotide, and the substrate arm of each of partzyme oligonucleotides B1 and B2 hybridises to the catalytic nucleic acid substrate A of a second PA polynucleotide, thereby forming catalytic nucleic acid B which cleaves the second PA polynucleotide to thereby release the catalytic nucleic acid A of the second PA polynucleotide which is consequently capable of movement through the permeable barrier;

and

detecting the cleavage of any said PA and/or PB polynucleotide indicates the presence of the target in the sample.

[0016c] In another aspect, the present disclosure refers to a composition for the detection of a target in a sample, the composition comprising:

a population of polynucleotide A (PA) and a population of polynucleotide B (PB),
wherein:

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,

each PB polynucleotide comprises a catalytic nucleic acid B and a catalytic nucleic acid substrate B,

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify the catalytic nucleic acid substrate A, and

the catalytic nucleic acid B is capable of catalytically modifying the catalytic nucleic acid substrate A and is unable to catalytically modify the catalytic nucleic acid substrate B,

the PA and/or PB are mobile in the composition;

a selectively permeable barrier capable of separating the PA from the PB;

and

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNazyme.

[0016d] In another aspect, the present disclosure refers to a composition comprising:

(i) a population of polynucleotide A (PA), a population of polynucleotide B (PB) and a population of polynucleotide C (PC), wherein

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A, and is mobile in the composition,

each PB polynucleotide comprises a partzyme oligonucleotide B1 and a catalytic nucleic acid substrate B,

each PC polynucleotide comprises a partzyme oligonucleotide B2,

wherein:

a substrate arm of the partzyme oligonucleotide B1 is capable of hybridising to the catalytic nucleic acid substrate A,

a substrate arm of the partzyme oligonucleotide B2 is capable of hybridising to the catalytic nucleic acid substrate A, and

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify the catalytic nucleic acid substrate A;

(ii) an assembly facilitator oligonucleotide capable of hybridising to a sensor arm of partzyme oligonucleotide B1 and to a sensor arm of partzyme oligonucleotide B2;

(iii) a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, wherein hybridisation of the partzyme oligonucleotide B1 substrate arm and the partzyme oligonucleotide B2 substrate arm to the catalytic nucleic acid substrate A, and hybridisation of the partzyme oligonucleotide B1 sensor arm and the partzyme oligonucleotide B2 sensor arm to the assembly facilitator, forms a catalytic nucleic acid B capable of cleaving the catalytic nucleic acid substrate A; and wherein:

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate B capable of cleavage by catalytic nucleic acid A; or

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate A;

and

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.

[0017] The present disclosure thus addresses at least one of the disadvantages evident in existing target molecule detection and/or signal amplification assays by encompassing, at least in part, the following listed examples 1-59:

[0018] Example 1: A method for determining a presence of a target in a sample, the method comprising:

- providing a reaction mix comprising:

- a population of polynucleotide A (PA) and a population of polynucleotide B (PB),
 - wherein

- each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,

- each PB polynucleotide comprises a catalytic nucleic acid B and a catalytic nucleic acid substrate B,

- the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B, and

- the catalytic nucleic acid B is capable of catalytically modifying the catalytic nucleic acid substrate A;

- a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, and the PA and/or PB is/are mobile in the reaction mix; and

- (i) two oligonucleotides each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide; or

- (ii) a catalytic enzyme D capable of cleaving one or more strands of a nucleic acid duplex formed by hybridisation of the target to the PA;

- contacting:

- (i) the PA, the PB, and the oligonucleotide(s) with the sample, and using the selectively permeable barrier to separate the PA from the PB, wherein:

- if the target is present in the sample, each of the two oligonucleotides hybridises to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one further hybridises to the target to thereby form the catalytic nucleic acid C which cleaves the PA polynucleotide to thereby release the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier; or

- (ii) the PA, the PB, and the catalytic enzyme D with the sample, and using the selectively permeable barrier to separate the PA from the PB, wherein:

if the target is present in the sample, the target hybridises with a PA polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site releasing the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

- wherein:

the released catalytic nucleic acid A moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate B of a PB polynucleotide, and cleaves the PB polynucleotide to thereby release the catalytic nucleic acid B of the PB polynucleotide which is consequently capable of movement through the permeable barrier;

the released catalytic nucleic acid B moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate A of a second PA polynucleotide, and cleaves the second PA polynucleotide to thereby release the catalytic nucleic acid A of the second PA polynucleotide which is consequently capable of movement through the permeable barrier;

and

detecting the cleavage of any said PA and/or PB polynucleotide indicates the presence of the target in the sample.

[0019] Example 2: The method according to example 1, wherein:

the catalytic nucleic acid A is unable to catalytically modify catalytic nucleic acid substrate A, and/or

the catalytic nucleic acid B of the second polynucleotide is unable to catalytically modify the catalytic nucleic acid substrate B.

[0020] Example 3: The method according to example 1 or example 2, wherein the catalytic nucleic acid C is not capable of cleaving the catalytic nucleic acid substrate B of the PB polynucleotide.

[0021] Example 4: The method according to any one of examples 1 to 3, wherein any of the PA and/or PB that are mobile in the reaction mix polynucleotides comprises:

an attached component and/or,

a region of self-complementarity providing a secondary structure, and/or

a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier.

[0022] Example 5: The method according to example 4, wherein the attached component is:

an aptamer hybridised to a ligand, or
 a bead (e.g. a magnetic bead), or
 a microparticle, or
 a nanoparticle, or
 a charged moiety.

[0023] Example 6: The method according to example 4 or example 5, wherein:

the cleavage of the catalytic nucleic acid substrate A releases the attached component or the region of self-complementarity from the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier, and/or

the cleavage of the catalytic nucleic acid substrate B releases the attached component or the region of self-complementarity from the catalytic nucleic acid B of the PB polynucleotide which is consequently capable of movement through the permeable barrier.

[0024] Example 7: The method according to any one of examples 1 to 6, wherein the catalytic nucleic acid A and/or the catalytic nucleic acid B is a DNAzyme or a ribozyme.

