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(71) Applicant(s)
Avidity Biosciences, Inc.

(72) Inventor(s)
LEVIN, Arthur A;GEALL, Andrew John;COCHRAN, Michael Caramian;HUANG, Hanhua;DOPPALAPUDI, Venkata Ramana;BURKE, Rob;DARIMONT, Beatrice Diana;SHI, Yunyu;JOHNS, Rachel

(74) Agent / Attorney
FB Rice Pty Ltd, L 23 44 Market St, Sydney, NSW, 2000, AU

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(71) Applicant: **AVIDITY BIOSCIENCES LLC** [US/US]; 10975 North Torrev Pines Road. Suite 150, La Jolla, California 92037 (US).

(72) Inventors: **LEVIN, Arthur A**; 10975 North Torrev Pines Road. Suite 150, La Jolla, California 92037 (US). **GEALL, Andrew John**; 10975 North Torrev Pines Road. Suite 150, La Jolla, California 92037 (US). **COCHRAN, Michael Caramian**; 10975 North Torrev Pines Road. Suite 150, La Jolla, California 92037 (US). **HUANG, Hanhua**; 10975 North Torrev Pines Road. Suite 150, La Jolla, California 92037 (US). **DOPPALAPUDI, Venkata Ramana**; 10975 North Torrev Pines Road. Suite 150, La Jolla, California 92037 (US). **BURKE, Rob**; 10975 North Torrev Pines Road. Suite 150, La Jolla, California 92037 (US). **DARIMONT, Beatrice Diana**; 10975 North Torrey Pines Road, Suite 150, La Jolla, California 92037 (US). **SHI, Yunyu**; 10975 North Torrey Pines Road, Suite 150, La Jolla, California 92037 (US). **JOHNS, Rachel**; 10975 North Torrey Pines Road, Suite 150, La Jolla, California 92037 (US).

(74) Agent: **ZHANG, Adrianna P.**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304 (US).

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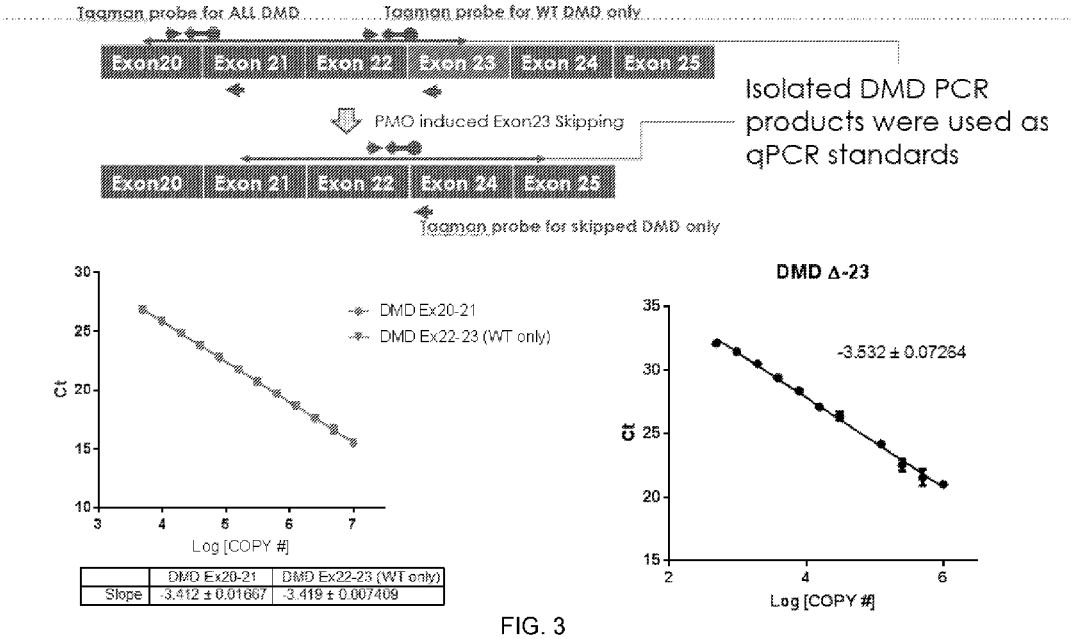

	DMD Ex20-21	DMD Ex22-23 (WT only)
Slope	-3.412 ± 0.01667	-3.419 ± 0.007409

FIG. 3

(57) Abstract: Disclosed herein are molecules and pharmaceutical compositions that induce an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion. Also described herein include methods for treating a disease or disorder that comprises a molecule or a pharmaceutical composition that induces an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

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[Continued on next page]

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NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND METHODS OF INDUCING EXON SKIPPING

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/561,939, filed September 22, 2017, and U.S. Provisional Application No. 62/696,766, filed July 11, 2018, each of which is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0002] Modulation of RNA function is a developing area of therapeutic interest. Drugs that affect mRNA stability like antisense oligonucleotides and short interfering RNAs are one way to modulate RNA function. Another group of oligonucleotides can modulate RNA function by altering the processing of pre-mRNA to include or exclude specific regions of pre-mRNAs from the ultimate gene product: the encoded protein. As such, oligonucleotide therapeutics represent a means of modulating protein expression in disease states and as such have utility as therapeutics.

SUMMARY OF THE DISCLOSURE

[0003] Disclosed herein, in certain embodiments, are molecules and pharmaceutical compositions for modulating RNA processing. In some embodiments, also disclosed herein are molecules and pharmaceutical compositions for the treatment of a muscular dystrophy.

[0004] Disclosed herein, in certain embodiments, are methods of treating a disease or disorder caused by an incorrectly spliced mRNA transcript in a subject in need thereof, the method comprising: administering to the subject a polynucleic acid molecule conjugate; wherein the polynucleic acid molecule conjugate is conjugated to a cell targeting binding moiety; wherein the polynucleotide optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; wherein the polynucleic acid molecule conjugate induces insertion, deletion, duplication, or alteration in the incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion in the incorrectly spliced mRNA transcript to generate a fully processed mRNA transcript; and wherein the fully processed mRNA transcript encodes a functional protein, thereby treating the disease or disorder in the subject. In some embodiments, the disease or disorder is further characterized by one or more mutations in the mRNA. In some embodiments, the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In some embodiments, the disease or disorder is muscular dystrophy. In some embodiments, the disease or disorder is Duchenne muscular dystrophy. In some embodiments, the exon skipping is of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some embodiments, the exon skipping is of exon 23 of the *DMD* gene. In some embodiments, the polynucleic acid molecule conjugate is of Formula (I):

A-X-B

Formula I

wherein,

A is a binding moiety;

B is a polynucleotide; and

X is a bond or first linker.

In some embodiments, the polynucleic acid molecule conjugate is of Formula (II):

A-X-B-Y-C

Formula II

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or first linker; and

Y is a bond or second linker.

In some embodiments, the polynucleic acid molecule conjugate is of Formula (III):

A-X-C-Y-B

Formula III

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or first linker; and

Y is a bond or second linker.

In some embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), or a peptide nucleic acid (PNA). In some embodiments, the at least one 2' modified nucleotide comprises a morpholino. In some embodiments, the at least one inverted basic moiety is at least one terminus. In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleic acid molecule is at least from about 10 to about 30 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least one of: from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100%

modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 90% modification, from about 20% to about 90% modification, from about 30% to about 90% modification, from about 40% to about 90% modification, from about 50% to about 90% modification, from about 60% to about 90% modification, from about 70% to about 90% modification, and from about 80% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 80% modification, from about 20% to about 80% modification, from about 30% to about 80% modification, from about 40% to about 80% modification, from about 50% to about 80% modification, from about 60% to about 80% modification, and from about 70% to about 80% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 70% modification, from about 20% to about 70% modification, from about 30% to about 70% modification, from about 40% to about 70% modification, from about 50% to about 70% modification, and from about 60% to about 70% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 60% modification, from about 20% to about 60% modification, from about 30% to about 60% modification, from about 40% to about 60% modification, and from about 50% to about 60% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 50% modification, from about 20% to about 50% modification, from about 30% to about 50% modification, and from about 40% to about 50% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 40% modification, from about 20% to about 40% modification, and from about 30% to about 40% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 30% modification, and from about 20% to about 30% modification. In some embodiments, the polynucleic acid molecule comprises from about 10% to about 20% modification. In some embodiments, the polynucleic acid molecule comprises from about 15% to about 90%, from about 20% to about 80%, from about 30% to about 70%, or from about 40% to about 60% modifications. In some embodiments, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification. In some embodiments, the polynucleic acid molecule comprises at least about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications. In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modified nucleotides. In some embodiments, the polynucleic acid molecule comprises a single strand. In some embodiments, the polynucleic acid molecule comprises two or more strands. In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second

polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group. In some embodiments, X is a bond. In some embodiments, X is a C_1-C_6 alkyl group. In some embodiments, Y is a C_1-C_6 alkyl group. In some embodiments, X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some embodiments, Y is a homobifuctional linker or a heterobifunctional linker. In some embodiments, the binding moiety is an antibody or binding fragment thereof. In some embodiments, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some embodiments, C is polyethylene glycol. In some embodiments, C has a molecular weight of about 5000 Da. In some embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some embodiments, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group. In some embodiments, methods further comprise D. In some embodiments, D is conjugated to C or to A. In some embodiments, D is conjugated to the molecule conjugate of Formula (II) according to Formula (IV):

$$(A\text{-}X\text{-}B\text{-}Y\text{-}C_c)\text{-}L\text{-}D$$

Formula IV

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or first linker;

Y is a bond or second linker;

L is a bond or third linker;

D is an endosomolytic moiety; and

c is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or an inverted abasic moiety; and D is conjugated anywhere on A, B, or C.

In some embodiments, D is INF7 or melittin. In some embodiments, L is a C_1-C_6 alkyl group. In some embodiments, L is a homobifuctional linker or a heterobifunctional linker. In some embodiments,

methods further comprise at least a second binding moiety A. In some embodiments, the at least second binding moiety A is conjugated to A, to B, or to C.

[0005] Disclosed herein, in some embodiments, are methods of inducing an insertion, deletion, duplication, or alteration in the incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion in the incorrectly spliced mRNA transcript, the method comprising: contacting a target cell with a polynucleic acid molecule conjugate, wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; hybridizing the polynucleic acid molecule conjugate to the incorrectly spliced mRNA transcript within the target cell to induce an insertion, deletion, duplication, or alteration in the incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion, wherein the incorrectly spliced mRNA transcript is capable of encoding a functional form of a protein; and translating the functional form of a protein from a fully processed mRNA transcript of the previous step. In some embodiments, the target cell is a target cell of a subject. In some embodiments, the incorrectly spliced mRNA transcript further induces a disease or disorder. In some embodiments, the disease or disorder is further characterized by one or more mutations in the mRNA. In some embodiments, the disease or disorder comprises a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In some embodiments, the disease or disorder is muscular dystrophy. In some embodiments, the disease or disorder is Duchenne muscular dystrophy. In some embodiments, the exon skipping is of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some embodiments, the exon skipping is of exon 23 of the *DMD* gene. In some embodiments, the polynucleic acid molecule conjugate is of Formula (I):

A-X-B

Formula I

wherein,

A is a binding moiety;

B is a polynucleotide; and

X is a bond or first linker.

In some embodiments, the polynucleic acid molecule conjugate is of Formula (II):

A-X-B-Y-C

Formula II

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or first linker; and

Y is a bond or second linker.

In some embodiments, the polynucleic acid molecule conjugate is of Formula (III):

A-X-C-Y-B

Formula III

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or first linker; and

Y is a bond or second linker.

In some embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA), ethylene nucleic acid (ENA), peptide nucleic acid (PNA). In some embodiments, the at least one 2' modified nucleotide comprises a morpholino. In some embodiments, the at least one inverted basic moiety is at least one terminus. In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the polynucleic acid molecule is at least from about 10 to about 30 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least one of: from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length. In some embodiments, the polynucleic acid molecule is at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 90% modification, from about 20% to about 90% modification, from about 30% to about 90% modification, from about 40% to about 90% modification, from about 50% to about 90% modification, from about 60% to about 90% modification, from about 70% to about 90% modification, and from about 80% to about 100% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 80% modification, from about 20% to about 80% modification, from about 30% to about 80% modification, from about 40% to about 80% modification, from about 50% to about 80% modification, from about 60% to about 80% modification, and from about 70% to about 80% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 70% modification, from about 20% to about 70% modification, from about 30% to about 70% modification, from about 40% to about 70% modification, from about 50% to about 70% modification, and from about 60% to about 70% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 60% modification, from about 20% to

about 60% modification, from about 30% to about 60% modification, from about 40% to about 60% modification, and from about 50% to about 60% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 50% modification, from about 20% to about 50% modification, from about 30% to about 50% modification, and from about 40% to about 50% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 40% modification, from about 20% to about 40% modification, and from about 30% to about 40% modification. In some embodiments, the polynucleic acid molecule comprises at least one of: from about 10% to about 30% modification, and from about 20% to about 30% modification. In some embodiments, the polynucleic acid molecule comprises from about 10% to about 20% modification. In some embodiments, the polynucleic acid molecule comprises from about 15% to about 90%, from about 20% to about 80%, from about 30% to about 70%, or from about 40% to about 60% modifications. In some embodiments, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification. In some embodiments, the polynucleic acid molecule comprises at least about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications. In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modified nucleotides. In some embodiments, the polynucleic acid molecule comprises a single strand. In some embodiments, the polynucleic acid molecule comprises two or more strands. In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X and Y are independently a bond, a degradable linker, a non-degradable linker, a cleavable linker, or a non-polymeric linker group. In some embodiments, X is a bond. In some embodiments, X is a C_1-C_6 alkyl group. In some embodiments, Y is a C_1-C_6 alkyl group. In some embodiments, X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some embodiments, Y is a homobifuctional linker or a heterobifunctional linker. In some embodiments, the binding moiety is an antibody or binding fragment thereof. In some embodiments, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some embodiments, C is polyethylene glycol. In some embodiments, C has a molecular weight of about 5000 Da. In some embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3'

end of B. In some embodiments, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group. In some embodiments, methods further comprise D. In some embodiments, D is conjugated to C or to A. In some embodiments, D is conjugated to the molecule conjugate of Formula (II) according to Formula (IV):

$$(A\text{-}X\text{-}B\text{-}Y\text{-}C_c)\text{-}L\text{-}D$$

Formula IV

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or first linker;

Y is a bond or second linker;

L is a bond or third linker;

D is an endosomolytic moiety; and

c is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or an inverted abasic moiety; and D is conjugated anywhere on A, B, or C.

