SUMMARY

[0006] In some embodiments, an aptamer comprising at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine is provided, wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different. In some embodiments, the first 5-position modified pyrimidine is a 5-position modified uridine and wherein the second 5-position modified pyrimidine is a 5-position modified cytidine. In some embodiments, the first 5-position modified pyrimidine is a 5-position modified cytidine and wherein the second 5-position modified pyrimidine is a 5-position modified uridine. In some embodiments, the 5-position modified uridine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety. In some embodiments, the 5-position modified cytidine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, and a morpholino moiety.

[0006a] In one aspect, there is provided an aptamer comprising at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine, wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines;

wherein the first 5-position modified pyrimidine is a 5-position modified uridine and wherein the second 5-position modified pyrimidine is a 5-position modified cytidine; or

wherein the first 5-position modified pyrimidine is a 5-position modified cytidine and wherein the second 5-position modified pyrimidine is a 5-position modified uridine;

wherein the 5-position modified uridine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety; and wherein the 5-position modified cytidine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, and a morpholino moiety.

[0006b] In certain embodiments, the moiety is covalently linked to the 5-position of the base via a linker comprising a group selected from an amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker. In some embodiments, the 5-position modified cytidine is selected from NapdC, 2NapdC, TyrdC, and PPdC. In some embodiments, the 5-position modified uridine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a NapdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a PPdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one second 5-position modified pyrimidine is a TyrdU. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a polymerase enzyme. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a KOD DNA polymerase.

[0007] In some embodiments, the aptamer binds a target protein selected from PCSK9, PSMA, ErbB1, ErbB2, FXN, KDM2A, IGF1R, pIGF1R, a1-Antritrypsin, CD99, MMP28 and PPIB.

[0008] In some embodiments, the aptamer comprises a region at the 5' end of the aptamer that is at least 10, at least 15, at least 20, at least 25 or at least 30 nucleotides in length,

or 5 to 30, 10 to 30, 15 to 30, 5 to 20, or 10 to 20 nucleotides in length, wherein the region at the 5' end of the aptamer lacks 5-position modified pyrimidines. In some embodiments, the aptamer comprises a region at the 3' end of the aptamer that is at least 10, at least 15, at least 20, at least 25 or at least 30 nucleotides in length, or 5 to 30, 10 to 30, 15 to 30, 5 to 20, or 10 to 20 nucleotides in length, wherein the region at the 3' end of the aptamer lacks 5-position modified pyrimidines. In some embodiments, the aptamer is 20 to 100, or 20 to 90, or 20 to 80, or 20 to 70, or 20 to 60, or 20 to 50, or 30 to 100, or 30 to 90, or 30 to 80, or 30 to 70, or 30 to 60, or 30 to 50, or 40 to 100, or 40 to 90, or 40 to 80, or 40 to 70, or 40 to 60, or 40 to 50 nucleotides in length.

[0009] In some embodiments, the aptamer has improved nuclease stability compared to an aptamer of the same length and nucleobase sequence that comprises an unmodified pyrimidine in place of each of the first 5-position modified pyrimidines and/or an unmodified pyrimidine in place of each of the second 5-position modified pyrimidine. In some embodiments, the aptamer has a longer half-life in human serum compared to an aptamer of the same length and nucleobase sequence that comprises an unmodified pyrimidine in place of each of the first 5-position modified pyrimidines or an unmodified pyrimidine in place of each of the second 5-position modified pyrimidine.

[0010] In some embodiments, a composition comprising a plurality of polynucleotides is provided, wherein each polynucleotide comprises at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine, wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different.

[0010a] In one aspect, there is provided a composition comprising a plurality of polynucleotides, wherein each polynucleotide comprises at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine, wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines;

wherein the first 5-position modified pyrimidine is a 5-position modified uridine and wherein the second 5-position modified pyrimidine is a 5-position modified cytidine; or

wherein the first 5-position modified pyrimidine is a 5-position modified cytidine and wherein the second 5-position modified pyrimidine is a 5-position modified uridine;

wherein the 5-position modified uridine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety; and wherein the 5-position modified cytidine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, and a morpholino moiety.