[0025] Example 8: The method according to any one of examples 1 to 7, wherein:

the catalytic nucleic acid A and/or the catalytic nucleic acid B is selected from the group consisting of an 8-17 DNAzyme, a 10-23 DNAzyme, a 9-86 DNAzyme, a 12-91 DNAzyme, a GR-5 DNAzyme, a 17E DNAzyme, an RFD-EC1 DNAzyme, an F-8 DNAzyme, a 39-E DNAzyme, an E2 DNAzyme, an Mg5 DNAzyme, an A43 DNAzyme, a DAB22 DNAzyme, a PS2.M DNAzyme, a hammerhead ribozyme, an L-histidine-dependent DNAzyme, and an HRP DNAzyme.

[0026] Example 9: The method according to any one of examples 1 to 8, wherein:

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.

[0027] Example 10: The method according to any one of examples 1 to 9, wherein:

(i) the catalytic nucleic acid A is a 10-23 DNAzyme and the catalytic nucleic acid B is an 8-17 DNAzyme; and

the catalytic nucleic acid substrate A is an 8-17 DNAzyme substrate and the catalytic nucleic acid substrate B is an 10-23 DNAzyme substrate; or

(ii) the catalytic nucleic acid A is a 8-17 DNAzyme and the catalytic nucleic acid B is an 10-23 DNAzyme; and

the catalytic nucleic acid substrate A is an 10-23 DNAzyme substrate and the catalytic nucleic acid substrate B is an 8-17 DNAzyme substrate.

[0028] Example 11: The method according to any one of examples 1 to 10, wherein:

the two oligonucleotides are provided, each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form the catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide;

the target comprises or consist of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

the catalytic nucleic acid C comprises an aptamer portion capable of binding to the target;

the method further comprises contacting the sample with an inhibitor that hybridises to the aptamer to thereby render catalytic nucleic acid C inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

when present in the sample the target binds to the aptamer displacing the inhibitor and rendering the catalytic nucleic acid C active, thereby facilitating cleavage of the catalytic nucleic acid substrate A of the PA polynucleotide.

[0029] Example 12: The method according to any one of examples 1 to 11, wherein:

the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),
and

the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target.

[0030] Example 13: The method according to any one of examples 1 to 10, wherein:

the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),

the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target,

the target comprises or consists of a polynucleotide; and

the two partzyme oligonucleotides each hybridise to the polynucleotide during the self-assembly to form the MNAzyme.

[0031] Example 14: The method according to example 12, wherein:

the target comprises or consists of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison,

small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

at least one of the two partzyme oligonucleotides comprises a DNA, RNA or peptide aptamer portion capable hybridising specifically to the target;

the method further comprises contacting the sample with an MNzyme assembly facilitator oligonucleotide facilitating self-assembly of the two partzyme oligonucleotides into an MNzyme, and an inhibitor molecule that hybridises to the aptamer rendering the MNzyme catalytically inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

the target hybridises to the aptamer portion removing the inhibitor and rendering the MNzyme catalytically active.

[0032] Example 15: The method according to any one of examples 1 to 10, wherein:

the target is a polynucleotide,

the catalytic enzyme D is an endonuclease or an exonuclease.

[0033] Example 16: The method according to any one of examples 1 to 15, wherein

the release of the catalytic nucleic acid A from the catalytic nucleic acid substrate A of the PA polynucleotide and/or the release of the catalytic nucleic acid B from the catalytic nucleic acid substrate B of the PB polynucleotide separates a fluorophore from a quencher thereby providing a signal to facilitate the detection of the target.

[0034] Example 17: The method according to any one of examples 1 to 16, wherein the detection comprises

isolating the released catalytic nucleic acid A and/or the released catalytic nucleic acid B from the reaction mix;

applying the isolated catalytic nucleic acid(s) to a second reaction mix comprising catalytic nucleic acid substrate A and/or catalytic nucleic acid substrate B under conditions suitable for cleavage of the catalytic nucleic acid substrates by the catalytic nucleic acids; and

detecting cleavage of the catalytic nucleic acid substrates by the catalytic nucleic acids.

[0035] Example 18: The method according to any one of examples 1 to 17, wherein the detection comprises quantifying the released catalytic nucleic acid A and/or released catalytic nucleic acid B in real time.

[0036] Example 19: The method according to any one of examples 1 to 18, comprising:

the cleavage of fewer than five, fewer than six, fewer than seven, fewer than eight, fewer than nine, fewer than ten, fewer than fifteen, or fewer than twenty, of the PA polynucleotides, and/or

the cleavage of fewer than five, fewer than six, fewer than seven, fewer than eight, fewer than nine, fewer than ten, fewer than fifteen, or fewer than twenty, of the PB polynucleotides, to facilitate the detection of the target.

[0037] Example 20: A method for determining a presence of a target in a sample, the method comprising:

- (a) providing a reaction mix comprising:
 - (i) a population of polynucleotide A (PA), a population of polynucleotide B (PB) and a population of polynucleotide C (PC), wherein
 - each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,
 - each PB polynucleotide comprises a partzyme oligonucleotide B1 and a catalytic nucleic acid substrate B,
 - each PC polynucleotide comprises a partzyme oligonucleotide B2,
 wherein:
 - a substrate arm of the partzyme oligonucleotide B1 is capable of hybridising to the catalytic nucleic acid substrate A,
 - a substrate arm of the partzyme oligonucleotide B2 is capable of hybridising to the catalytic nucleic acid substrate A, and
 - the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B;
 - (ii) an assembly facilitator oligonucleotide capable of hybridising to a sensor arm of partzyme oligonucleotide B1 and to a sensor arm of partzyme oligonucleotide B2;
 - (iii) a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, and the PA, PB and PC and are mobile in the reaction mix; and either one of:
 - (iv) two partzyme oligonucleotides C1 and C2 each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide;
 - or
 - (v) a catalytic enzyme D capable of cleaving one or more strands of a nucleic acid duplex formed by hybridisation of the target to the PA;
- (b) contacting:

(i) the partzyme oligonucleotides C1 and C2 with the sample, and using the selectively permeable barrier to separate the PB from the PA, wherein:

if the target is present in the sample, the substrate arm of each of the partzyme oligonucleotides C1 and C2 hybridises to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one of partzyme oligonucleotides C1 and C2 hybridises to the target forming the catalytic nucleic acid C which cleaves the PA polynucleotide to thereby release the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

or

(ii) the catalytic enzyme D with the sample, and using the selectively permeable barrier to separate the PB from the PA, wherein:

if the target is present in the sample, the target hybridises with a PA polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site releasing the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

wherein:

the released catalytic nucleic acid A moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate B of a PB polynucleotide, and cleaves the PB polynucleotide to thereby release the partzyme oligonucleotide B1 of the PB polynucleotide which is consequently capable of movement through the permeable barrier;

the released partzyme oligonucleotide B1 moves through the permeable barrier, the sensor arm of each of partzyme oligonucleotides B1 and B2 hybridises to the assembly facilitator oligonucleotide, and the substrate arm of each of partzyme oligonucleotides B1 and B2 hybridises to the catalytic nucleic acid substrate A of a second PA polynucleotide, thereby forming catalytic nucleic acid B which cleaves the second PA polynucleotide to thereby release the catalytic nucleic acid A of the second PA polynucleotide which is consequently capable of movement through the permeable barrier;

and

detecting the cleavage of any said PA and/or PB polynucleotide indicates the presence of the target in the sample.

[0038] Example 21: The method according to example 20, wherein:

each PC polynucleotide comprises a partzyme oligonucleotide B2 and a catalytic nucleic acid substrate B that is cleavable by catalytic nucleic acid A,

the released catalytic nucleic acid A cleaves the PC polynucleotide to thereby release the partzyme oligonucleotide B2 of the PC polynucleotide, and

detecting the cleavage of any PC polynucleotide indicates the presence of the target in the sample.

[0039] Example 22: The method according to example 20, wherein

each PC polynucleotide comprises a partzyme oligonucleotide B2 and a catalytic nucleic acid substrate A, and

if the target is present in the sample, the substrate arm of each of the partzyme oligonucleotides C1 and C2 hybridises to the catalytic nucleic acid substrate A of the PC polynucleotide and at least one of the partzyme oligonucleotides C1 and C2 hybridises to the target forming the catalytic nucleic acid C which cleaves the PC polynucleotide to thereby release the partzyme oligonucleotide B2 of the PC polynucleotide.

[0040] Example 23: The method according to example 20, wherein if the target is present in the sample, the target hybridises with a PC polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A of the PC polynucleotide, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site to thereby release the partzyme oligonucleotide B2 of the PC polynucleotide.

[0041] Example 24: The method according to any one of examples 20 to 22, wherein any of the PA, PB and/or PC polynucleotides that are mobile in the reaction mix comprises:

an attached component and/or,

a region of self-complementarity providing a secondary structure, and/or

a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier

[0042] Example 25: The method according to example 24, wherein the attached component is:

an aptamer hybridised to a ligand, or

a bead (e.g. a magnetic bead), or a microparticle,

or a nanoparticle, or a charged moiety.

[0043] Example 26: The method according to example 24 or example 25, wherein:

the cleavage of the catalytic nucleic acid substrate A releases the attached component or the region of self-complementarity from the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier,

and/or the cleavage of the catalytic nucleic acid substrate B releases the attached component or the region of self-complementarity from the PB polynucleotide which is consequently capable of movement through the permeable barrier.

[0044] Example 27: The method according to any one of examples 20 to 26, wherein:

the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),
and

the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target.

[0045] Example 28: The method according to example 27, wherein:

the target comprises or consists of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

at least one of the two partzyme oligonucleotides comprises a DNA, RNA or peptide aptamer portion capable hybridising specifically to the target;

the method further comprises contacting the sample with an MNAzyme assembly facilitator oligonucleotide facilitating self-assembly of the two partzyme oligonucleotides into an MNAzyme, and an inhibitor molecule that hybridises to the aptamer rendering the MNAzyme catalytically inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

the target hybridises to the aptamer portion removing the inhibitor and rendering the MNAzyme catalytically active.

[0046] Example 29: The method according to example 27, wherein:

the target comprises or consists of a polynucleotide; and

the two partzyme oligonucleotides each hybridise to the polynucleotide during the self-assembly to form the MNAzyme.

[0047] Example 30: The method according to any one of examples 1 to 29, wherein:

the reaction mix further comprises a population of the catalytic nucleic acid substrate A which is mobile in the reaction mix, each catalytic nucleic acid substrate A of the population comprising a fluorophore molecule and a quencher molecule;

and the catalytic nucleic acid B and/or the catalytic nucleic acid C cleaves members of the population of catalytic nucleic acid substrate A, separating the fluorophore from the quencher to thereby providing a signal facilitating the detection of the target.

[0048] Example 31: A composition for the detection of a target in a sample, the composition comprising:

a population of polynucleotide A (PA) and a population of polynucleotide B (PB), wherein

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,

each PB polynucleotide comprises a catalytic nucleic acid B and a catalytic nucleic acid substrate B,

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B, and

the catalytic nucleic acid B is capable of catalytically modifying the catalytic nucleic acid substrate A,

a selectively permeable barrier capable of separating the PA from the PB.

[0049] Example 32: A composition comprising:

(i) a population of polynucleotide A (PA), a population of polynucleotide B (PB) and a population of polynucleotide C (PC), wherein

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,

each PB polynucleotide comprises a partzyme oligonucleotide B1 and a catalytic nucleic acid substrate B,

each PC polynucleotide comprises a partzyme oligonucleotide B2,

wherein:

a substrate arm of the partzyme oligonucleotide B1 is capable of hybridising to the catalytic nucleic acid substrate A,

a substrate arm of the partzyme oligonucleotide B2 is capable of hybridising to the catalytic nucleic acid substrate A, and

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B;

(ii) an assembly facilitator oligonucleotide capable of hybridising to a sensor arm of partzyme oligonucleotide B1 and to a sensor arm of partzyme oligonucleotide B2;

(iii) a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier,

wherein hybridisation of the partzyme oligonucleotide B1 substrate arm and the partzyme oligonucleotide B2 substrate arm to the catalytic nucleic acid substrate A, and hybridisation of the partzyme oligonucleotide B1 sensor arm and the partzyme oligonucleotide B2 sensor arm to the assembly facilitator, forms a catalytic nucleic acid B capable of cleaving the catalytic nucleic acid substrate A.