In some embodiments, D is INF7 or melittin. In some embodiments, L is a C_1-C_6 alkyl group. In some embodiments, L is a homobifuctional linker or a heterobifunctional linker. In some embodiments, methods further comprise at least a second binding moiety A. In some embodiments, the at least second binding moiety A is conjugated to A, to B, or to C. In some embodiments, the method is an *in vivo* method. In some embodiments, the method is an *in vitro* method. In some embodiments, the subject is a human.

[0006] Disclosed herein, in certain embodiments, are pharmaceutical compositions comprising: a molecule obtained by any one of the methods disclosed herein and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is formulated as a nanoparticle formulation. In some embodiments, the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.

[0007] Disclosed herein, in certain embodiments, are compositions comprising a polynucleic acid molecule conjugate, wherein the polynucleic acid molecule conjugate comprises a polynucleotide comprising a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 45-963. Disclosed herein, in certain embodiments, are compositions comprising a polynucleic acid molecule conjugate, wherein the polynucleic acid molecule conjugate comprises a polynucleotide comprising a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 45-963. In certain embodiments, the polynucleic acid molecule conjugate is of Formula (I):

$$A\text{-}X\text{-}B$$

Formula I

wherein,

A is a binding moiety;

B is the polynucleotide; and

X is a bond or first linker.

In certain embodiments, the polynucleic acid molecule conjugate is of Formula (II):

A-X-B-Y-C

Formula II

wherein,

A is a binding moiety;

B is the polynucleotide;

C is a polymer;

X is a bond or first linker; and

Y is a bond or second linker.

In certain embodiments, the polynucleic acid molecule conjugate is of Formula (III):

A-X-C-Y-B

Formula III

wherein,

A is a binding moiety;

B is the polynucleotide;

C is a polymer;

X is a bond or first linker; and

Y is a bond or second linker.

[0008] In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In certain embodiments, the at least one 2' modified nucleotide comprises a morpholino.

[0009] Disclosed herein, in certain embodiments, is a polynucleic acid conjugate comprising a target cell binding moiety binding to at least one polynucleic acid molecule that hybridizes to a target region of a pre-mRNA transcript of *DMD* gene, wherein the at least one polynucleic acid molecule induces splicing out of an exon from a pre-mRNA transcript to generate a mRNA transcript that encodes a functional dystrophin protein. In some embodiments, the functional dystrophin protein is a truncated form of the dystrophin protein. In some embodiments, the target region is at an exon-intron junction, wherein the exon is the exon that is to be spliced out. In some embodiments, the exon is exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55. In some embodiments, the exon-intron junction is located at the 5' of the exon that is to be spliced out. In some embodiments, the target region is an intronic region upstream of

the exon-intron junction. In some embodiments, the target region is about 500, 450, 400, 350, 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides upstream of the exon-intron junction. In some embodiments, the exon-intron junction is located at the 3' of the exon that is to be spliced out. In some embodiments, the target region is an intronic region downstream of the exon-intron junction. In some embodiments, the target region is about 500, 450, 400, 350, 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides downstream of the exon-intron junction. In some embodiments, the target cell binding moiety binds to two or more, three or more, four or more, five or more, six or more, or eight or more polynucleic acid molecules. In some embodiments, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some embodiments, the polynucleic acid molecule comprises about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a sequence selected from SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule further comprises 1, 2, 3, or 4 mismatches. In some embodiments, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1056-1094, 1147-1162, or 1173-1211. In some embodiments, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1173-1211. In some embodiments, the binding moiety comprises an antibody. In some embodiments, the antibody comprises an anti-transferrin antibody. In some embodiments, the binding moiety comprises a plasma protein. In some embodiments, the polynucleic acid conjugate comprises $A-(X^1-B)_n$; Formula (V), wherein, A comprises the binding moiety; B consists of the polynucleic acid molecule; X^1 consists of a bond or first non-polymeric linker; and n is an averaged value selected from 1-12. In some embodiments, the polynucleic acid molecule comprises a passenger strand and a guide strand. In some embodiments, the guide strand comprises at least one modified internucleotide linkage, at least one inverted abasic moiety, at least one 5'-vinylphosphonate modified non-natural nucleotide, or a combination thereof. In some embodiments, the guide strand comprises about 2, 3, 4, 5, 6, 7, 8, or 9 phosphorothioate-modified non-natural nucleotides. In some embodiments, the guide strand comprises 1 phosphorothioate-modified non-natural nucleotide. In some embodiments, the phosphorothioate modified non-natural nucleotide is located at an internucleotide linkage of the polynucleotide. In some embodiments, the at least one 5'-vinylphosphonate modified non-natural nucleotide is located about 1, 2, 3, 4, or 5 bases away from the 5' terminus of the guide strand. In some embodiments, the at least one 5'-vinylphosphonate modified non-natural

nucleotide is further modified at the 2'-position. In some embodiments, the 2'-modification is selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the passenger strand comprises at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorodiamidate morpholino oligomer-modified non-natural nucleotides. In some embodiments, the passenger strand comprises 100% phosphorodiamidate morpholino oligomer-modified non-natural nucleotides. In some embodiments, the passenger strand is shorter in length than the guide strand, thereby generating a 5' overhang, a 3' overhang, or a combination thereof. In some embodiments, the passenger strand is equal in length to the guide strand, thereby generating a blunt end at each terminus of the polynucleic acid molecule. In some embodiments, the polynucleic acid molecule is a phosphorodiamidate morpholino oligomer/RNA hetero-duplex. In some embodiments, the passenger strand comprises at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more peptide nucleic acid-modified non-natural nucleotides. In some embodiments, the passenger strand comprises 100% peptide nucleic acid-modified non-natural nucleotides. In some embodiments, the passenger strand is shorter in length than the guide strand, thereby generating a 5' overhang, a 3' overhang, or a combination thereof. In some embodiments, the passenger strand is equal in length to the guide strand, thereby generating a blunt end at each terminus of the polynucleic acid molecule. In some embodiments, the polynucleic acid molecule is a peptide nucleic acid/RNA hetero-duplex. In some embodiments, the passenger strand is conjugated to A- X^1. In some embodiments, A- X^1 is conjugated to the 5' end of the passenger strand. In some embodiments, A- X^1 is conjugated to the 3' end of the passenger strand. In some embodiments, X^1 is a bond. In some embodiments, X^1 is a C_1-C_6 alkyl group. In some embodiments, X^1 is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some embodiments, the polynucleic acid conjugate further comprises C. In some embodiments, C is polyethylene glycol. In some embodiments, C is directly conjugated to B via X^2. In some embodiments, X^2 consists of a bond or second non-polymeric linker. In some embodiments, X^2 is a bond. In some embodiments, X^2 is a C_1-C_6 alkyl group. In some embodiments, X^2 is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some embodiments, the passenger strand is conjugated to A- X^1 and X^2-C. In some embodiments, A- X^1 is conjugated to the 5' end of the passenger strand and X^2-C is conjugated to the 3' end of the passenger strand. In some embodiments, X^2-C is conjugated to the 5' end of the passenger strand and A- X^1 is conjugated to the 3' end of the passenger strand. In some embodiments, the polynucleic acid conjugate comprises: A-X^1-(B-X^2-C)$_n$; Formula (VI), wherein, A comprises the binding moiety; B consists of the polynucleic acid molecule; C consists of a polymer; X^1 consists a bond or first non-polymeric linker; X^2 consists of a bond or second non-polymeric linker; and n is an averaged value selected from 1-12. In some embodiments, the polynucleic acid conjugate further comprises D. In some embodiments, D is an endosomolytic moiety.

[0010] Disclosed herein, in certain embodiments, is a polynucleic acid molecule comprising at least 23 contiguous bases of a base sequence selected from SEQ ID NOs: 1056-1058 or 1087-1089, wherein the polynucleic acid molecule comprises no more than 50 nucleotides in length.

[0011] Disclosed herein, in certain embodiments, is a polynucleic acid molecule comprising SEQ ID NOs: 1056-1058, wherein the polynucleic acid molecule comprises no more than 50 nucleotides in length.

[0012] Disclosed herein, in certain embodiments, is a polynucleic acid molecule comprising SEQ ID NOs: 1087-1089, wherein the polynucleic acid molecule comprises no more than 50 nucleotides in length.

[0013] Disclosed herein, in certain embodiments, is a pharmaceutical composition, comprising: a polynucleic acid conjugate described herein or a polynucleic acid molecule described herein; and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is formulated for systemic delivery. In some embodiments, the pharmaceutical composition is formulated for parenteral administration.

[0014] Disclosed herein, in certain embodiments, is a method of treating a disease or condition characterized with a defective mRNA in a subject in need thereof, comprising: administering to the subject a polynucleic acid conjugate described herein or a polynucleic acid molecule described herein to induce skipping of an exon that leads to the defective mRNA to generate a processed mRNA encoding a functional protein, thereby treating the disease or condition in the subject. In some embodiments, the disease or condition is a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In some embodiments, the neuromuscular disease is a muscular dystrophy. In some embodiments, the muscular dystrophy is Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some embodiments, the subject is a human.

[0015] Disclosed herein, in certain embodiments, is a method of treating a muscular dystrophy in a subject in need thereof, comprising: administering to the subject a polynucleic acid conjugate described herein or a polynucleic acid molecule described herein, thereby treating the muscular dystrophy in the subject. In some embodiments, the muscular dystrophy is Duchenne muscular dystrophy. In some embodiments, the subject is a human.

[0016] Disclosed herein, in certain embodiments, is a kit comprising a polynucleic acid conjugate described herein or a polynucleic acid molecule described herein.

[0017] Disclosed herein, in certain embodiments, are kits comprising a molecule obtained by any one of the methods disclosed herein.

[0017A] In a further embodiment, the present invention provides a polynucleic acid conjugate comprising an anti-human transferrin receptor antibody or its binding fragment thereof and at least one polynucleic acid molecule, wherein the polynucleic acid molecule hybridizes to a target region in exon 44 or exon 45 or at or around an exon-intron junction of exon 44 or exon 45, wherein the polynucleic

acid molecule comprises at least one phosphorodiamidate morpholino oligonucleotide (PMO) modified non-natural nucleotide and induces splicing out of the exon 44 or the exon 45 from a pre-mRNA transcript to generate an mRNA transcript that encodes a functional dystrophin protein in a human muscle cell.

[0017B] In a further embodiment, the present invention provided a method of treating a disease or condition characterized with a defective DMD mRNA in a subject in need thereof, wherein the method comprises administering to the subject the polynucleic acid conjugate described herein, wherein the polynucleic acid conjugate induces skipping of exon 44 or exon 45 that leads to the defective DMD mRNA to generate a processed DMD mRNA encoding the functional dystrophin protein, thereby treating the disease or condition in the subject.

[0017C] In a further embodiment, the present invention provides a method of inducing exon skipping of exon 44 or 45 in a targeted pre-mRNA transcript of a DMD gene in a human muscle cell, comprising: a) contacting the human muscle cell with a polynucleic acid conjugate described herein; b) hybridizing the polynucleic acid conjugate to exon 44 or exon 45 of the targeted pre-mRNA transcript; and c) translating an mRNA transcript produced from the targeted pre-mRNA transcript processed in step b) in the muscle cell to generate a functional dystrophin protein.

[0017D] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0017E] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

DESCRIPTION OF THE DRAWINGS

[0018] **Fig. 1** depicts a phosphorodiamidate morpholino oligomer (PMO) sequence with end nucleotides expanded.

[0019] **Fig. 2A** depicts a phosphorothioate antisense oligonucleotide (PS ASO) sequence with end nucleotides expanded.

[0020] **Fig. 2B** depicts a fully expanded phosphorothioate antisense oligonucleotide (PS ASO) sequence.

[0021] **Fig. 3** depicts methods used to quantify skipped DMD mRNA in total RNA using Taqman qPCR.

[0022] **Fig. 4** depicts a chromatogram of anti-CD71 mAb-PMO reaction mixture produced with hydrophobic interaction chromatography (HIC) method 2.

[0023] **Fig. 5A** depicts a chromatogram of anti-CD71 mAb produced using size exclusion chromatography (SEC) method 1.

[0024] **Fig. 5B** depicts a chromatogram of anti-CD71 mAb-PMO DAR 1,2 produced using size exclusion chromatography (SEC) method 1.