[0010b] In some embodiments, each polynucleotide comprises a fixed region at the 5' end of the polynucleotide. In some embodiments, the fixed region at the 5' end of each polynucleotide is at least 10, at least 15, at least 20, at least 25 or at least 30 nucleotides in length, or 5 to 30, 10 to 30, 15 to 30, 5 to 20, or 10 to 20 nucleotides in length. In some embodiments, each polynucleotide comprises a fixed region at the 3' end of the polynucleotide. In some embodiments, the fixed region at the 3' end of the polynucleotide is at least 10 , at least 15, at least 20, at least 25 or at least 30 nucleotides in length, or 5 to 30, 10 to 30, 15 to 30, 5 to 20, or 10 to 20 nucleotides in length. In some embodiments, the first 5-position modified pyrimidine is a 5-position modified uridine and wherein the second 5-position modified pyrimidine is a 5-position modified cytidine. In some embodiments, the first 5-position modified pyrimidine is a 5-position modified cytidine and wherein the second 5-position modified pyrimidine is a 5-position modified uridine. In some embodiments, the 5-position modified uridine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, a

tryptophanyl moiety, an indole moiety and a morpholino moiety. In some embodiments, the 5-position modified cytidine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety and a morpholino moiety. In certain embodiments, the moiety is covalently linked to the 5-position of the base via a linker comprising a group selected from an amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker. In some embodiments, the 5-position modified cytidine is selected from NapdC, 2NapdC, TyrdC, and PPdC. In some embodiments, the 5-position modified uridine is selected from NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a NapdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a PPdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TrydU, TrpdU, and ThrdU. In some embodiments, the at least one second 5-position modified pyrimidine is a TyrdU. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a polymerase enzyme. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a KOD DNA polymerase.

[0011] In some embodiments, each polynucleotide of the composition comprises a random region. In some embodiments, the random region is 20 to 100, or 20 to 90, or 20 to 80, or 20 to 70, or 20 to 60, or 20 to 50, or 20 to 40, or 30 to 100, or 30 to 90, or 30 to 70, or 30 to 60, or 30 to 50, or 30 to 40 nucleotides in length. In some embodiments, each polynucleotide is 20 to 100, or 20 to 90, or 20 to 80, or 20 to 70, or 20 to 60, or 20 to 50, or 30 to 100, or 30 to 90, or 30 to 80, or 30 to 70, or 30 to 60, or 30 to 50, or 40 to 100, or 40 to 90, or 40 to 80, or 40 to 70, or 40 to 60, or 40 to 50 nucleotides in length.

[0011a] In one aspect, there is provided a composition comprising an aptamer and a target, wherein the aptamer and the target are capable of forming a complex, and wherein the aptamer is an aptamer as described herein.

[0012] In some embodiments, a composition is provided, comprising a first aptamer, a second aptamer, and a target, wherein the first aptamer comprises at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine; wherein the second aptamer comprises at least one third 5-position modified pyrimidine or wherein the second aptamer comprises at least one third 5-position modified pyrimidine and at least one fourth 5-position modified pyrimidine; wherein the first aptamer, second aptamer and the target are capable of forming a trimer complex; and wherein the first 5-position modified

pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines.

[0012a] In one aspect, there is provided a composition comprising a first aptamer, a second aptamer, and a target,

wherein the first aptamer comprises at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine;

wherein the second aptamer comprises at least one third 5-position modified pyrimidine or wherein the second aptamer comprises at least one third 5-position modified pyrimidine and at least one fourth 5-position modified pyrimidine;

wherein the first aptamer, second aptamer and the target are capable of forming a trimer complex; and

wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines; and

wherein the first aptamer is an aptamer as described herein.