[0050] Example 33: The composition according to example 32, wherein:

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate B capable of cleavage by catalytic nucleic acid A; or

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate A.

[0051] Example 34: The composition according to example 31, wherein any of the PA and/or PB comprises:

an attached component and/or,

a region of self-complementarity providing a secondary structure, and/or

a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier.

[0052] Example 35: The composition according to example 32 or example 33, wherein any of the PC comprises:

an attached component and/or,

a region of self-complementarity providing a secondary structure, and/or

a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier.

[0053] Example 36: The composition according to example 34 or example 35, wherein the attached component is:

an aptamer hybridised to a ligand, or

a bead (e.g. a magnetic bead), or

a microparticle, or

a nanoparticle, or

a charged moiety.

[0054] Example 37: The composition according to any one of examples 31 to 36, wherein:

the catalytic nucleic acid A is unable to catalytically modify catalytic nucleic acid substrate A, and/or

the catalytic nucleic acid B of the second polynucleotide is unable to catalytically modify the catalytic nucleic acid substrate B.

[0055] Example 38: The composition of any one of examples 31 to 37, further comprising two oligonucleotides each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide.

[0056] Example 39: The composition according to example 38, wherein:

the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),
and

the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target.

[0057] Example 40: The composition of any one of examples 31 to 36, further comprising:

a catalytic enzyme D capable of cleaving one or more stands of a nucleic acid duplex formed by hybridisation of the target to the PA;

wherein:

at least a portion of the PA polynucleotide shares sequence complementarity with the target such that it is capable of hybridising with the target to form a nucleic acid duplex comprising a recognition and cleavage site for the catalytic enzyme D, and

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A of the PA polynucleotide.

[0058] Example 41: The composition according to example 40, wherein:

the target is a polynucleotide,

the catalytic enzyme D is an endonuclease or an exonuclease.

[0059] Example 42: The composition according to example 38, wherein the target comprises or consists of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

the catalytic nucleic acid C comprises an aptamer portion capable of binding to the target;

the composition further comprises an inhibitor that hybridises to the aptamer; and

the inhibitor has lower binding affinity for the aptamer portion than the target.

a selectively permeable barrier capable of separating the PA from the PB.

[0060] Example 43: The composition according to example 38 or example 39, wherein:

the target comprises or consists of a polynucleotide; and

the two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target can each hybridise to the polynucleotide of the target and the catalytic nucleic acid A substrate of the PA polynucleotide, and thereby self-assemble to form the MNAzyme.

[0061] Example 44: The composition according to example 39, wherein:

the target comprises or consist of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

at least one of the two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target comprises a DNA, RNA or peptide aptamer portion capable hybridising specifically to the target;

the composition further comprises an MNAzyme assembly facilitator oligonucleotide to which the two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target can each hybridise facilitating self-assembly of the MNAzyme;

the composition further comprises an inhibitor molecule that can hybridise to the aptamer thereby rendering the MNAzyme catalytically inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

the target can hybridise to the aptamer portion to remove the inhibitor and render the MNAzyme catalytically active.

[0062] Example 45: The composition according to any one of examples 31 to 44, further comprising catalytic nucleic acid A released by cleavage of the PA polynucleotide by the catalytic nucleic acid C and/or by the catalytic nucleic acid B.

[0063] Example 46: The composition according to any one of examples 31 to 45, further comprising catalytic nucleic acid B released by cleavage of the PB polynucleotide by the catalytic nucleic acid A.

[0064] Example 47: The composition according to any one of examples 31 to 46, wherein:

the catalytic nucleic acid A and/or the catalytic nucleic acid B is a DNAzyme or a ribozyme.

[0065] Example 48: The composition according to any one of examples 31 to 47, wherein:

the catalytic nucleic acid A and/or the catalytic nucleic acid B is selected from the group consisting of an 8-17 DNAzyme, a 10-23 DNAzyme, a 9-86 DNAzyme, a 12-91 DNAzyme, a GR-5 DNAzyme, a 17E DNAzyme, an RFD-EC1 DNAzyme, an F-8 DNAzyme, a 39-E DNAzyme, an E2 DNAzyme, an Mg5 DNAzyme, an A43 DNAzyme, a DAB22 DNAzyme, a PS2.M DNAzyme, a hammerhead ribozyme, an L-histidine-dependent DNAzyme, and an HRP DNAzyme.

[0066] Example 49: The composition according to any one of examples 31 to 48, wherein:

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.

[0067] Example 50: The composition according to any one of examples 31 to 49, wherein:

(i) the catalytic nucleic acid A is a 10-23 DNAzyme and the catalytic nucleic acid B is an 8-17 DNAzyme; and

the catalytic nucleic acid substrate A is an 8-17 DNAzyme substrate and the catalytic nucleic acid substrate B is a 10-23 DNAzyme substrate; or

(ii) the catalytic nucleic acid A is an 8-17 DNAzyme and the catalytic nucleic acid B is a 10-23 DNAzyme; and

the catalytic nucleic acid substrate A is a 10-23 DNAzyme substrate and the catalytic nucleic acid substrate B is an 8-17 DNAzyme substrate.

[0068] Example 51: The composition according to any one of examples 31 to 50, wherein the PA and/or PB and/or PC are mobile in the composition.

[0069] Example 52: The composition according to any one of examples 31 to 51, wherein the PA polynucleotides and/or PB polynucleotides and/or the PC polynucleotides each comprise a fluorophore and a quencher.

[0070] Example 53: The composition according to any one of examples 31 to 52, further comprising a population of the catalytic nucleic acid substrate A which is mobile in the composition, each catalytic nucleic acid substrate A of the population comprising a fluorophore molecule and a quencher molecule, wherein the catalytic nucleic acid B is capable of cleaving members of the population of catalytic nucleic acid substrate A, separating the fluorophore from the quencher to thereby providing a signal facilitating the detection of the target.