[0025] **Fig. 5C** depicts a chromatogram of anti-CD71 mAb-PMO DAR >2 produced using size exclusion chromatography (SEC) method 1.

[0026] **Fig. 6A** depicts a chromatogram of anti-CD71 mAb produced using hydrophobic interaction chromatography (HIC) method 2.

[0027] **Fig. 6B** depicts a chromatogram of purified anti-CD71 mAb-PMO DAR 1,2 conjugate produced using hydrophobic interaction chromatography (HIC) method 2.

[0028] **Fig. 6C** depicts a chromatogram of purified anti-CD71 mAb-PMO DAR >2 conjugate produced using hydrophobic interaction chromatography (HIC) method 2.

[0029] **Fig. 7A** depicts a chromatogram of fast protein liquid chromatography (FPLC) purification of anti-CD71 Fab-PMO using hydrophobic interaction chromatography (HIC) method 3.

[0030] **Fig. 7B** depicts a chromatogram of anti-CD71 Fab produced using SEC method 1.

[0031] **Fig. 7C** depicts a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using SEC method 1.

[0032] **Fig. 7D** depicts a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using SEC method 1.

[0033] **Fig. 7E** depicts a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using SEC method 1.

[0034] **Fig. 7F** depicts a chromatogram of anti-CD71 Fab produced using HIC method 4.

[0035] **Fig. 7G** depicts a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using HIC method 4.

[0036] **Fig. 7H** depicts a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using HIC method 4.

[0037] **Fig. 7I** depicts a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using HIC method 4.

[0038] **Fig. 8A** depicts a chromatogram of anti-CD71 mAb-PS ASO reaction mixture produced with SAX method 2.

[0039] **Fig. 8B** depicts a chromatogram of anti-CD71 mAb produced using SEC method 1.

[0040] **Fig. 8C** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SEC method 1.

[0041] **Fig. 8D** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SEC method 1.

[0042] **Fig. 8E** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SEC method 1.

[0043] **Fig. 8F** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SAX method 2.

[0044] **Fig. 8G** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SAX method 2.

[0045] **Fig. 8H** depicts a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SAX method 2.

[0046] **Fig. 9** depicts an agarose gel from nested PCR detecting exon 23 skipping in differentiated C2C12 cells using PMO and anti-CD71 mAb-PMO conjugate.

[0047] **Fig. 10** depicts an agarose gel from nested PCR detecting exon 23 skipping in differentiated C2C12 cells using PMO, anti-CD71 mAb-PMO, and anti-CD71 Fab-PMO conjugates.

[0048] **Fig. 11** depicts an agarose gel from nested PCR detecting exon 23 skipping in differentiated C2C12 cells PMO, ASO, conjugated anti-CD71 mAb-ASO of DAR1 ("ASC-DAR1"), conjugated anti-CD71 mAb-ASO of DAR2 ("ASC-DAR2"), and conjugated anti-CD71 mAb-ASO of DAR3 ("ASC-DAR3").

[0049] **Fig. 12A** depicts an agarose gel from nested PCR detecting exon 23 skipping in gastrocnemius muscle of wild- type mice administered a single intravenous injection of anti-CD71 mAb-PMO conjugate.

[0050] **Fig. 12B** is a graph of quantification of PCR products from gastrocnemius muscle.

[0051] **Fig. 12C** is a graph of quantification of *in vivo* exon skipping using Taqman qPCR from gastrocnemius muscle from wild-type mice.

[0052] **Fig. 13A** depicts an agarose gel from nested PCR detecting exon 23 skipping in heart muscle from wild-type mice after a single intravenous injection.

[0053] **Fig. 13B** is a graph of quantification of PCR products from heart muscle.

[0054] **Fig. 14** depicts sequencing data of DNA fragments from skipped and wild-type PCR products.

[0055] **Fig. 15** illustrates exon skipping activity of exon-skipping PMOs at different lengths targeting exon 45 in the human DMD pre-mRNA in transfected primary human skeletal muscle cells.

[0056] **Fig. 16** illustrates binding of hTfR1.mAb-PMO conjugates to human Transferrin Receptor in vitro.

[0057] **Fig. 17** illustrates exon skipping activity of hTfR1.mAb-PMO conjugates in primary human skeletal muscle cells.

[0058] **Fig. 18** illustrates exon skipping activity of hTfR1.mAb-PMO conjugates in myotubes of primary and immortalized human skeletal muscle cells.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0059] Nucleic acid (e.g., RNAi) therapy is a targeted therapy with high selectivity and specificity. However, in some instances, nucleic acid therapy is also hindered by poor intracellular uptake, insufficient intracellular concentrations in target cells, and low efficacy. To address these issues, various modifications of the nucleic acid composition are explored, such as for example, novel linkers for better stabilizing and/or lower toxicity, optimization of binding moiety for increased target specificity and/or target delivery, and nucleic acid polymer modifications for increased stability and/or reduced off-target effect.

[0060] In some instances, one such area where oligonucleotide is used is for treating muscular dystrophy. Muscular dystrophy encompasses several diseases that affect the muscle. Duchenne muscular dystrophy is a severe form of muscular dystrophy and caused by mutations in the *DMD* gene. In some instances, mutations in the *DMD* gene disrupt the translational reading frame and results in non-functional dystrophin protein.

[0061] Described herein, in certain embodiments, are methods and compositions relating nucleic acid therapy to induce an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion, which is used to restore the translational reading frame. In some embodiments, also described herein include methods and compositions for treating a disease or disorder characterized by an incorrectly processed mRNA transcript, in which after removal of an exon, the mRNA is capable of encoding a functional protein, thereby treating the disease or disorder. In additional embodiments, described herein include pharmaceutical compositions and kits for treating the same.

RNA Processing

[0062] RNA has a central role in regulation of gene expression and cell physiology. Proper processing of RNA is important for translational of functional protein. Alterations in RNA processing such as a result of incorrect splicing of RNA can result in disease. For example, mutations in a splice site causes exposure of a premature stop codon, a loss of an exon, or inclusion of an intron. In some instances, alterations in RNA processing results in an insertion, deletion, or duplication. In some instances, alterations in RNA processing results in an insertion, deletion, or duplication of an exon. Alterations in RNA processing, in some cases, results in an insertion, deletion, or duplication of an intron.

Exon Skipping

[0063] Exon skipping is a form of RNA splicing. In some cases, exon skipping occurs when an exon is skipped over or is spliced out of the processed mRNA. As a result of exon skipping, the processed mRNA does not contain the skipped exon. In some instances, exon skipping results in expression of an altered product.

[0064] In some instances, antisense oligonucleotides (AONs) are used to induce exon skipping. In some instances, AONs are short nucleic acid sequences that bind to specific mRNA or pre-mRNA sequences. For example, AONs bind splice sites or exonic enhancers. In some instances, binding of AONs to specific mRNA or pre-mRNA sequences generates double-stranded regions. In some instances, formation of double-stranded regions occurs at sites where the spliceosome or proteins associated with the spliceosome would normally bind and causes exons to be skipped. In some instances, skipping of exons results in restoration of the transcript reading frame and allows for production of a partially functional protein.

Exon Inclusion

[0065] In some instances, a mutation in RNA results in exon skipping. In some cases, a mutation is at least one of at the splice site, near the splice site, and at a distance from the splice site. In some instances, the mutations result in at least one of inactivating or weakening the splice site, disrupting exon splice enhancer or intron splice enhancer, and creating an exon splice silencer or intron splice enhancer. Mutations in some instances alter RNA secondary structure. In some cases, a mutation alters a RNA secondary structure result in disrupting the accessibility of signals important for exon recognition.

[0066] In some instances, use of AONs results in inclusion of the skipped exon. In some instances, the AONs bind to at least one of a splice site, a site near a splice site, and a site distant to a splice site. In some cases, AONs bind at site in the RNA to prevent disruption of an exon splice enhancer or intron splice enhancer. In some instances, AONs bind at site in the RNA to prevent creation of an exon splice silencer or intron splice silencer.

Indications

[0067] In some embodiments, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of a disease or disorder characterized with a defective mRNA. In some embodiments, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of disease or disorder by inducing an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

[0068] A large percentage of human protein-coding genes are alternatively spliced. In some instances, a mutation results in improperly spliced or partially spliced mRNA. For example, a mutation is in at least one of a splice site in a protein coding gene, a silencer or enhancer sequence, exonic sequences, or intronic sequences. In some instances, a mutation results in gene dysfunction. In some instances, a mutation results in a disease or disorder.

[0069] In some instances, a disease or disorder resulting from improperly spliced or partially spliced mRNA includes, but not limited to, a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease.

[0070] In some instances, genetic diseases or disorders include an autosomal dominant disorder, an autosomal recessive disorder, X-linked dominant disorder, X-linked recessive disorder, Y-linked disorder, mitochondrial disease, or multifactorial or polygenic disorder.

[0071] In some instances, cardiovascular disease such as hypercholesterolemia results from improperly spliced or partially spliced mRNA. In hypercholesterolemia, it has been shown that a single nucleotide polymorphism in exon 12 of the low density lipoprotein receptor (LDLR) promotes exon skipping.

[0072] In some instances, improperly spliced or partially spliced mRNA results in cancer. For example, improperly spliced or partially spliced mRNA affects cellular processes involved in cancer including, but not limited to, proliferation, motility, and drug response. In some instances is a solid cancer or a hematologic cancer. In some instances, the cancer is bladder cancer, lung cancer, brain cancer, melanoma, breast cancer, Non-Hodgkin lymphoma, cervical cancer, ovarian cancer, colorectal cancer, pancreatic cancer, esophageal cancer, prostate cancer, kidney cancer, skin cancer, leukemia, thyroid cancer, liver cancer, or uterine cancer.

[0073] Improperly spliced or partially spliced mRNA in some instances causes a neuromuscular disease or disorder. Exemplary neuromuscular diseases include muscular dystrophy such as Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some instances, muscular dystrophy is genetic. In some instances, muscular dystrophy is caused by a spontaneous mutation. Becker muscular dystrophy and Duchenne muscular dystrophy have been shown to involve mutations in the *DMD* gene, which encodes the protein dystrophin. Facioscapulohumeral muscular dystrophy has been shown to involve mutations in double homeobox, 4 (*DUX4*) gene.

[0074] In some instances, improperly spliced or partially spliced mRNA causes Duchenne muscular dystrophy. Duchenne muscular dystrophy results in severe muscle weakness and is caused by mutations in the *DMD* gene that abolishes the production of functional dystrophin. In some instances, Duchenne muscular dystrophy is a result of a mutation in an exon in the *DMD* gene. In some instances, Duchenne muscular dystrophy is a result of a mutation in at least one of exon 1, 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78 and 79 in the *DMD* gene. In some instances, Duchenne muscular dystrophy is a result of a mutation in at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 in the *DMD* gene. In some instances, Duchenne muscular dystrophy is a result of a mutation in at least one of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, and 55 in the *DMD* gene. In some instances, multiple exons are mutated. For example, mutation of exons 48-50 is common in Duchenne muscular dystrophy

patients. In some instances, Duchenne muscular dystrophy is a result of mutation of exon 51. In some instances, Duchenne muscular dystrophy is a result of mutation of exon 23. In some instances, a mutation involves a deletion of one or multiple exons. In some instances, a mutation involves a duplication of one or multiple exons. In some instances, a mutation involves a point mutation in an exon. For example, it has been shown that some patients have a nonsense point mutation in exon 51 of the *DMD* gene.

[0075] In some instances, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of muscular dystrophy. In some instances, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some instances, a polynucleic acid molecule or a pharmaceutical composition described herein is used for the treatment of Duchenne muscular dystrophy.

Polynucleic Acid Molecule

[0076] In some embodiments, a polynucleic acid molecule described herein that induces an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion. In some instances, the polynucleic acid molecule restores the translational reading frame. In some instances, the polynucleic acid molecule results in a functional and truncated protein.

[0077] In some instances, a polynucleic acid molecule targets an mRNA sequence. In some instances, the polynucleic acid molecule targets a splice site. In some instances, the polynucleic acid molecule targets a *cis*-regulatory element. In some instances, the polynucleic molecule targets a *trans*-regulatory element. In some instances, the polynucleic acid molecule targets exonic splice enhancers or intronic splice enhancers. In some instances, the polynucleic acid molecule targets exonic splice silencers or intronic splice silencers.

[0078] In some instances, a polynucleic acid molecule targets a sequence found in introns or exons. For example, the polynucleic acid molecule targets a sequence found in an exon that mediates splicing of said exon. In some instances, the polynucleic acid molecule targets an exon recognition sequence. In some instances, the polynucleic acid molecule targets a sequence upstream of an exon. In some instances, the polynucleic acid molecule targets a sequence downstream of an exon.

[0079] As described above, a polynucleic acid molecule targets an incorrectly processed mRNA transcript which results in a disease or disorder not limited to a neuromuscular disease, a genetic disease, cancer, a hereditary disease, or a cardiovascular disease. In some cases, a polynucleic acid molecule targets an incorrectly processed mRNA transcript which results in a neuromuscular disease or disorder. In some cases, a neuromuscular disease or disorder is Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some cases, a polynucleic acid molecule targets an incorrectly processed mRNA transcript which results in Duchenne muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, or myotonic dystrophy. In some cases, a polynucleic

acid molecule targets an incorrectly processed mRNA transcript which results in Duchenne muscular dystrophy.