[0013] In some embodiments, the first 5-position modified pyrimidine is a 5-position modified uridine and wherein the second 5-position modified pyrimidine is a 5-position modified cytidine. In some embodiments, the first 5-position modified pyrimidine is a 5-position modified cytidine and wherein the second 5-position modified pyrimidine is a 5-position modified uridine. In some embodiments, the 5-position modified uridine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety. In some embodiments, the 5-position modified cytidine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety and a morpholino moiety. In certain embodiments, the moiety is covalently linked to the 5-position of the base via a linker comprising a group selected from an amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker. In some embodiments, the 5-position modified cytidine is selected from NapdC, 2NapdC, TyrdC, and PPdC. In some embodiments, the 5-position modified uridine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a NapdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a PPdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one second 5-position modified pyrimidine is a TyrdU. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a polymerase enzyme. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a KOD DNA polymerase.

[0014] In some embodiments, the third 5-position modified pyrimidine is selected from a 5-position modified cytidine and a 5-position modified pyrimidine. In some embodiments, the third 5-position modified pyrimidine and the fourth 5-position modified pyrimidine are different 5-position modified pyrimidines. In some embodiments, third 5-position modified pyrimidine is a 5-position modified cytidine and the fourth 5-position modified pyrimidine is a 5-position modified uridine. In some embodiments, the third 5-position modified cytidine is selected from BndC, PEdC, PPdC, NapdC, 2NapdC, NEdC, 2NEdC, and TyrdC. In some

embodiments, the 5-position modified uridine is selected from BNdU, NapdU, PEdU, IbdU, FBndU, 2NapdU, NEdU, MBndU, BFdU, BTdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU.

[0015] In some embodiments, the target is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

[0015a] In one aspect, there is provided a method comprising:

(a) contacting an aptamer capable of binding to a target molecule with a sample;

(b) incubating the aptamer with the sample to allow an aptamer-target complex to form;

(c) enriching for the aptamer-target complex in the sample and

(c) detecting for the presence of the aptamer, aptamer-target complex or target molecule, wherein the detection of the aptamer, aptamer-target complex or target molecule indicates that the target molecule is present in the sample, and wherein the lack of detection of the aptamer, aptamer-target complex or target molecule indicates that the target molecule is not present in the sample;

wherein the aptamer is an aptamer as described herein.

[0016] In some embodiments, a method is provided, comprising:

(a) contacting an aptamer capable of binding to a target molecule with a sample;

(b) incubating the aptamer with the sample to allow an aptamer-target complex to form;

(c) enriching for the aptamer-target complex in the sample and

(c) detecting for the presence of the aptamer, aptamer-target complex or target molecule, wherein the detection of the aptamer, aptamer-target complex or target molecule indicates that the target molecule is present in the sample, and wherein the lack of detection of the aptamer, aptamer-target complex or target molecule indicates that the target molecule is not present in the sample;

wherein the aptamer is a dual-modified aptamer provided herein. In some embodiments, the method comprises at least one additional step selected from: adding a competitor molecule to the sample; capturing the aptamer-target complex on a solid support; and adding a competitor molecule and diluting the sample; wherein the at least one additional step occurs after step (a) or step (b). In some embodiments, the competitor molecule is selected from a polyanionic competitor. In some embodiments, the polyanionic competitor is selected from an oligonucleotide, polydextran, DNA, heparin and dNTPs. In some embodiments, polydextran is dextran sulfate; and DNA is herring sperm DNA or salmon sperm DNA. In some embodiments, the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue. In some embodiments, the sample is selected from whole blood, leukocytes, peripheral blood mononuclear cells, plasma, serum, sputum, breath, urine, semen, saliva, meningial fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, synovial fluid, joint aspirate, cells, a cellular extract, stool, tissue, a tissue biopsy, and cerebrospinal fluid.