[0071] Example 54: The composition according to example 38 or example 39, further comprising a population of the catalytic nucleic acid substrate A which is mobile in the composition, each catalytic nucleic acid substrate A of the population comprising a fluorophore molecule and a quencher molecule, wherein the catalytic nucleic acid C is capable of cleaving members of the population of catalytic nucleic acid substrate A, separating the fluorophore from the quencher to thereby providing a signal facilitating the detection of the target.

[0072] Example 55: A polynucleotide comprising

- a catalytic nucleic acid or a component thereof;
 - a catalytic nucleic acid substrate;
 - and any one or more of:
 - (i) an attached component,
 - (ii) a region of self-complementarity providing a secondary structure,
 - (iii) a charged moiety,
 capable of preventing permeation of the polynucleotide across a selectively permeable barrier;
- wherein the catalytic nucleic acid is not capable of catalytically modifying the catalytic nucleic acid substrate.

[0073] Example 56: The polynucleotide according to example 55, wherein the attached component is:

an aptamer hybridised to a ligand, or

- a bead (e.g. a magnetic bead), or
- a microparticle, or
- a nanoparticle, or
- a charged moiety.

[0074] Example 57: The polynucleotide according to example 55 or example 56, wherein the catalytic nucleic acid is a DNAzyme or a ribozyme.

[0075] Example 58: The polynucleotide according to example 57, wherein the DNAzyme is a 10-23 DNAzyme or an 8-17 DNAzyme.

[0076] Example 59: The polynucleotide according to example 55 or example 56, wherein the component thereof is a partzyme oligonucleotide component of an MNAzyme.

Brief Description of the Figures

[0077] Preferred embodiments of the present invention will now be described by way of example only, with reference to the accompanying figures wherein:

CLAIMS

1. A method for determining a presence of a target in a sample, the method comprising:
 - providing a reaction mix comprising:

a population of polynucleotide A (PA) and a population of polynucleotide B (PB),
wherein

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,

each PB polynucleotide comprises a catalytic nucleic acid B and a catalytic nucleic acid substrate B,

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify catalytic nucleic acid substrate A, and

the catalytic nucleic acid B is capable of catalytically modifying the catalytic nucleic acid substrate A and is unable to catalytically modify the catalytic nucleic acid substrate B;

a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, and the PA and/or PB is/are mobile in the reaction mix; and

- (i) two oligonucleotides each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide, at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide; or
 - (ii) a catalytic enzyme D capable of cleaving one or more strands of a nucleic acid duplex formed by hybridisation of the target to the PA;
- contacting:

(i) the PA, the PB, and the oligonucleotide(s) with the sample, and using the selectively permeable barrier to separate the PA from the PB, wherein:

if the target is present in the sample, each of the two oligonucleotides hybridises to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one further hybridises to the target to thereby form the catalytic nucleic acid C which cleaves the PA polynucleotide to thereby release the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier; or

(ii) the PA, the PB, and the catalytic enzyme D with the sample, and using the selectively permeable barrier to separate the PA from the PB, wherein:

if the target is present in the sample, the target hybridises with a PA polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site releasing the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

- wherein:

the released catalytic nucleic acid A moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate B of a PB polynucleotide, and cleaves the PB polynucleotide to thereby release the catalytic nucleic acid B of the PB polynucleotide which is consequently capable of movement through the permeable barrier;

the released catalytic nucleic acid B moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate A of a second PA polynucleotide, and cleaves the second PA polynucleotide to thereby release the catalytic nucleic acid A of the second PA polynucleotide which is consequently capable of movement through the permeable barrier;

detecting the cleavage of any said PA and/or PB polynucleotide indicates the presence of the target in the sample;

and

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.

2. The method according to claim 1, wherein the catalytic nucleic acid C is not capable of cleaving the catalytic nucleic acid substrate B of the PB polynucleotide.

3. The method according to claim 1 or claim 2, wherein any of the PA and/or PB that are mobile in the reaction mix polynucleotides comprises:

an attached component and/or,

a region of self-complementarity providing a secondary structure, and/or

a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier.

4. The method according to claim 3, wherein the attached component is:
an aptamer hybridised to a ligand, or
a bead (e.g. a magnetic bead), or
a microparticle, or
a nanoparticle, or
a charged moiety.

5. The method according to claim 3 or claim 4, wherein:
the cleavage of the catalytic nucleic acid substrate A releases the attached component or the region of self-complementarity from the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier, and/or
the cleavage of the catalytic nucleic acid substrate B releases the attached component or the region of self-complementarity from the catalytic nucleic acid B of the PB polynucleotide which is consequently capable of movement through the permeable barrier.

6. The method according to any one of claims 1 to 5, wherein:
the catalytic nucleic acid A and/or the catalytic nucleic acid B is selected from the group consisting of an 8-17 DNAzyme, a 10-23 DNAzyme, a 9-86 DNAzyme, a 12-91 DNAzyme, a GR-5 DNAzyme, a 17E DNAzyme, an RFD-EC1 DNAzyme, an F-8 DNAzyme, a 39-E DNAzyme, an E2 DNAzyme, an Mg5 DNAzyme, an A43 DNAzyme, a DAB22 DNAzyme, a PS2.M DNAzyme, an L-histidine-dependent DNAzyme, and an HRP DNAzyme.

7. The method according to any one of claims 1 to 6, wherein:
 - (i) the catalytic nucleic acid A is a 10-23 DNAzyme and the catalytic nucleic acid B is an 8-17 DNAzyme; and
the catalytic nucleic acid substrate A is an 8-17 DNAzyme substrate and the catalytic nucleic acid substrate B is an 10-23 DNAzyme substrate; or
 - (ii) the catalytic nucleic acid A is a 8-17 DNAzyme and the catalytic nucleic acid B is an 10-23 DNAzyme; and
the catalytic nucleic acid substrate A is an 10-23 DNAzyme substrate and the catalytic nucleic acid substrate B is an 8-17 DNAzyme substrate.

8. The method according to any one of claims 1 to 7, wherein:

the two oligonucleotides are provided, each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form the catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide;

the target comprises or consist of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

the catalytic nucleic acid C comprises an aptamer portion capable of binding to the target;

the method further comprises contacting the sample with an inhibitor that hybridises to the aptamer to thereby render catalytic nucleic acid C inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

when present in the sample the target binds to the aptamer displacing the inhibitor and rendering the catalytic nucleic acid C active, thereby facilitating cleavage of the catalytic nucleic acid substrate A of the PA polynucleotide.