[0080] In some instances, a polynucleic acid molecule targets an exon that is mutated in the *DMD* gene that causes Duchenne muscular dystrophy. Exemplary exons that are mutated in the *DMD* gene that causes Duchenne muscular dystrophy include, but not limited to, exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78. In some instances, the polynucleic acid molecule targets a sequence adjacent to a mutated exon. For example, if there is a deletion of exon 50, the polynucleic acid molecule targets a sequence in exon 51 so that exon 51 is skipped. In another instance, if there is a mutation in exon 23, the polynucleic acid molecule targets a sequence in exon 22 so that exon 23 is skipped.

[0081] In some instances, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, or 78 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 8 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 23 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 35 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 43 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 44 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 45 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 48 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 49 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 50 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 51 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 52 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 53 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a region that is at the exon-intron junction of exon 55 of the *DMD* gene.

[0082] In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon junction or the 3' exon-intron junction of at least one of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon junction or the 3' exon-intron junction of at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon junction or the 3' exon-intron junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene.

[0083] In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of at least one of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78 of the *DMD* gene (e.g., the 5' intron-exon junction of exon 3 is the junction intron 2-exon 3). In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene (e.g., the 5' intron-exon junction of exon 3 is the junction intron 2-exon 3). In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 8 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 23 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 35 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 43 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 44 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 45 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 50 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 51 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 52 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 53 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 5' intron-exon junction of exon 55 of the *DMD* gene.

[0084] In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of at least one of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78 of the *DMD* gene (e.g., the 3' exon-intron junction of exon 3 is the junction exon 3-intron 3). In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene (e.g., the 3' exon-intron junction of exon 3 is the junction exon 3-intron 3). In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 8 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 23 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 35 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 43 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 44 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 45 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 50 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 51 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 52 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 53 of the *DMD* gene. In some cases, the polynucleic acid molecule hybridizes to a target region that is at the 3' exon-intron junction of exon 55 of the *DMD* gene.

[0085] In some instances, a polynucleic acid molecule described herein targets a splice site of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 23 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 35 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 43 of the

DMD gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 44 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 45 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 48 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a splice site of exon 51 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 52 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 53 of the *DMD* gene. In some cases, a polynucleic acid molecule described herein targets a splice site of exon 55 of the *DMD* gene. As used herein, a splice site includes a canonical splice site, a cryptic splice site or an alternative splice site that is capable of inducing an insertion, deletion, duplication, or alteration in an incorrectly spliced mRNA transcript to induce exon skipping or exon inclusion.

[0086] In some embodiments, a polynucleic acid molecule described herein target a partially spliced mRNA sequence comprising additional exons involved in Duchenne muscular dystrophy such as exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63.

[0087] In some instances, the polynucleic acid molecule hybridizes to a target region that is proximal to the exon-intron junction. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, or 78 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 23 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 35 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400

nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 43 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 44 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 45 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 48 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 51 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 52 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 53 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nt, 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt upstream (or from the 5') of exon 55 of the *DMD* gene.

[0088] In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to at least one of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene.

[0089] In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, or 78 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 23 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 35 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 43 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 44 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 45 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 48 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 51 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000

nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 52 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 53 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets a region at least 1000 nucleotides (nt), 500 nt, 400 nt, 300 nt, 200 nt, 100 nt, 80 nt, 60 nt, 50 nt, 40 nt, 30 nt, 20 nt, 10 nt, or 5 nt downstream (or from the 3') of exon 55 of the *DMD* gene.

[0090] In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to at least one of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to at least one of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene.

[0091] In some instances, a polynucleic acid molecule described herein targets an internal region within exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, or 78 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 8 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 23 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 35 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 43 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 44 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 45 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 48 of the *DMD* gene. In some instances, a polynucleic acid

molecule described herein targets an internal region within exon 49 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 50 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 51 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 52 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 53 of the *DMD* gene. In some instances, a polynucleic acid molecule described herein targets an internal region within exon 55 of the *DMD* gene.

[0092] In some instances, the polynucleic acid molecule hybridizes to a target region that is within at least one of exon 2, 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, and 78 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is within at least one of exon 3, 4, 5, 6, 7, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is within at least one of exon 8, 23, 35, 43, 44, 45, 50, 51, 52, 53, or 55 of the *DMD* gene.

[0093] In some embodiments, a polynucleic acid molecule described herein targets a partially spliced mRNA sequence comprising exon 44 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to exon 44. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to exon 44. In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to exon 44. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to exon 44.

[0094] In some instances, the polynucleic acid molecule hybridizes to a target region that is within exon 44 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon 44 junction or the 3' exon 44-intron junction.

[0095] In some embodiments, a polynucleic acid molecule described herein targets a partially spliced mRNA sequence comprising exon 45 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to exon 45. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to exon 45. In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to exon 45. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to exon 45.

[0096] In some instances, the polynucleic acid molecule hybridizes to a target region that is within exon 45 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon 45 junction or the 3' exon 45-intron junction.

[0097] In some embodiments, a polynucleic acid molecule described herein targets a partially spliced mRNA sequence comprising exon 51 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to exon 51. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to exon 51.

[0098] In some instances, the polynucleic acid molecule hybridizes to a target region that is within exon 51 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon 51 junction or the 3' exon 51-intron junction.

[0099] In some embodiments, a polynucleic acid molecule described herein targets a partially spliced mRNA sequence comprising exon 53 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is upstream (or 5') to exon 53. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp upstream (or 5') to exon 53. In some instances, the polynucleic acid molecule hybridizes to a target region that is downstream (or 3') to exon 53. In some instances, the polynucleic acid molecule hybridizes to a target region that is about 5, 10, 15, 20, 50, 100, 200, 300, 400 or 500 bp downstream (or 3') to exon 53.

[0100] In some instances, the polynucleic acid molecule hybridizes to a target region that is within exon 53 of the *DMD* gene. In some instances, the polynucleic acid molecule hybridizes to a target region that is at either the 5' intron-exon 53 junction or the 3' exon 53-intron junction.

[0101] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having

at least 97% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to a target sequence of interest. In some embodiments, the polynucleic acid molecule consists of a target sequence of interest.

[0102] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence of interest.

[0103] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 964-1285. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 964-1285.

[0104] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a

sequence having at least 50% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1056-1094. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1056-1094.

[0105] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1147-1162. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1147-1162.

[0106] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1173-1211. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1173-1211.

[0107] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the

polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1056-1076. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1056-1076.

[0108] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1077-1094. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1077-1094.

[0109] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence

identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1056-1058. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1056-1058.

[0110] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1087-1089. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1087-1089.

[0111] In some embodiments, the polynucleic acid molecule at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 964-1285. In some instances, the polynucleic acid molecule at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1056-1094, 1147-1162, or 1173-1211. In some instances, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1056-1076. In some instances, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1077-1094. In some instances, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1147-1162. In some instances, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1173-1211. In some instances, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1056-1058. In some

instances, the polynucleic acid molecule comprises at least 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or more contiguous bases of a base sequence selected from SEQ ID NOs: 1087-1089. In some cases, the polynucleic acid molecule further comprises 1, 2, 3, or 4 mismatches.

[0112] In some embodiments, the polynucleic acid molecule comprises a guide strand and a passenger strand. In some instances, the guide strand comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 964-1285. In some cases, the guide strand comprises a sequence selected from SEQ ID NOs: 964-1285.

[0113] In some embodiments, the polynucleic acid molecule described herein comprises RNA or DNA. In some cases, the polynucleic acid molecule comprises RNA. In some instances, RNA comprises short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), double-stranded RNA (dsRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), or heterogeneous nuclear RNA (hnRNA). In some instances, RNA comprises shRNA. In some instances, RNA comprises miRNA. In some instances, RNA comprises dsRNA. In some instances, RNA comprises tRNA. In some instances, RNA comprises rRNA. In some instances, RNA comprises hnRNA. In some instances, the RNA comprises siRNA. In some instances, the polynucleic acid molecule comprises siRNA. In some instances, the polynucleic acid molecule is an antisense oligonucleotide (ASO).

[0114] In some embodiments, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 30, from about 15 to about 30, from about 18 to about 30, from about 18 to about 25, form about 18 to about 24, from about 19 to about 23, from about 19 to about 30, from about 19 to about 25, form about 19 to about 24, from about 19 to about 23, from about 20 to about 30, from about 20 to about 25, from about 20 to about 24, from about 20 to about 23, or from about 20 to about 22 nucleotides in length.

[0115] In some embodiments, the polynucleic acid molecule is about 50 nucleotides in length. In some instances, the polynucleic acid molecule is about 45 nucleotides in length. In some instances, the polynucleic acid molecule is about 40 nucleotides in length. In some instances, the polynucleic acid molecule is about 35 nucleotides in length. In some instances, the polynucleic acid molecule is about 30 nucleotides in length. In some instances, the polynucleic acid molecule is about 25 nucleotides in length. In some instances, the polynucleic acid molecule is about 20 nucleotides in length. In some instances, the polynucleic acid molecule is about 19 nucleotides in length. In some instances, the polynucleic acid molecule is about 18 nucleotides in length. In some instances, the polynucleic acid molecule is about 17 nucleotides in length. In some instances, the polynucleic acid molecule is about 16 nucleotides in length. In some instances, the polynucleic acid molecule is about 15 nucleotides in length. In some instances, the polynucleic acid molecule is about 14 nucleotides in length. In some instances, the polynucleic acid molecule is about 13 nucleotides in length. In some instances, the polynucleic acid molecule is about 12 nucleotides in length. In some instances, the polynucleic acid molecule is about 11 nucleotides in length. In some instances, the polynucleic acid molecule is about 10 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 50 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 45 nucleotides in length. In some instances,

the polynucleic acid molecule is between about 10 and about 40 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 35 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 25 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 20 nucleotides in length. In some instances, the polynucleic acid molecule is between about 15 and about 25 nucleotides in length. In some instances, the polynucleic acid molecule is between about 15 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 12 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 19 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 20 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 19 and about 25 nucleotides in length. In some instances, the polynucleic acid molecule is between about 20 and about 25 nucleotides in length.

[0116] In some embodiments, the polynucleic acid molecule is at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, or 50 nucleotides in length. In some instances, the polynucleic acid molecule is at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In some instances, the polynucleic acid molecule is at least 15 nucleotides in length. In some instances, the polynucleic acid molecule is at least 18 nucleotides in length. In some instances, the polynucleic acid molecule is at least 19 nucleotides in length. In some instances, the polynucleic acid molecule is at least 20 nucleotides in length. In some instances, the polynucleic acid molecule is at least 21 nucleotides in length. In some instances, the polynucleic acid molecule is at least 22 nucleotides in length. In some instances, the polynucleic acid molecule is at least 23 nucleotides in length. In some instances, the polynucleic acid molecule is at least 24 nucleotides in length. In some instances, the polynucleic acid molecule is at least 25 nucleotides in length. In some instances, the polynucleic acid molecule is at least 30 nucleotides in length.

[0117] In some embodiments, the polynucleic acid molecule is about 50 nucleotides in length. In some instances, the polynucleic acid molecule is about 45 nucleotides in length. In some instances, the polynucleic acid molecule is about 40 nucleotides in length. In some instances, the polynucleic acid molecule is about 35 nucleotides in length. In some instances, the polynucleic acid molecule is about 30 nucleotides in length. In some instances, the polynucleic acid molecule is about 29 nucleotides in length. In some instances, the polynucleic acid molecule is about 28 nucleotides in length. In some instances, the polynucleic acid molecule is about 27 nucleotides in length. In some instances, the polynucleic acid molecule is about 26 nucleotides in length. In some instances, the polynucleic acid molecule is about 25 nucleotides in length. In some instances, the polynucleic acid molecule is about 24 nucleotides in length. In some instances, the polynucleic acid molecule is about 23 nucleotides in length. In some instances, the polynucleic acid molecule is about 22 nucleotides in length. In some instances, the polynucleic acid molecule is about 21 nucleotides in length. In some instances, the polynucleic acid molecule is about 20 nucleotides in length. In some instances, the polynucleic acid molecule is about 19 nucleotides in length. In some instances, the polynucleic acid molecule is about 18 nucleotides in length. In some instances,

the polynucleic acid molecule is about 17 nucleotides in length. In some instances, the polynucleic acid molecule is about 16 nucleotides in length. In some instances, the polynucleic acid molecule is about 15 nucleotides in length. In some instances, the polynucleic acid molecule is about 14 nucleotides in length. In some instances, the polynucleic acid molecule is about 13 nucleotides in length. In some instances, the polynucleic acid molecule is about 12 nucleotides in length. In some instances, the polynucleic acid molecule is about 11 nucleotides in length. In some instances, the polynucleic acid molecule is about 10 nucleotides in length.

[0118] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide. In some instances, the polynucleic acid molecule comprises a second polynucleotide. In some instances, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide is a sense strand or passenger strand. In some instances, the second polynucleotide is an antisense strand or guide strand.

[0119] In some embodiments, the polynucleic acid molecule is a first polynucleotide. In some embodiments, the first polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 30, from about 18 to about 25, form about 18 to about 24, from about 19 to about 23, from about 19 to about 30, from about 19 to about 25, form about 19 to about 24, from about 19 to about 23, from about 20 to about 30, from about 20 to about 25, from about 20 to about 24, from about 20 to about 23, or from about 20 to about 22 nucleotides in length.