[0017] In some embodiments, a method for detecting a target in a sample is provided, comprising

a) contacting the sample with a first aptamer to form a mixture, wherein the first aptamer is capable of binding to the target to form a first complex;

b) incubating the mixture under conditions that allow for the first complex to form;

c) contacting the mixture with a second aptamer, wherein the second aptamer is capable of binding the first complex to form a second complex;

d) incubating the mixture under conditions that allow for the second complex to form;

e) detecting for the presence or absence of the first aptamer, the second aptamer, the target, the first complex or the second complex in the mixture, wherein the presence of the first aptamer, the second aptamer, the target, the first complex or the second complex indicates that the target is present in the sample;

wherein the first aptamer comprises at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine;

wherein the second aptamer comprises at least one third 5-position modified pyrimidine, or wherein the second aptamer comprises at least one third 5-position modified pyrimidine and at least one fourth 5-position modified pyrimidine;

wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines.

[0017a] In one aspect, there is provided a method for detecting a target in a sample comprising

a) contacting the sample with a first aptamer to form a mixture, wherein the first aptamer is capable of binding to the target to form a first complex;

b) incubating the mixture under conditions that allow for the first complex to form;

c) contacting the mixture with a second aptamer, wherein the second aptamer is capable of binding the first complex to form a second complex;

d) incubating the mixture under conditions that allow for the second complex to form;

e) detecting for the presence or absence of the first aptamer, the second aptamer, the target, the first complex or the second complex in the mixture, wherein the presence of the first aptamer, the second aptamer, the target, the first complex or the second complex indicates that the target is present in the sample;

wherein the first aptamer is an aptamer as described herein;

wherein the second aptamer comprises at least one third 5-position modified pyrimidine, or wherein the second aptamer comprises at least one third 5-position modified pyrimidine and at least one fourth 5-position modified pyrimidine.

[0018] In some embodiments, the first 5-position modified pyrimidine is a 5-position modified uridine and wherein the second 5-position modified pyrimidine is a 5-position modified cytidine. In some embodiments, the first 5-position modified pyrimidine is a 5-position modified cytidine and wherein the second 5-position modified pyrimidine is a 5-position modified uridine. In some embodiments, the 5-position modified uridine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety. In some embodiments, the 5-position modified cytidine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety and a morpholino moiety. In certain embodiments, the moiety is covalently linked to the 5-position of the base via a linker comprising a group selected from an amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker. In some embodiments, the 5-position modified cytidine is selected from NapdC, 2NapdC, TyrdC, and PPdC. In some embodiments, the 5-position modified uridine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a NapdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, and ThrdU. In some embodiments, the at least one first 5-position modified pyrimidine is a PPdC and the at least one second 5-position modified pyrimidine is selected from NapdU, 2NapdU, PPdU, MOEdU, TyrdU, TrpdU, and ThrdU. In some embodiments,

the at least one second 5-position modified pyrimidine is a TyrdU. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a polymerase enzyme. In some embodiments, the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are capable of being incorporated by a KOD DNA polymerase.

[0019] In some embodiments, the third 5-position modified pyrimidine is selected from a 5-position modified cytidine and a 5-position modified pyrimidine. In some embodiments, the third 5-position modified pyrimidine and the fourth 5-position modified pyrimidine are different 5-position modified pyrimidines. In some embodiments, third 5-position modified pyrimidine is a 5-position modified cytidine and the fourth 5-position modified pyrimidine is a 5-position modified uridine. In some embodiments, the third 5-position modified cytidine is selected from BndC, PEdC, PPdC, NapdC, 2NapdC, NEdC, 2NEdC, and TyrdC. In some embodiments, the 5-position modified uridine is selected from BNdU, NapdU, PedU, IbdU, FbndU, 2NapdU, NedU, MbndU, BfdU, BtdU, PpdU, MOEdU, TyrdU, TrpdU, and ThrdU.

[0020] In some embodiments, the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue. In some embodiments, the first aptamer, second aptamer and the target are capable of forming a trimer complex.

[0021] In some embodiments, a method for identifying one or more aptamers capable of binding to a target molecule is provided, comprising:

(a) contacting a library of aptamers with the target molecule to form a mixture, and allowing for the formation of an aptamer-target complex, wherein the aptamer-target complex forms when an aptamer has affinity for the target molecule;

(b) partitioning the aptamer-target complex from the remainder of the mixture (or enriching for the aptamer-target complex);

(c) dissociating the aptamer-target complex; and

(d) identifying the one or more aptamers capable of binding to the target molecule;

wherein the library of aptamers comprises a plurality of polynucleotides, wherein each polynucleotide comprises at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine, wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines.