9. The method according to any one of claims 1 to 8, wherein:

the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),
and

the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target.

10. The method according to any one of claims 1 to 7, wherein:

the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),

the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target,

the target comprises or consists of a polynucleotide; and

the two partzyme oligonucleotides each hybridise to the polynucleotide during the self-assembly to form the MNAzyme.

11. The method according to claim 9, wherein:

the target comprises or consists of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

at least one of the two partzyme oligonucleotides comprises a DNA, RNA or peptide aptamer portion capable hybridising specifically to the target;

the method further comprises contacting the sample with an MNzyme assembly facilitator oligonucleotide facilitating self-assembly of the two partzyme oligonucleotides into an MNzyme, and an inhibitor molecule that hybridises to the aptamer rendering the MNzyme catalytically inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

the target hybridises to the aptamer portion removing the inhibitor and rendering the MNzyme catalytically active.

12. The method according to any one of claims 1 to 7, wherein:

the target is a polynucleotide,

the catalytic enzyme D is an endonuclease or an exonuclease.

13. The method according to any one of claims 1 to 12, wherein

the release of the catalytic nucleic acid A from the catalytic nucleic acid substrate A of the PA polynucleotide and/or the release of the catalytic nucleic acid B from the catalytic nucleic acid substrate B of the PB polynucleotide separates a fluorophore from a quencher thereby providing a signal to facilitate the detection of the target.

14. The method according to any one of claims 1 to 13, wherein the detection comprises

isolating the released catalytic nucleic acid A and/or the released catalytic nucleic acid B from the reaction mix;

applying the isolated catalytic nucleic acid(s) to a second reaction mix comprising catalytic nucleic acid substrate A and/or catalytic nucleic acid substrate B under conditions suitable for cleavage of the catalytic nucleic acid substrates by the catalytic nucleic acids; and

detecting cleavage of the catalytic nucleic acid substrates by the catalytic nucleic acids.

15. The method according to any one of claims 1 to 14, wherein the detection comprises quantifying the released catalytic nucleic acid A and/or released catalytic nucleic acid B in real time.
16. The method according to any one of claims 1 to 15, comprising:
- the cleavage of fewer than five, fewer than six, fewer than seven, fewer than eight, fewer than nine, fewer than ten, fewer than fifteen, or fewer than twenty, of the PA polynucleotides, and/or
 - the cleavage of fewer than five, fewer than six, fewer than seven, fewer than eight, fewer than nine, fewer than ten, fewer than fifteen, or fewer than twenty, of the PB polynucleotides,
 - to facilitate the detection of the target.
17. A method for determining a presence of a target in a sample, the method comprising:
- (a) providing a reaction mix comprising:
 - (i) a population of polynucleotide A (PA), a population of polynucleotide B (PB) and a population of polynucleotide C (PC), wherein
 - each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,
 - each PB polynucleotide comprises a partzyme oligonucleotide B1 and a catalytic nucleic acid substrate B,
 - each PC polynucleotide comprises a partzyme oligonucleotide B2,
 wherein:
 - a substrate arm of the partzyme oligonucleotide B1 is capable of hybridising to the catalytic nucleic acid substrate A,
 - a substrate arm of the partzyme oligonucleotide B2 is capable of hybridising to the catalytic nucleic acid substrate A, and
 - the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify the catalytic nucleic acid substrate A;
 - (ii) an assembly facilitator oligonucleotide capable of hybridising to a sensor arm of partzyme oligonucleotide B1 and to a sensor arm of partzyme oligonucleotide B2;

(iii) a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, and the PA, PB and PC and are mobile in the reaction mix; and either one of:

(iv) two partzyme oligonucleotides C1 and C2 each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide;

or

(v) a catalytic enzyme D capable of cleaving one or more strands of a nucleic acid duplex formed by hybridisation of the target to the PA;

and

(b) contacting:

(i) the partzyme oligonucleotides C1 and C2 with the sample, and using the selectively permeable barrier to separate the PB from the PA, wherein:

if the target is present in the sample, the substrate arm of each of the partzyme oligonucleotides C1 and C2 hybridises to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one of partzyme oligonucleotides C1 and C2 hybridises to the target forming the catalytic nucleic acid C which cleaves the PA polynucleotide to thereby release the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

or

(ii) the catalytic enzyme D with the sample, and using the selectively permeable barrier to separate the PB from the PA, wherein:

if the target is present in the sample, the target hybridises with a PA polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site releasing the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier;

wherein:

the released catalytic nucleic acid A moves through the permeable barrier, hybridises to the catalytic nucleic acid substrate B of a PB polynucleotide, and cleaves the PB

polynucleotide to thereby release the partzyme oligonucleotide B1 of the PB polynucleotide which is consequently capable of movement through the permeable barrier;

the released partzyme oligonucleotide B1 moves through the permeable barrier, the sensor arm of each of partzyme oligonucleotides B1 and B2 hybridises to the assembly facilitator oligonucleotide, and the substrate arm of each of partzyme oligonucleotides B1 and B2 hybridises to the catalytic nucleic acid substrate A of a second PA polynucleotide, thereby forming catalytic nucleic acid B which cleaves the second PA polynucleotide to thereby release the catalytic nucleic acid A of the second PA polynucleotide which is consequently capable of movement through the permeable barrier;

and

detecting the cleavage of any said PA and/or PB polynucleotide indicates the presence of the target in the sample.

18. The method according to claim 17, wherein:

each PC polynucleotide comprises a partzyme oligonucleotide B2 and a catalytic nucleic acid substrate B that is cleavable by catalytic nucleic acid A,

the released catalytic nucleic acid A cleaves the PC polynucleotide to thereby release the partzyme oligonucleotide B2 of the PC polynucleotide, and

detecting the cleavage of any PC polynucleotide indicates the presence of the target in the sample.