[0120] In some instances, the first polynucleotide is about 50 nucleotides in length. In some instances, the first polynucleotide is about 45 nucleotides in length. In some instances, the first polynucleotide is about 40 nucleotides in length. In some instances, the first polynucleotide is about 35 nucleotides in length. In some instances, the first polynucleotide is about 30 nucleotides in length. In some instances, the first polynucleotide is about 25 nucleotides in length. In some instances, the first polynucleotide is about 20 nucleotides in length. In some instances, the first polynucleotide is about 19 nucleotides in length. In some instances, the first polynucleotide is about 18 nucleotides in length. In some instances, the first polynucleotide is about 17 nucleotides in length. In some instances, the first polynucleotide is about 16 nucleotides in length. In some instances, the first polynucleotide is about 15 nucleotides in length. In some instances, the first polynucleotide is about 14 nucleotides in length. In some instances, the first polynucleotide is about 13 nucleotides in length. In some instances, the first polynucleotide is about 12 nucleotides in length. In some instances, the first polynucleotide is about 11 nucleotides in length. In some instances, the first polynucleotide is about 10 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 50 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 45 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 40 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 35 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 30 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 25 nucleotides in length. In some instances, the first

polynucleotide is between about 10 and about 20 nucleotides in length. In some instances, the first polynucleotide is between about 15 and about 25 nucleotides in length. In some instances, the first polynucleotide is between about 15 and about 30 nucleotides in length. In some instances, the first polynucleotide is between about 12 and about 30 nucleotides in length.

[0121] In some embodiments, the polynucleic acid molecule is a second polynucleotide. In some embodiments, the second polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 30, from about 18 to about 25, form about 18 to about 24, from about 19 to about 23, from about 19 to about 30, from about 19 to about 25, form about 19 to about 24, from about 19 to about 23, from about 20 to about 30, from about 20 to about 25, from about 20 to about 24, from about 20 to about 23, or from about 20 to about 22 nucleotides in length.

[0122] In some instances, the second polynucleotide is about 50 nucleotides in length. In some instances, the second polynucleotide is about 45 nucleotides in length. In some instances, the second polynucleotide is about 40 nucleotides in length. In some instances, the second polynucleotide is about 35 nucleotides in length. In some instances, the second polynucleotide is about 30 nucleotides in length. In some instances, the second polynucleotide is about 25 nucleotides in length. In some instances, the second polynucleotide is about 20 nucleotides in length. In some instances, the second polynucleotide is about 19 nucleotides in length. In some instances, the second polynucleotide is about 18 nucleotides in length. In some instances, the second polynucleotide is about 17 nucleotides in length. In some instances, the second polynucleotide is about 16 nucleotides in length. In some instances, the second polynucleotide is about 15 nucleotides in length. In some instances, the second polynucleotide is about 14 nucleotides in length. In some instances, the second polynucleotide is about 13 nucleotides in length. In some instances, the second polynucleotide is about 12 nucleotides in length. In some instances, the second polynucleotide is about 11 nucleotides in length. In some instances, the second polynucleotide is about 10 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 50 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 45 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 40 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 35 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 30 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 25 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 20 nucleotides in length. In some instances, the second polynucleotide is between about 15 and about 25 nucleotides in length. In some instances, the second polynucleotide is between about 15 and about 30 nucleotides in length. In some instances, the second polynucleotide is between about 12 and about 30 nucleotides in length.

[0123] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the polynucleic acid molecule further comprises a blunt terminus, an overhang, or a combination thereof. In some instances, the blunt terminus is a 5' blunt

terminus, a 3' blunt terminus, or both. In some cases, the overhang is a 5' overhang, 3' overhang, or both. In some cases, the overhang comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, 4, 5, or 6 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, or 4 non-base pairing nucleotides. In some cases, the overhang comprises 1 non-base pairing nucleotide. In some cases, the overhang comprises 2 non-base pairing nucleotides. In some cases, the overhang comprises 3 non-base pairing nucleotides. In some cases, the overhang comprises 4 non-base pairing nucleotides.

[0124] In some embodiments, the sequence of the polynucleic acid molecule is at least 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 50% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 60% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 70% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 80% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 90% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 95% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 99% complementary to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule is 100% complementary to a target sequence described herein.

[0125] In some embodiments, the sequence of the polynucleic acid molecule has 5 or less mismatches to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule has 4 or less mismatches to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule has 3 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 2 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 1 or less mismatches to a target sequence described herein.

[0126] In some embodiments, the specificity of the polynucleic acid molecule that hybridizes to a target sequence described herein is a 95%, 98%, 99%, 99.5% or 100% sequence complementarity of the polynucleic acid molecule to a target sequence. In some instances, the hybridization is a high stringent hybridization condition.

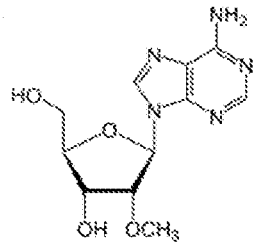
[0127] In some embodiments, the polynucleic acid molecule has reduced off-target effect. In some instances, "off-target" or "off-target effects" refer to any instance in which a polynucleic acid polymer directed against a given target causes an unintended effect by interacting either directly or indirectly with another mRNA sequence, a DNA sequence or a cellular protein or other moiety. In some instances, an "off-target effect" occurs when there is a simultaneous degradation of other transcripts due to partial

homology or complementarity between that other transcript and the sense and/or antisense strand of the polynucleic acid molecule.

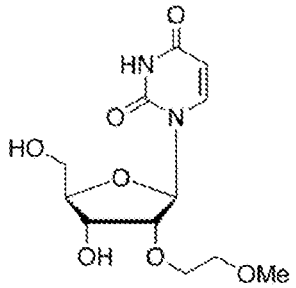
[0128] In some embodiments, the polynucleic acid molecule comprises natural or synthetic or artificial nucleotide analogues or bases. In some cases, the polynucleic acid molecule comprises combinations of DNA, RNA and/or nucleotide analogues. In some instances, the synthetic or artificial nucleotide analogues or bases comprise modifications at one or more of ribose moiety, phosphate moiety, nucleoside moiety, or a combination thereof.

[0129] In some embodiments, nucleotide analogues or artificial nucleotide base comprise a nucleic acid with a modification at a 2' hydroxyl group of the ribose moiety. In some instances, the modification includes an H, OR, R, halo, SH, SR, NH2, NHR, NR2, or CN, wherein R is an alkyl moiety. Exemplary alkyl moiety includes, but is not limited to, halogens, sulfurs, thiols, thioethers, thioesters, amines (primary, secondary, or tertiary), amides, ethers, esters, alcohols and oxygen. In some instances, the alkyl moiety further comprises a modification. In some instances, the modification comprises an azo group, a keto group, an aldehyde group, a carboxyl group, a nitro group, a nitroso, group, a nitrile group, a heterocycle (e.g., imidazole, hydrazino or hydroxylamino) group, an isocyanate or cyanate group, or a sulfur containing group (e.g., sulfoxide, sulfone, sulfide, or disulfide). In some instances, the alkyl moiety further comprises a hetero substitution. In some instances, the carbon of the heterocyclic group is substituted by a nitrogen, oxygen or sulfur. In some instances, the heterocyclic substitution includes but is not limited to, morpholino, imidazole, and pyrrolidino.

[0130] In some instances, the modification at the 2' hydroxyl group is a 2'-O-methyl modification or a 2'-O-methoxyethyl (2'-O-MOE) modification. In some cases, the 2'-O-methyl modification adds a methyl group to the 2' hydroxyl group of the ribose moiety whereas the 2'O-methoxyethyl modification adds a methoxyethyl group to the 2' hydroxyl group of the ribose moiety. Exemplary chemical structures of a 2'-O-methyl modification of an adenosine molecule and 2'O-methoxyethyl modification of an uridine are illustrated below.

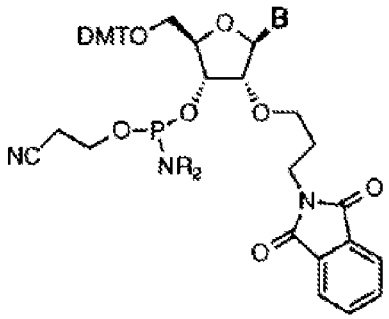


2'-O-methyl-adenosine



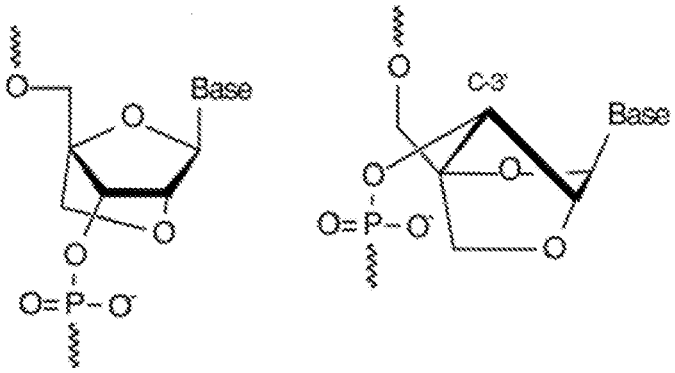
2'-O-methoxyethyl uridine

[0131] In some instances, the modification at the 2' hydroxyl group is a 2'-O-aminopropyl modification in which an extended amine group comprising a propyl linker binds the amine group to the 2' oxygen. In some instances, this modification neutralizes the phosphate derived overall negative charge of the oligonucleotide molecule by introducing one positive charge from the amine group per sugar and thereby improves cellular uptake properties due to its zwitterionic properties. An exemplary chemical structure of a 2'-O-aminopropyl nucleoside phosphoramidite is illustrated below.



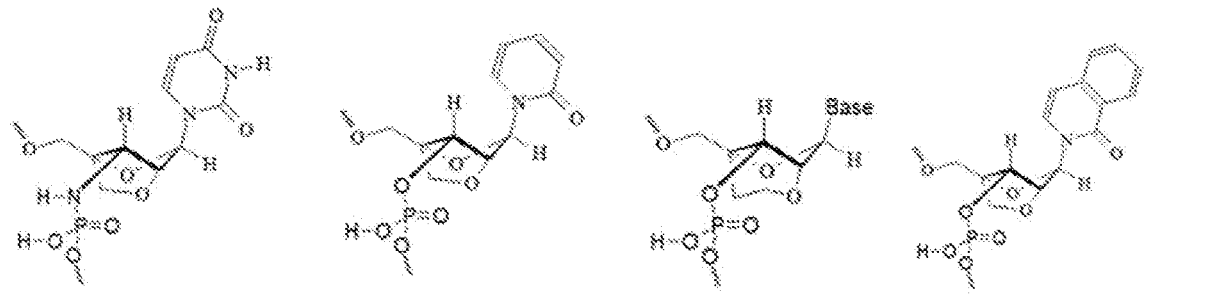
2'-O-aminopropyl nucleoside phosphoramidite

[0132] In some instances, the modification at the 2' hydroxyl group is a locked or bridged ribose modification (e.g., locked nucleic acid or LNA) in which the oxygen molecule bound at the 2' carbon is linked to the 4' carbon by a methylene group, thus forming a 2′-*C*,4′-*C*-oxy-methylene-linked bicyclic ribonucleotide monomer. Exemplary representations of the chemical structure of LNA are illustrated below. The representation shown to the left highlights the chemical connectivities of an LNA monomer. The representation shown to the right highlights the locked 3′-endo (^{3}E) conformation of the furanose ring of an LNA monomer.



LNA (Locked Nucleic Acids)

[0133] In some instances, the modification at the 2' hydroxyl group comprises ethylene nucleic acids (ENA) such as for example 2'-4'-ethylene-bridged nucleic acid, which locks the sugar conformation into a C_3'-endo sugar puckering conformation. ENA are part of the bridged nucleic acids class of modified nucleic acids that also comprises LNA. Exemplary chemical structures of the ENA and bridged nucleic acids are illustrated below.

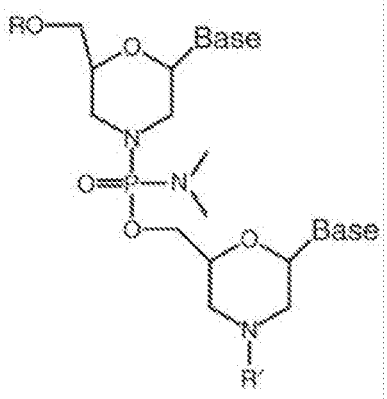


3'-amino-2',4'-BNA 2',4'-BNA-2-pyridone 2',4'-ENA 2',4'-BNA-1-isoquinolone

[0134] In some embodiments, additional modifications at the 2' hydroxyl group include 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA).

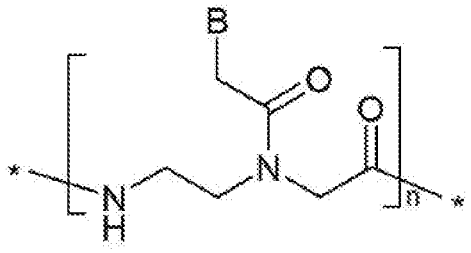
[0135] In some embodiments, nucleotide analogues comprise modified bases such as, but not limited to, 5-propynyluridine, 5-propynylcytidine, 6- methyladenine, 6-methylguanine, N, N, -dimethyladenine, 2-propyladenine, 2propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5- (2- amino) propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1- methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2- methylguanosine, 7-methylguanosine, 2, 2-dimethylguanosine, 5-methylaminoethyluridine, 5-methyloxyuridine, deazanucleotides such as 7-deaza- adenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O-and N-alkylated purines and pyrimidines such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4, 6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyi nucleotides, and alkylcarbonylalkylated nucleotides. Modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties, in some cases are or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles. The term nucleotide also includes what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine.

