

CLAIMS

1. A catalytic nucleic acid nanostructure comprising a DNAzyme/RNAzyme and a stimulus responsive element.
2. The catalytic nucleic acid nanostructure of claim 1, wherein the DNAzyme/RNAzyme is
5 selected from the group comprising:
a ribonuclease, such as ribonuclease 8-17, ribonuclease 10-23 or Dz10-66 deoxyribozyme;
a deoxyribonuclease, such as 10MD5 deoxyribozyme or 9NL27 deoxyribozyme;
a peroxidase, such as G-quadruplex Hemin;
an enzyme with ligation activity, such as E47 deoxyribozyme;
10 a phosphatase, such as 14WM9 deoxyribozyme;
an amide hydrolyser, such as AmideAm1 deoxyribozyme; and
an RNA branching enzyme, such as 9F7 deoxyribozyme or 7S11 deoxyribozyme.
3. The catalytic nucleic acid nanostructure of claim 1 or 2, wherein the stimulus responsive
15 element comprises a polymerase-responsive element that inhibits the DNAzyme/RNAzyme
activity in the presence of polymerase.
4. The catalytic nucleic acid nanostructure of claim 3, wherein the polymerase-responsive
element has an internal hairpin structure.
5. The catalytic nucleic acid nanostructure of claim 4, wherein polymerase elongation of the
polymerase-responsive element eliminates catalytic activity.
- 20 6. The catalytic nucleic acid nanostructure of any one of claims 1 to 5, wherein the
DNAzyme/RNAzyme activity is a peroxidase activity.
7. The catalytic nucleic acid nanostructure of claim 6, wherein activity of a peroxidase
substrate is detected by different modalities, including but not limited to colorimetric,
fluorescence, electrochemical or luminescence means.
- 25 8. A method of detecting polymerase activity in a test sample, comprising the steps of:
(a) providing a test sample;
(b) providing a composition comprising a catalytic nucleic acid nanostructure of any one
of claims 3 to 7;

(c) contacting the sample in a) with the composition in b) in the presence of DNAzyme/RNAzyme substrate and, optionally, signal development reagents;

(d) detecting signal development, wherein the intensity of signal is inverse to the amount of polymerase activity in the sample.

5 9. A method of detecting target molecules in a sample, comprising the steps of:

(a) providing a test sample;

10 (b) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer adapted to recognize said target molecule in the sample;

15 (c) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is adapted to recognize said target molecule in the sample with a higher affinity than the variable duplex region;

20 (d) contacting the test sample with the composition of (b) or (c), wherein target molecule binding to:

(i) the recognition sequence region of the aptamer in (b) promotes the formation of a stable aptamer-DNA polymerase enzyme complex, thereby inhibiting DNA polymerase enzyme activity; or

25 (ii) the inverter oligonucleotide in (c) destabilizes the recognition nanostructure, thereby releasing the DNA polymerase enzyme from inhibition by the DNA aptamer;

(e) providing a catalytic nucleic acid nanostructure of any one of claims 3 to 7;

(f) contacting the nanostructure from step (b) or (c) in the presence of DNAzyme/RNAzyme substrate and, optionally, signal development reagents;

(g) detecting signal development, wherein the intensity of signal indicates;

30 (i) presence of target molecule in the sample when using composition (b); or

(ii) the absence of target molecule in the sample when using composition (c).

10. A method of detecting target nucleic acids in a sample, comprising the steps of:

(a) providing a sample comprising nucleic acid;

(b) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to a target nucleic acid in the sample; or

(c) providing a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer and an inverter oligonucleotide, wherein the aptamer has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to, and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer-inverter duplex and has more than 10 nucleotides complementary to a target nucleic acid in the sample;

(d) contacting the sample comprising nucleic acid with the composition of (b) or (c), wherein target nucleic acid binding to:

(i) the variable sequence region of the aptamer in (b) promotes the formation of a stable aptamer-DNA polymerase enzyme complex, thereby inhibiting DNA polymerase enzyme activity; or

(ii) the inverter oligonucleotide in (c) destabilizes the recognition nanostructure, thereby releasing the DNA polymerase enzyme from inhibition by the DNA aptamer;

(e) providing a catalytic nucleic acid nanostructure of any one of claims 3 to 7;

(f) contacting the nanostructure from step (d) in the presence of DNAzyme/RNAzyme substrate and, optionally, signal development reagents;

(g) detecting signal development, wherein the intensity of signal indicates;

(i) presence of target nucleic acid in the sample when using composition (b); or

(ii) the absence of target nucleic acid in the sample when using composition (c).

11. A device comprising a catalytic nucleic acid nanostructure of any one of claims 1 to 7 immobilized on a surface.

12. The device of claim 11 comprising:

- (i) composition b) or composition c) comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, as defined in claim 10, at a first location;
- (ii) catalytic nucleic acid nanostructure of any one of claims 3 to 7, attached at a second location; and
- (iii) an intermediate stage for mixing of said detection nanostructures with sample nucleic acid to release active enzyme to said second location.

13. The device of claim 11 or 12, selected from a group comprising a microfluidic device and a lateral flow device.

14. The device of any one of claims 11 to 13, comprising an electrode.

15. A nucleic acid detection kit comprising;

- (a) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to a target nucleic acid; and/or
- (b) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer and an inverter oligonucleotide, wherein the aptamer has a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to, and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is at least one nucleotide longer than the aptamer-inverter duplex and has more than 10 nucleotides complementary to a target nucleic acid; optionally
- (c) a catalytic nucleic acid nanostructure of any one of claims 3 to 7; optionally
- (d) DNAzyme/RNAzyme substrate and, optionally,
- (e) signal development reagents.

16. A molecule detection kit comprising;

- (a) a composition comprising at least one DNA polymerase enzyme and at least one recognition nanostructure, wherein the recognition nanostructure comprises a DNA polymerase enzyme-specific DNA aptamer having a conserved sequence region and a variable sequence region, wherein the variable sequence region comprises an overhang segment which is at least 10 nucleotides complementary to and forms a duplex with, a portion of the inverter oligonucleotide, wherein the inverter oligonucleotide is adapted to

recognize said target molecule in the sample with a higher affinity than the variable duplex region;

(b) a catalytic nucleic acid nanostructure of any one of claims 3 to 7; optionally

(c) DNAzyme/RNAzyme substrate and, optionally,

5 (d) signal development reagents.