19. The method according to claim 17, wherein

each PC polynucleotide comprises a partzyme oligonucleotide B2 and a catalytic nucleic acid substrate A, and

if the target is present in the sample, the substrate arm of each of the partzyme oligonucleotides C1 and C2 hybridises to the catalytic nucleic acid substrate A of the PC polynucleotide and at least one of the partzyme oligonucleotides C1 and C2 hybridises to the target forming the catalytic nucleic acid C which cleaves the PC polynucleotide to thereby release the partzyme oligonucleotide B2 of the PC polynucleotide.

20. The method according to claim 17, wherein if the target is present in the sample, the target hybridises with a PC polynucleotide to form the nucleic acid duplex which comprises a recognition and cleavage site for the catalytic enzyme D,

the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A of the PC polynucleotide, and

the catalytic enzyme D binds to and cleaves the nucleic acid duplex at the cleavage site to thereby release the partzyme oligonucleotide B2 of the PC polynucleotide.

21. The method according to any one of claims 17 to 19, wherein any of the PA, PB and/or PC polynucleotides that are mobile in the reaction mix comprises:

an attached component and/or,

a region of self-complementarity providing a secondary structure, and/or

a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier

22. The method according to claim 21, wherein the attached component is:

an aptamer hybridised to a ligand, or

a bead (e.g. a magnetic bead), or a microparticle,

or a nanoparticle, or a charged moiety.

23. The method according to claim 21 or claim 22, wherein:

the cleavage of the catalytic nucleic acid substrate A releases the attached component or the region of self-complementarity from the catalytic nucleic acid A of the PA polynucleotide which is consequently capable of movement through the permeable barrier,

and/or the cleavage of the catalytic nucleic acid substrate B releases the attached component or the region of self-complementarity from the PB polynucleotide which is consequently capable of movement through the permeable barrier.

24. The method according to any one of claims 17 to 23, wherein:

the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),

and

the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target.

25. The method according to claim 24, wherein:

the target comprises or consists of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant,

poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

at least one of the two partzyme oligonucleotides comprises a DNA, RNA or peptide aptamer portion capable hybridising specifically to the target;

the method further comprises contacting the sample with an MNzyme assembly facilitator oligonucleotide facilitating self-assembly of the two partzyme oligonucleotides into an MNzyme, and an inhibitor molecule that hybridises to the aptamer rendering the MNzyme catalytically inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

the target hybridises to the aptamer portion removing the inhibitor and rendering the MNzyme catalytically active.

26. The method according to claim 24, wherein:

the target comprises or consists of a polynucleotide; and

the two partzyme oligonucleotides each hybridise to the polynucleotide during the self-assembly to form the MNzyme.

27. The method according to any one of claims 1 to 26, wherein:

the reaction mix further comprises a population of the catalytic nucleic acid substrate A which is mobile in the reaction mix, each catalytic nucleic acid substrate A of the population comprising a fluorophore molecule and a quencher molecule;

and the catalytic nucleic acid B and/or the catalytic nucleic acid C cleaves members of the population of catalytic nucleic acid substrate A, separating the fluorophore from the quencher to thereby providing a signal facilitating the detection of the target.

28. A composition for the detection of a target in a sample, the composition comprising: a population of polynucleotide A (PA) and a population of polynucleotide B (PB), wherein:

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A,

each PB polynucleotide comprises a catalytic nucleic acid B and a catalytic nucleic acid substrate B,

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify the catalytic nucleic acid substrate A, and

the catalytic nucleic acid B is capable of catalytically modifying the catalytic nucleic acid substrate A and is unable to catalytically modify the catalytic nucleic acid substrate B,

the PA and/or PB are mobile in the composition;

a selectively permeable barrier capable of separating the PA from the PB;

and

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.

29. A composition comprising:

(i) a population of polynucleotide A (PA), a population of polynucleotide B (PB) and a population of polynucleotide C (PC), wherein

each PA polynucleotide comprises a catalytic nucleic acid A and a catalytic nucleic acid substrate A, and is mobile in the composition,

each PB polynucleotide comprises a partzyme oligonucleotide B1 and a catalytic nucleic acid substrate B,

each PC polynucleotide comprises a partzyme oligonucleotide B2,

wherein:

a substrate arm of the partzyme oligonucleotide B1 is capable of hybridising to the catalytic nucleic acid substrate A,

a substrate arm of the partzyme oligonucleotide B2 is capable of hybridising to the catalytic nucleic acid substrate A, and

the catalytic nucleic acid A is capable of catalytically modifying the catalytic nucleic acid substrate B and is unable to catalytically modify the catalytic nucleic acid substrate A;

(ii) an assembly facilitator oligonucleotide capable of hybridising to a sensor arm of partzyme oligonucleotide B1 and to a sensor arm of partzyme oligonucleotide B2;

(iii) a selectively permeable barrier capable of physically separating the PA from the PB, wherein the PA and PB are unable to permeate the selectively permeable barrier, wherein hybridisation of the partzyme oligonucleotide B1 substrate arm and the partzyme oligonucleotide B2 substrate arm to the catalytic nucleic acid substrate A, and hybridisation

of the partzyme oligonucleotide B1 sensor arm and the partzyme oligonucleotide B2 sensor arm to the assembly facilitator, forms a catalytic nucleic acid B capable of cleaving the catalytic nucleic acid substrate A; and wherein:

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate B capable of cleavage by catalytic nucleic acid A; or

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate A;

and

the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.

30. The composition according to claim 29, wherein:

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate B capable of cleavage by catalytic nucleic acid A; or

each PC polynucleotide comprises a partzyme oligonucleotide B2 and said catalytic nucleic acid substrate A.

31. The composition according to claim 28, wherein any of the PA and/or PB comprises:
an attached component and/or,
a region of self-complementarity providing a secondary structure, and/or
a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier.

32. The composition according to claim 29 or claim 30, wherein any of the PC comprises:
an attached component and/or,
a region of self-complementarity providing a secondary structure, and/or
a charged moiety;

that prevents permeation of the polynucleotide across the selectively permeable barrier.

33. The composition according to claim 31 or claim 32, wherein the attached component is:

an aptamer hybridised to a ligand, or

a bead (e.g. a magnetic bead), or

a microparticle, or

a nanoparticle, or
a charged moiety.