[0136] In some embodiments, nucleotide analogues further comprise morpholinos, peptide nucleic acids (PNAs), methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, 1', 5'- anhydrohexitol nucleic acids (HNAs), or a combination thereof. Morpholino or phosphorodiamidate morpholino oligo (PMO) comprises synthetic molecules whose structure mimics natural nucleic acid structure by deviates from the normal sugar and phosphate structures. In some instances, the five member ribose ring is substituted with a six member morpholino ring containing four carbons, one nitrogen and one oxygen. In some cases, the ribose monomers are linked by a phosphordiamidate group instead of a phosphate group. In such cases, the backbone alterations remove all positive and negative charges making morpholinos neutral molecules capable of crossing cellular membranes without the aid of cellular delivery agents such as those used by charged oligonucleotides.



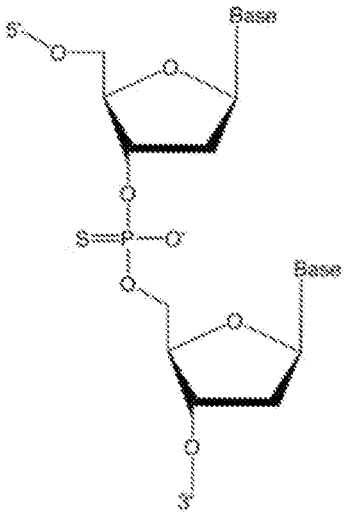
Morpholino

[0137] In some embodiments, peptide nucleic acid (PNA) does not contain sugar ring or phosphate linkage and the bases are attached and appropriately spaced by oligoglycine-like molecules, therefore, eliminating a backbone charge.

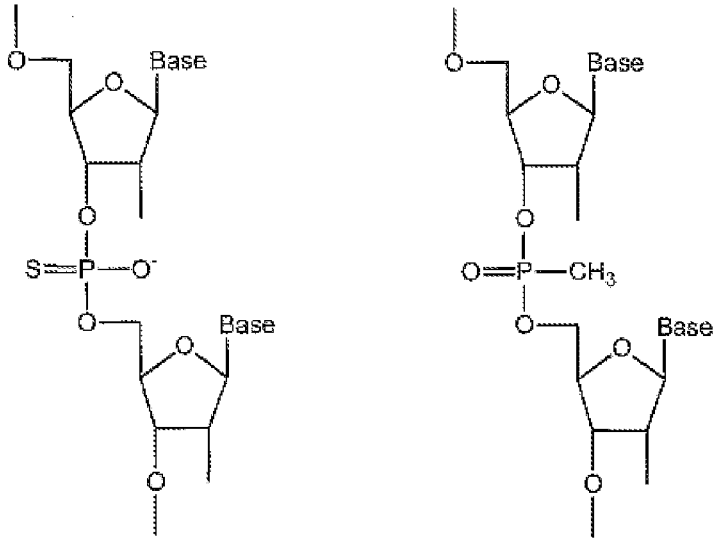


PNA

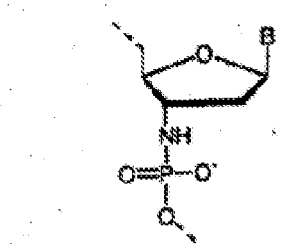
[0138] In some embodiments, one or more modifications optionally occur at the internucleotide linkage. In some instances, modified internucleotide linkage include, but is not limited to, phosphorothioates, phosphorodithioates, methylphosphonates, 5'- alkylenephosphonates, 5'-methylphosphonate, 3'-alkylene phosphonates, borontrifluoridates, borano phosphate esters and selenophosphates of 3'-5'linkage or 2'-5'linkage, phosphotriesters, thionoalkylphosphotriesters, hydrogen phosphonate linkages, alkyl phosphonates, alkylphosphonothioates, arylphosphonothioates, phosphoroselenoates, phosphorodiselenoates, phosphinates, phosphoramidates, 3'-alkylphosphoramidates, aminoalkylphosphoramidates, thionophosphoramidates, phosphoropiperazidates, phosphoroanilothioates, phosphoroanilidates, ketones, sulfones, sulfonamides, carbonates, carbamates, methylenehydrazos, methylenedimethylhydrazos, formacetals, thioformacetals, oximes, methyleneiminos, methylenemethyliminos, thioamidates, linkages with riboacetyl groups, aminoethyl glycine, silyl or siloxane linkages, alkyl or cycloalkyl linkages with or without heteroatoms of, for example, 1 to 10 carbons that are saturated or unsaturated and/or substituted and/or contain heteroatoms, linkages with morpholino structures, amides, polyamides wherein the bases are attached to the aza nitrogens of the backbone directly or indirectly, and combinations thereof. Phosphorothioate antisene oligonucleotides (PS ASO) are antisense oligonucleotides comprising a phosphorothioate linkage. An exemplary PS ASO is illustrated below.



[0139] In some instances, the modification is a methyl or thiol modification such as methylphosphonate or thiolphosphonate modification. Exemplary thiolphosphonate nucleotide (left) and methylphosphonate nucleotide (right) are illustrated below.

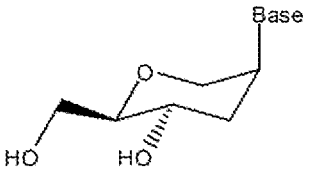


[0140] In some instances, a modified nucleotide includes, but is not limited to, 2'-fluoro N3-P5'-phosphoramidites illustrated as:



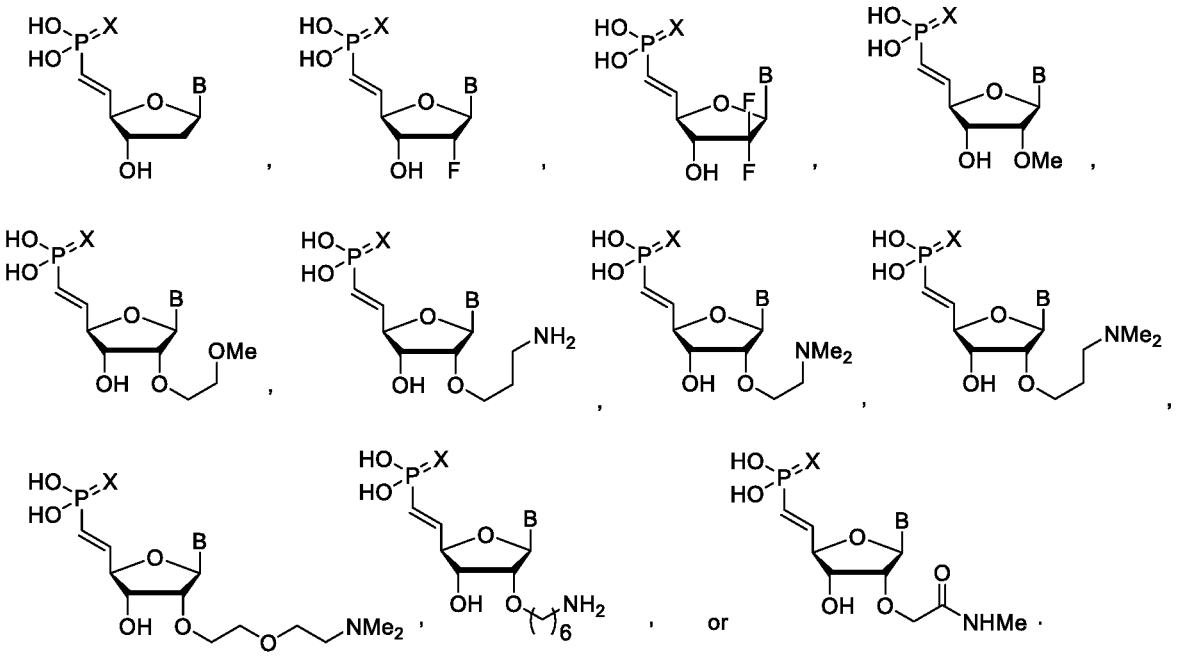
N3'-P5' Phosphoroamidate

[0141] In some instances, a modified nucleotide includes, but is not limited to, hexitol nucleic acid (or 1', 5'- anhydrohexitol nucleic acids (HNA)) illustrated as:



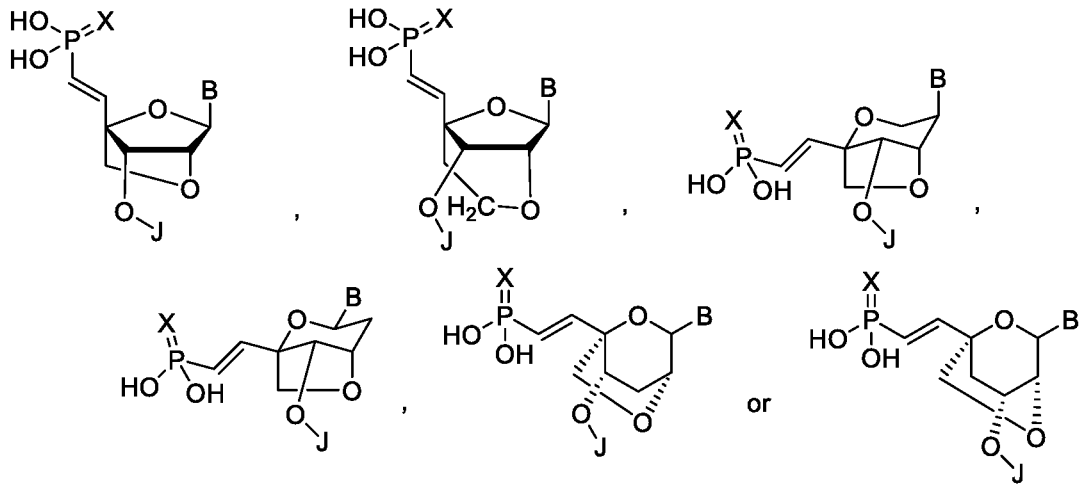
HNA

[0142] In some embodiments, a nucleotide analogue or artificial nucleotide base described above comprises a 5'-vinylphosphonate modified nucleotide nucleic acid with a modification at a 5' hydroxyl group of the ribose moiety. In some embodiments, the 5'-vinylphosphonate modified nucleotide is selected from the nucleotide provided below, wherein X is O or S; and B is a heterocyclic base moiety.



[0143] In some instances, the modification at the 2' hydroxyl group is a 2'-O-aminopropyl modification in which an extended amine group comprising a propyl linker binds the amine group to the 2' oxygen. In some instances, this modification neutralizes the phosphate-derived overall negative charge of the oligonucleotide molecule by introducing one positive charge from the amine group per sugar and thereby improves cellular uptake properties due to its zwitterionic properties.

[0144] In some instances, the 5'-vinylphosphonate modified nucleotide is further modified at the 2' hydroxyl group in a locked or bridged ribose modification (e.g., locked nucleic acid or LNA) in which the oxygen molecule bound at the 2' carbon is linked to the 4' carbon by a methylene group, thus forming a 2′-C,4′-C-oxy-methylene-linked bicyclic ribonucleotide monomer. Exemplary representations of the chemical structure of 5'-vinylphosphonate modified LNA are illustrated below, wherein X is O or S; B is a heterocyclic base moiety; and J is an internucleotide linking group linking to the adjacent nucleotide of the polynucleotide.



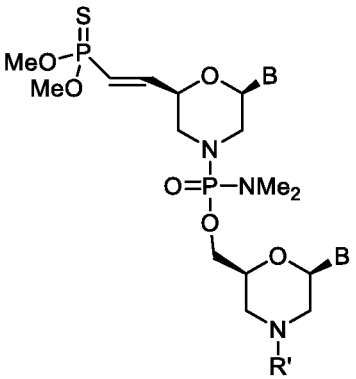
LNA (Locked Nucleic Acids)

[0145] In some embodiments, additional modifications at the 2' hydroxyl group include 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA).

[0146] In some embodiments, a nucleotide analogue comprises a modified base such as, but not limited to, 5-propynyluridine, 5-propynylcytidine, 6- methyladenine, 6-methylguanine, N, N, -dimethyladenine, 2-propyladenine, 2propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5- (2-amino) propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1- methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2- methylguanosine, 7-methylguanosine, 2, 2-dimethylguanosine, 5- methylaminoethyluridine, 5-methyloxyuridine, deazanucleotides (such as 7-deaza-adenosine, 6-azouridine, 6-azocytidine, or 6-azothymidine), 5-methyl-2-thiouridine, other thio bases (such as 2-thiouridine, 4-thiouridine, and 2-thiocytidine), dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O-and N-alkylated purines and pyrimidines (such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, or pyridine-2-one), phenyl and modified phenyl groups such as aminophenol or 2,4, 6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyi nucleotides, and alkylcarbonylalkylated nucleotides. 5'-Vinylphosphonate modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as 5'-vinylphosphonate modified nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties, in some cases are or are based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles. The term nucleotide also includes what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine.

[0147] In some embodiments, a 5'-vinylphosphonate modified nucleotide analogue further comprises a morpholino, a peptide nucleic acid (PNA), a methylphosphonate nucleotide, a thiolphosphonate

nucleotide, a 2'-fluoro N3-P5'-phosphoramidite, or a 1', 5'- anhydrohexitol nucleic acid (HNA). Morpholino or phosphorodiamidate morpholino oligo (PMO) comprises synthetic molecules whose structure mimics natural nucleic acid structure but deviates from the normal sugar and phosphate structures. In some instances, the five member ribose ring is substituted with a six member morpholino ring containing four carbons, one nitrogen, and one oxygen. In some cases, the ribose monomers are linked by a phosphordiamidate group instead of a phosphate group. In such cases, the backbone alterations remove all positive and negative charges making morpholinos neutral molecules capable of crossing cellular membranes without the aid of cellular delivery agents such as those used by charged oligonucleotides. A non-limiting example of a 5'-vinylphosphonate modified morpholino oligonucleotide is illustrated below, wherein X is O or S; and B is a heterocyclic base moiety.