[0021a] In one aspect, there is provided a method for identifying one or more aptamers capable of binding to a target molecule comprising:

(a) contacting a library of aptamers with the target molecule to form a mixture, and allowing for the formation of an aptamer-target complex, wherein the aptamer-target complex forms when an aptamer has affinity for the target molecule;

(b) partitioning the aptamer-target complex from the remainder of the mixture (or enriching for the aptamer-target complex);

(c) dissociating the aptamer-target complex; and

(d) identifying the one or more aptamers capable of binding to the target molecule;

wherein the library of aptamers comprises a plurality of polynucleotides, wherein each polynucleotide comprises at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine, wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines;

wherein the first 5-position modified pyrimidine is a 5-position modified uridine and wherein the second 5-position modified pyrimidine is a 5-position modified cytidine; or

wherein the first 5-position modified pyrimidine is a 5-position modified cytidine and wherein the second 5-position modified pyrimidine is a 5-position modified uridine;

wherein the 5-position modified uridine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety; and wherein the 5-position modified cytidine comprises a moiety at the 5-position selected from a naphthyl moiety, a benzyl moiety, a tyrosyl moiety, and a morpholino moiety.

[0021b] In some embodiments, steps (a), (b) and/or (c) are repeated at least one time, two times, three times, four times, five times, six times, seven times, eight times, nine times or ten times.

[0022] In some embodiments, the one or more aptamers capable of binding to the target molecule are amplified. In some embodiments, the mixture comprises a polyanionic

[0032] In some embodiments, the aptamer inhibits PCSK9 binding to LDL-R. In some embodiments, the aptamer inhibits PCSK9 binding to LDL-R with an IC_{50} of less than 30 nM, less than 20 nM, or less than 15 nM.

[0033] In some embodiments, a method of lowering cholesterol in a subject is provided, comprising administering to a subject in need thereof an aptamer that binds PCSK9. In some embodiments, the aptamer that binds PCSK9 is an aptamer provided herein. In some embodiments, the cholesterol is low-density lipoprotein (LDL) cholesterol (LDL-C). In some embodiments, the subject has heterozygous familial hypercholesterolemia or clinical atherosclerotic cardiovascular disease (CVD).

[0034] The foregoing and other objects, features, and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] Fig. 1. Selection of nucleic acid aptamers modified with DNA libraries containing C5- position modified uridine and cytidine triphosphates. Schematics of selection with two modified bases. Outline of selection method in which 30N randomized chemically synthesized master antisense biotinylated template library was used to enzymatically synthesize various modified and unmodified libraries by primer extension reactions.

[0036] Fig. 2. Binding affinities of 40mer (30N+5+5) aptamers to PCSK9 generated using various modified libraries. Aptamers with affinities $\geq$ 1 nM are highlighted in gray shade and aptamers shown at 320 nM affinity represent no detectable binding at 32 nM top concentration on binding curve. Black line on each of the library indicates median value for the all aptamers in that library.

[0037] Fig. 3. The affinity and percent copy number for single modified, either dU or dC vs two modified dC with dU. Each dot represents one of the aptamers with affinity values shown on Y-axis and their percent copy number on X-axis. Red dots are single modified aptamers and green dots (open and filled) are two modified aptamers. Filled green dots represent some Nap-dC/Tyr-dU and PP-dC/Tyr-dU aptamers.

[0038] Fig. 4A-B. Truncability of single base modified and two base modified aptamers. (**A**) All high affinity 40mer sequences were further truncated to 30mer to their random region length only removing 5 nucleotides from each of the 5' and 3' ends. The percentage of aptamers that retain or have improved binding affinity to PCSK9 is plotted on Y-axis. (**B**) Affinity comparisons and truncability of single base modified and two base modified