34. The composition of any one of claims 28 to 33, further comprising two oligonucleotides each capable of hybridising specifically to the catalytic nucleic acid substrate A of a PA polynucleotide and at least one being capable of hybridising specifically to the target, to thereby form a catalytic nucleic acid C capable of cleaving the catalytic nucleic acid substrate A of the PA polynucleotide.

35. The composition according to claim 34, wherein:
the catalytic nucleic acid C is a multi component nucleic acid enzyme (MNAzyme),
and
the two oligonucleotides are two partzyme oligonucleotides capable of self-assembling to form the MNAzyme only in the presence of the target.

36. The composition of any one of claims 28 to 33, further comprising:
a catalytic enzyme D capable of cleaving one or more stands of a nucleic acid duplex formed by hybridisation of the target to the PA;
wherein:
at least a portion of the PA polynucleotide shares sequence complementarity with the target such that it is capable of hybridising with the target to form a nucleic acid duplex comprising a recognition and cleavage site for the catalytic enzyme D, and
the cleavage site for the catalytic enzyme D is not within the catalytic nucleic acid A of the PA polynucleotide.

37. The composition according to claim 36, wherein:
the target is a polynucleotide,
the catalytic enzyme D is an endonuclease or an exonuclease.

38. The composition according to claim 34, wherein the target comprises or consists of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

the catalytic nucleic acid C comprises an aptamer portion capable of binding to the target;

the composition further comprises an inhibitor that hybridises to the aptamer; and
the inhibitor has lower binding affinity for the aptamer portion than the target.

a selectively permeable barrier capable of separating the PA from the PB.

39. The composition according to claim 34 or claim 35, wherein:

the target comprises or consists of a polynucleotide; and

the two partzyme oligonucleotides capable of self-assembling to form the MNzyme only in the presence of the target can each hybridise to the polynucleotide of the target and the catalytic nucleic acid A substrate of the PA polynucleotide, and thereby self-assemble to form the MNzyme.

40. The composition according to claim 35, wherein:

the target comprises or consist of a protein, analyte, glycoprotein, lipid, lipoprotein, cell, virus, bacterium, archeon, fungus, antibody, metabolite, pathogen, toxin, contaminant, poison, small molecule, polymer, metal ion, metal salt, prion, or any derivative, portion or combination thereof;

at least one of the two partzyme oligonucleotides capable of self-assembling to form the MNzyme only in the presence of the target comprises a DNA, RNA or peptide aptamer portion capable hybridising specifically to the target;

the composition further comprises an MNzyme assembly facilitator oligonucleotide to which the two partzyme oligonucleotides capable of self-assembling to form the MNzyme only in the presence of the target can each hybridise facilitating self-assembly of the MNzyme;

the composition further comprises an inhibitor molecule that can hybridise to the aptamer thereby rendering the MNzyme catalytically inactive;

the inhibitor has lower binding affinity for the aptamer portion than the target; and

the target can hybridise to the aptamer portion to remove the inhibitor and render the MNzyme catalytically active.

41. The composition according to any one of claims 28 to 40, further comprising catalytic nucleic acid A released by cleavage of the PA polynucleotide by the catalytic nucleic acid C and/or by the catalytic nucleic acid B.

42. The composition according to any one of claims 28 to 41, further comprising catalytic nucleic acid B released by cleavage of the PB polynucleotide by the catalytic nucleic acid A.
43. The composition according to any one of claims 28 to 42, wherein:
the catalytic nucleic acid A and/or the catalytic nucleic acid B is a DNAzyme or a ribozyme.
44. The composition according to any one of claims 28 to 43, wherein:
the catalytic nucleic acid A and/or the catalytic nucleic acid B is selected from the group consisting of an 8-17 DNAzyme, a 10-23 DNAzyme, a 9-86 DNAzyme, a 12-91 DNAzyme, a GR-5 DNAzyme, a 17E DNAzyme, an RFD-EC1 DNAzyme, an F-8 DNAzyme, a 39-E DNAzyme, an E2 DNAzyme, an Mg5 DNAzyme, an A43 DNAzyme, a DAB22 DNAzyme, a PS2.M DNAzyme, a hammerhead ribozyme, an L-histidine-dependent DNAzyme, and an HRP DNAzyme.
45. The composition according to any one of claims 28 to 44, wherein:
the catalytic nucleic acid A and the catalytic nucleic acid B are each a different variety of DNAzyme.
46. The composition according to any one of claims 28 to 45, wherein:
(i) the catalytic nucleic acid A is a 10-23 DNAzyme and the catalytic nucleic acid B is an 8-17 DNAzyme; and
the catalytic nucleic acid substrate A is an 8-17 DNAzyme substrate and the catalytic nucleic acid substrate B is a 10-23 DNAzyme substrate; or
(ii) the catalytic nucleic acid A is an 8-17 DNAzyme and the catalytic nucleic acid B is a 10-23 DNAzyme; and
the catalytic nucleic acid substrate A is a 10-23 DNAzyme substrate and the catalytic nucleic acid substrate B is an 8-17 DNAzyme substrate.
47. The composition according to any one of claims 28 to 46, wherein the PB and/or PC are mobile in the composition.

48. The composition according to any one of claims 28 to 47, wherein the PA polynucleotides and/or PB polynucleotides and/or the PC polynucleotides each comprise a fluorophore and a quencher.

49. The composition according to any one of claims 28 to 48, further comprising a population of the catalytic nucleic acid substrate A which is mobile in the composition, each catalytic nucleic acid substrate A of the population comprising a fluorophore molecule and a quencher molecule, wherein the catalytic nucleic acid B is capable of cleaving members of the population of catalytic nucleic acid substrate A, separating the fluorophore from the quencher to thereby providing a signal facilitating the detection of the target.

50. The composition according to claim 34 or claim 35, further comprising a population of the catalytic nucleic acid substrate A which is mobile in the composition, each catalytic nucleic acid substrate A of the population comprising a fluorophore molecule and a quencher molecule, wherein the catalytic nucleic acid C is capable of cleaving members of the population of catalytic nucleic acid substrate A, separating the fluorophore from the quencher to thereby providing a signal facilitating the detection of the target.