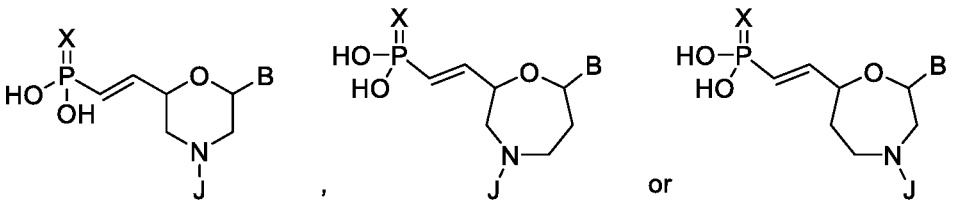


[0148] In some embodiments, a 5'-vinylphosphonate modified morpholino or PMO described above is a PMO comprising a positive or cationic charge. In some instances, the PMO is PMO*plus* (Sarepta). PMO*plus* refers to phosphorodiamidate morpholino oligomers comprising any number of (1-piperazino)phosphinylideneoxy, (1-(4-(omega -guanidino-alkanoyl))-piperazino)phosphinylideneoxy linkages (e.g., as such those described in PCT Publication No. WO2008/036127. In some cases, the PMO is a PMO described in U.S. Patent No. 7943762.

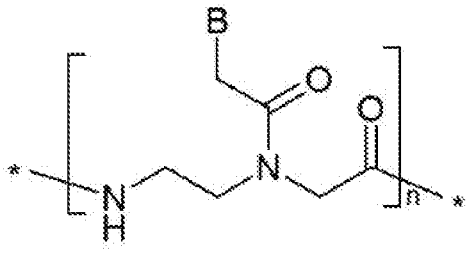
[0149] In some embodiments, a morpholino or PMO described above is a PMO-X (Sarepta). In some cases, PMO-X refers to phosphorodiamidate morpholino oligomers comprising at least one linkage or at least one of the disclosed terminal modifications, such as those disclosed in PCT Publication No. WO2011/150408 and U.S. Publication No. 2012/0065169.

[0150] In some embodiments, a morpholino or PMO described above is a PMO as described in Table 5 of U.S. Publication No. 2014/0296321.

[0151] Exemplary representations of the chemical structure of 5'-vinylphosphonate modified nucleic acids are illustrated below, wherein X is O or S; B is a heterocyclic base moiety; and J is an internucleotide linkage.



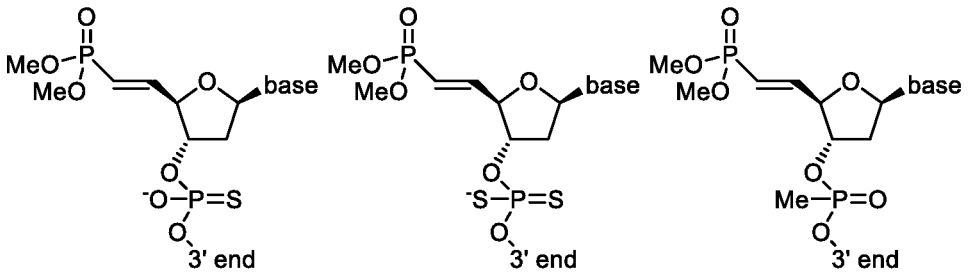
[0152] In some embodiments, peptide nucleic acid (PNA) does not contain sugar ring or phosphate linkage and the bases are attached and appropriately spaced by oligoglycine-like molecules, therefore, eliminating a backbone charge.



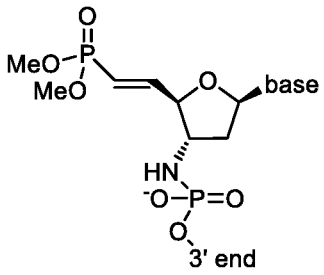
PNA

[0153] In some embodiments, one or more modifications of the 5'-vinylphosphonate modified oligonucleotide optionally occur at the internucleotide linkage. In some instances, modified internucleotide linkage includes, but is not limited to, phosphorothioates; phosphorodithioates; methylphosphonates; 5'- alkylenephosphonates; 5'-methylphosphonate; 3'-alkylene phosphonates; borontrifluoridates; borano phosphate esters and selenophosphates of 3'-5'linkage or 2'-5'linkage; phosphotriesters; thionoalkylphosphotriesters; hydrogen phosphonate linkages; alkyl phosphonates; alkylphosphonothioates; arylphosphonothioates; phosphoroselenoates; phosphorodiselenoates; phosphinates; phosphoramidates; 3'- alkylphosphoramidates; aminoalkylphosphoramidates; thionophosphoramidates; phosphoropiperazidates; phosphoroanilothioates; phosphoroanilidates; ketones; sulfones; sulfonamides; carbonates; carbamates; methylenehydrazos; methylenedimethylhydrazos; formacetals; thioformacetals; oximes; methyleneiminos; methylenemethyliminos; thioamidates; linkages with riboacetyl groups; aminoethyl glycine; silyl or siloxane linkages; alkyl or cycloalkyl linkages with or without heteroatoms of, for example, 1 to 10 carbons that are saturated or unsaturated and/or substituted and/or contain heteroatoms; linkages with morpholino structures, amides, or polyamides wherein the bases are attached to the aza nitrogens of the backbone directly or indirectly; and combinations thereof.

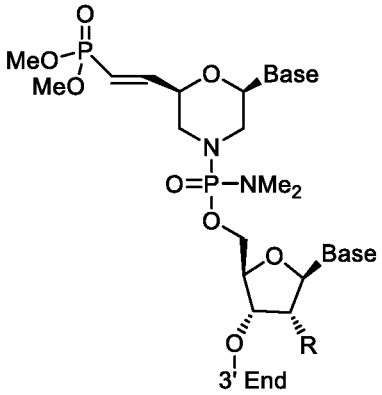
[0154] In some instances, the modification is a methyl or thiol modification such as methylphosphonate or thiolphosphonate modification. Exemplary thiolphosphonate nucleotide (left), phosphorodithioates (center) and methylphosphonate nucleotide (right) are illustrated below.



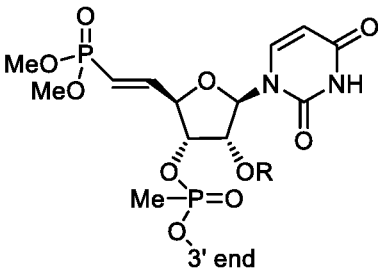
[0155] In some instances, a 5'-vinylphosphonate modified nucleotide includes, but is not limited to, phosphoramidites illustrated as:



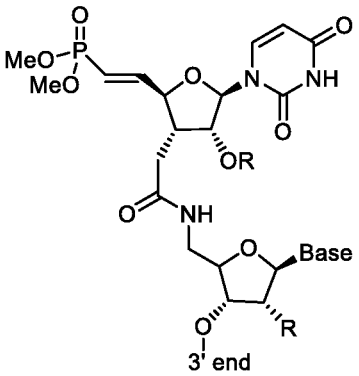
[0156] In some instances, the modified internucleotide linkage is a phosphorodiamidate linkage. A non-limiting example of a phosphorodiamidate linkage with a morpholino system is shown below.



[0157] In some instances, the modified internucleotide linkage is a methylphosphonate linkage. A non-limiting example of a methylphosphonate linkage is shown below.



[0158] In some instances, the modified internucleotide linkage is a amide linkage. A non-limiting example of an amide linkage is shown below.



[0159] In some instances, a 5'-vinylphosphonate modified nucleotide includes, but is not limited to, the modified nucleic acid illustrated below.

[0160] In some embodiments, one or more modifications comprise a modified phosphate backbone in which the modification generates a neutral or uncharged backbone. In some instances, the phosphate

backbone is modified by alkylation to generate an uncharged or neutral phosphate backbone. As used herein, alkylation includes methylation, ethylation, and propylation. In some cases, an alkyl group, as used herein in the context of alkylation, refers to a linear or branched saturated hydrocarbon group containing from 1 to 6 carbon atoms. In some instances, exemplary alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n- pentyl, isopentyl, neopentyl, hexyl, isohexyl, 1, 1 -dimethylbutyl, 2,2-dimethylbutyl, 3.3- dimethylbutyl, and 2-ethylbutyl groups. In some cases, a modified phosphate is a phosphate group as described in U.S. Patent No. 9481905.

[0161] In some embodiments, additional modified phosphate backbones comprise methylphosphonate, ethylphosphonate, methylthiophosphonate, or methoxyphosphonate. In some cases, the modified phosphate is methylphosphonate. In some cases, the modified phosphate is ethylphosphonate. In some cases, the modified phosphate is methylthiophosphonate. In some cases, the modified phosphate is methoxyphosphonate.

[0162] In some embodiments, one or more modifications further optionally include modifications of the ribose moiety, phosphate backbone and the nucleoside, or modifications of the nucleotide analogues at the 3' or the 5' terminus. For example, the 3' terminus optionally include a 3' cationic group, or by inverting the nucleoside at the 3'-terminus with a 3'-3' linkage. In another alternative, the 3'-terminus is optionally conjugated with an aminoalkyl group, e.g., a 3' C5-aminoalkyl dT. In an additional alternative, the 3'-terminus is optionally conjugated with an abasic site, *e.g.*, with an apurinic or apyrimidinic site. In some instances, the 5'-terminus is conjugated with an aminoalkyl group, e.g., a 5'-O-alkylamino substituent. In some cases, the 5'-terminus is conjugated with an abasic site, *e.g.*, with an apurinic or apyrimidinic site.

[0163] In some embodiments, the polynucleic acid molecule comprises one or more of the artificial nucleotide analogues described herein. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9,

10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methyl modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O- methoxyethyl (2'-O-MOE) modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of thiolphosphonate nucleotides.

[0164] In some embodiments, the polynucleic acid molecule comprises a plurality of phosphorodiamidate morpholino oligomers or a plurality of peptide nucleic acid-modified non-natural nucleotides, and optionally comprises at least one inverted abasic moiety. In some instances, the polynucleic acid molecule comprises at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorodiamidate morpholino oligomer-modified non-natural nucleotides. In some instances, the polynucleic acid molecule comprises 100% phosphorodiamidate morpholino oligomer-modified non-natural nucleotides.

[0165] In some instances, the polynucleic acid molecule comprises at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more peptide nucleic acid-modified non-natural nucleotides. In some instances, the polynucleic acid molecule comprises 100% peptide nucleic acid-modified non-natural nucleotides.

[0166] In some embodiments, the polynucleic acid molecule comprises one or more nucleotide analogs in which each nucleotide analog is in a stereochemically isomeric form. In such instance, the polynucleic acid molecule is a chiral molecule. In some cases, the nucleotide analog comprises a backbone stereochemistry. In additional cases, the nucleotide analog comprises a chiral analog as described in U.S. Patent 9,982,257, 9,695,211, or 9,605,019.

[0167] In some instances, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification.

[0168] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 90% modification, from about 20% to about 90% modification, from about 30% to about 90% modification, from about 40% to about 90% modification, from about 50% to about 90% modification, from about 60% to about 90% modification, from about 70% to about 90% modification, and from about 80% to about 100% modification.

[0169] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 80% modification, from about 20% to about 80% modification, from about 30% to about 80% modification, from about 40% to about 80% modification, from about 50% to about 80% modification, from about 60% to about 80% modification, and from about 70% to about 80% modification.

[0170] In some instances, the polynucleic acid molecule comprises at least one of: from about 10% to about 70% modification, from about 20% to about 70% modification, from about 30% to about 70%

modification, from about 40% to about 70% modification, from about 50% to about 70% modification, and from about 60% to about 70% modification.

[0171] In some instances, the polynucleic acid molecule comprises at least one of: from about 10% to about 60% modification, from about 20% to about 60% modification, from about 30% to about 60% modification, from about 40% to about 60% modification, and from about 50% to about 60% modification.

[0172] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 50% modification, from about 20% to about 50% modification, from about 30% to about 50% modification, and from about 40% to about 50% modification.

[0173] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 40% modification, from about 20% to about 40% modification, and from about 30% to about 40% modification.

[0174] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 30% modification, and from about 20% to about 30% modification.

[0175] In some cases, the polynucleic acid molecule comprises from about 10% to about 20% modification.

[0176] In some cases, the polynucleic acid molecule comprises from about 15% to about 90%, from about 20% to about 80%, from about 30% to about 70%, or from about 40% to about 60% modifications.

[0177] In additional cases, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification.

[0178] In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications.

[0179] In some instances, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modified nucleotides.

[0180] In some instances, from about 5 to about 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 10% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 15% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 20% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 25% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 30% of a polynucleic acid molecule comprises the artificial

nucleotide analogues described herein. In some instances, about 35% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 40% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 45% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 50% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 55% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 60% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 65% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 70% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 75% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 80% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 85% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 90% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 95% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 96% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 97% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 98% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 99% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 100% of a polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 2’-O-methyl, 2’-O-methoxyethyl (2’-O-MOE), 2’-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2’-fluoro N3-P5’-phosphoramidites, or a combination thereof.

[0181] In some embodiments, the polynucleic acid molecule comprises from about 1 to about 25 modifications in which the modification comprises an artificial nucleotide analogues described herein. In some embodiments, a polynucleic acid molecule comprises about 1 modification in which the modification comprises an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 2 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 3 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 4 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some

embodiments, a polynucleic acid molecule comprises about 5 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 6 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 7 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 8 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 9 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 10 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 11 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 12 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 13 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 14 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 15 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 16 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 17 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 18 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 19 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 20 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 21 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 22 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 23 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 24 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, a polynucleic acid molecule comprises about 25 modifications in which the modifications comprise an artificial nucleotide analogue described herein.

[0182] In some embodiments, a polynucleic acid molecule is assembled from two separate polynucleotides wherein one polynucleotide comprises the sense strand and the second polynucleotide

comprises the antisense strand of the polynucleic acid molecule. In other embodiments, the sense strand is connected to the antisense strand via a linker molecule, which in some instances is a polynucleotide linker or a non-nucleotide linker.

[0183] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein pyrimidine nucleotides in the sense strand comprises 2′-O-methylpyrimidine nucleotides and purine nucleotides in the sense strand comprise 2′-deoxy purine nucleotides. In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein pyrimidine nucleotides present in the sense strand comprise 2′-deoxy-2′-fluoro pyrimidine nucleotides and wherein purine nucleotides present in the sense strand comprise 2′-deoxy purine nucleotides.

[0184] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the pyrimidine nucleotides when present in said antisense strand are 2′-deoxy-2′-fluoro pyrimidine nucleotides and the purine nucleotides when present in said antisense strand are 2′-O-methyl purine nucleotides.

[0185] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the pyrimidine nucleotides when present in said antisense strand are 2′-deoxy-2′-fluoro pyrimidine nucleotides and wherein the purine nucleotides when present in said antisense strand comprise 2′-deoxy-purine nucleotides.

[0186] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the sense strand includes a terminal cap moiety at the 5′-end, the 3′-end, or both of the 5′ and 3′ ends of the sense strand. In other embodiments, the terminal cap moiety is an inverted deoxy abasic moiety.

[0187] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, wherein the antisense strand comprises a phosphate backbone modification at the 3′ end of the antisense strand. In some instances, the phosphate backbone modification is a phosphorothioate. In some cases, the passenger strand comprises more phosphorothioate modifications than the guide strand. In other cases, the guide strand comprises more phosphorothioate modifications than the passenger strand. In additional cases, the passenger strand comprises about 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate modifications. In additional cases, the guide strand comprises about 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate modifications.

[0188] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, wherein the antisense strand comprises a glyceryl modification at the 3′ end of the antisense strand.

[0189] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of the sense

strand; and in which the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2′-deoxy, 2′-O-methyl and/or 2′-deoxy-2′-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends, being present in the same or different strand.

[0190] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the sense strand comprises about 1 to about 25, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3-end, the 5′-end, or both of the 3′- and 5′-ends of the sense strand; and in which the antisense strand comprises about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2′-deoxy, 2′-O-methyl and/or 2′-deoxy-2′-fluoro nucleotides, with or without about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends, being present in the same or different strand.

[0191] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap

molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2′-deoxy, 2′-O-methyl and/or 2′-deoxy-2′-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′ and 5′-ends, being present in the same or different strand.

[0192] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the antisense strand comprises about 1 to about 25 or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2′-deoxy, 2′-O-methyl, 2′-deoxy-2′-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2′-deoxy, 2′-O-methyl and/or 2′-deoxy-2′-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends, being present in the same or different strand.

[0193] In some embodiments, a polynucleic acid molecule is a duplex polynucleic acid molecule with one or more of the following properties: a greater hepatocyte stability, reduced overall charge, reduced hepatocyte uptake, or extended pharmacokinetics. In some embodiments, the duplex polynucleic acid molecule comprises a passenger strand (e.g., a sense strand) and a guide strand (e.g., an antisense strand) comprising a plurality of modifications.

[0194] In some embodiments, the duplex polynucleic acid molecule comprises a guide strand (e.g., an antisense strand) with one or more of the modification described above, and a passenger strand (e.g., a sense strand) with a plurality of phosphorodiamidate morpholino oligomers or a plurality of peptide nucleic acid-modified non-natural nucleotides.

[0195] In some embodiments, a polynucleic acid molecule described herein is a chemically-modified short interfering nucleic acid molecule having about 1 to about 25, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more phosphorothioate internucleotide linkages in each strand of the polynucleic acid molecule.

[0196] In another embodiment, a polynucleic acid molecule described herein comprises 2′-5′ internucleotide linkages. In some instances, the 2′-5′ internucleotide linkage(s) is at the 3′-end, the 5′-end, or both of the 3′- and 5′-ends of one or both sequence strands. In addition instances, the 2′-5′ internucleotide linkage(s) is present at various other positions within one or both sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the polynucleic acid molecule comprise a 2′-5′ internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the polynucleic acid molecule comprise a 2′-5′ internucleotide linkage.

[0197] In some embodiments, a polynucleic acid molecule is a single stranded polynucleic acid molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the polynucleic acid molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the polynucleic acid are 2′-deoxy-2′-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2′-deoxy-2′-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2′-deoxy-2′-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the polynucleic acid are 2′-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2′-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2′-deoxy purine nucleotides), and a terminal cap modification, that is optionally present at the 3′-end, the 5′-end, or both of the 3′ and 5′-ends of the antisense sequence, the polynucleic acid molecule optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2′-deoxynucleotides at the 3′-end of the polynucleic acid molecule, wherein the terminal nucleotides further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the polynucleic acid molecule optionally further comprises a terminal phosphate group, such as a 5′-terminal phosphate group.

[0198] In some cases, one or more of the artificial nucleotide analogues described herein are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribunuclease such as DNase, or exonuclease such as 5’-3’ exonuclease and 3’-5’ exonuclease when compared to natural polynucleic acid molecules. In some instances, artificial nucleotide analogues comprising 2’-O-methyl, 2’-O-methoxyethyl (2’-O-MOE), 2’-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2’-fluoro N3-P5’-phosphoramidites, or combinations thereof are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribunuclease such as DNase, or exonuclease such as 5’-3’ exonuclease and 3’-5’ exonuclease. In some instances, 2’-O-methyl modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5’-3’ exonuclease or 3’-5’ exonuclease resistance). In some instances, 2’O-methoxyethyl (2’-O-MOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5’-3’ exonuclease or 3’-5’ exonuclease resistance). In some instances, 2’-O-

aminopropyl modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-deoxy modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, LNA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, ENA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, HNA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, morpholinos is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, PNA modified polynucleic acid molecule is resistant to nucleases (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, methylphosphonate nucleotides modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, thiolphosphonate nucleotides modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, the 5' conjugates described herein inhibit 5'-3' exonucleolytic cleavage. In some instances, the 3' conjugates described herein inhibit 3'-5' exonucleolytic cleavage.

[0199] In some embodiments, one or more of the artificial nucleotide analogues described herein have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. The one or more of the artificial nucleotide analogues comprising 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-0-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, or 2'-fluoro N3-P5'-phosphoramidites have increased binding affinity toward their mRNA target relative to

an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-deoxy modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, LNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, ENA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, PNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, HNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, morpholino modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, methylphosphonate nucleotides modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, thiolphosphonate nucleotides modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some cases, the increased affinity is illustrated with a lower Kd, a higher melt temperature (Tm), or a combination thereof.

[0200] In some embodiments, a polynucleic acid molecule described herein is a chirally pure (or stereo pure) polynucleic acid molecule, or a polynucleic acid molecule comprising a single enantiomer. In some instances, the polynucleic acid molecule comprises L-nucleotide. In some instances, the polynucleic acid molecule comprises D-nucleotides. In some instance, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of its mirror enantiomer. In some cases, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of a racemic mixture. In some instances, the polynucleic acid molecule is a polynucleic acid molecule described in: U.S. Patent Publication Nos: 2014/194610 and 2015/211006; and PCT Publication No.: WO2015107425.

[0201] In some embodiments, a polynucleic acid molecule described herein is further modified to include an aptamer conjugating moiety. In some instances, the aptamer conjugating moiety is a DNA aptamer conjugating moiety. In some instances, the aptamer conjugating moiety is Alphamer (Centauri Therapeutics), which comprises an aptamer portion that recognizes a specific cell-surface target and a portion that presents a specific epitopes for attaching to circulating antibodies. In some instance, a polynucleic acid molecule described herein is further modified to include an aptamer conjugating moiety as described in: U.S. Patent Nos: 8,604,184, 8,591,910, and 7,850,975.

[0202] In additional embodiments, a polynucleic acid molecule described herein is modified to increase its stability. In some embodiment, the polynucleic acid molecule is RNA (e.g., siRNA). In some instances, the polynucleic acid molecule is modified by one or more of the modifications described above to increase its stability. In some cases, the polynucleic acid molecule is modified at the 2' hydroxyl position, such as by 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-0-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modification or by a locked or bridged ribose conformation (e.g., LNA or ENA). In some cases, the polynucleic acid molecule is modified by 2'-O-methyl and/or 2'-O-methoxyethyl ribose. In some cases, the polynucleic acid molecule also includes morpholinos, PNAs, HNA, methylphosphonate nucleotides, thiolphosphonate nucleotides, and/or 2'-fluoro N3-P5'-phosphoramidites to increase its stability. In some instances, the polynucleic acid molecule is a chirally pure (or stereo pure) polynucleic acid molecule. In some instances, the chirally pure (or stereo pure) polynucleic acid molecule is modified to increase its stability. Suitable modifications to the RNA to increase stability for delivery will be apparent to the skilled person.

[0203] In some embodiments, a polynucleic acid molecule describe herein has RNAi activity that modulates expression of RNA encoded by a gene involved in muscular dystrophy such as, but not limited to, *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*. In some instances, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*, wherein one of the strands of the double-stranded siRNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or RNA encoded by at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or a

portion thereof, and wherein the second strand of the double-stranded siRNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or RNA encoded by at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA* or a portion thereof. In some cases, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*, wherein each strand of the siRNA molecule comprises about 15 to 25, 18 to 24, or 19 to about 23 nucleotides, and wherein each strand comprises at least about 14, 17, or 19 nucleotides that are complementary to the nucleotides of the other strand. In some cases, a polynucleic acid molecule describe herein is a double-stranded siRNA molecule that down-regulates expression of at least one of *DMD*, *DUX4*, *DYSF*, *EMD*, or *LMNA*, wherein each strand of the siRNA molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand. In some instances, the RNAi activity occurs within a cell. In other instances, the RNAi activity occurs in a reconstituted in vitro system.

[0204] In some embodiments, a polynucleic acid molecule describe herein has RNAi activity that modulates expression of RNA encoded by the *DMD* gene. In some instances, a polynucleic acid molecule describe herein is a single-stranded siRNA molecule that down-regulates expression of *DMD*, wherein the single-stranded siRNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of *DMD* or RNA encoded by *DMD* or a portion thereof. In some cases, a polynucleic acid molecule describe herein is a single-stranded siRNA molecule that down-regulates expression of *DMD*, wherein the siRNA molecule comprises about 15 to 25, 18 to 24, or 19 to about 23 nucleotides. In some cases, a polynucleic acid molecule describe herein is a single-stranded siRNA molecule that down-regulates expression of *DMD*, wherein the siRNA molecule comprises about 19 to about 23 nucleotides. In some instances, the RNAi activity occurs within a cell. In other instances, the RNAi activity occurs in a reconstituted in vitro system.

[0205] In some instances, the polynucleic acid molecule is a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. In some instances, the polynucleic acid molecule is assembled from two separate polynucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (e.g., each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19, 20, 21, 22, 23, or more base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the polynucleic acid molecule is assembled from a single

oligonucleotide, where the self-complementary sense and antisense regions of the polynucleic acid molecule are linked by means of a nucleic acid based or non-nucleic acid-based linker(s).

[0206] In some cases, the polynucleic acid molecule is a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. In other cases, the polynucleic acid molecule is a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide is processed either in vivo or in vitro to generate an active polynucleic acid molecule capable of mediating RNAi. In additional cases, the polynucleic acid molecule also comprises a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such polynucleic acid molecule does not require the presence within the polynucleic acid molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide further comprises a terminal phosphate group, such as a 5′-phosphate (see for example Martinez et al., 2002, *Cell.,* 110, 563-574 and Schwarz et al., 2002, *Molecular Cell,* 10, 537-568), or 5′,3′-diphosphate.

[0207] In some instances, an asymmetric is a linear polynucleic acid molecule comprising an antisense region, a loop portion that comprises nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complimentary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin polynucleic acid molecule comprises an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 nucleotides) and a loop region comprising about 4 to about 8 nucleotides, and a sense region having about 3 to about 18 nucleotides that are complementary to the antisense region. In some cases, the asymmetric hairpin polynucleic acid molecule also comprises a 5′-terminal phosphate group that is chemically modified. In additional cases, the loop portion of the asymmetric hairpin polynucleic acid molecule comprises nucleotides, non-nucleotides, linker molecules, or conjugate molecules.

[0208] In some embodiments, an asymmetric duplex is a polynucleic acid molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complimentary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex polynucleic acid molecule comprises an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 nucleotides) and a sense region having about 3 to about 18 nucleotides that are complementary to the antisense region.

[0209] In some cases, an universal base refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research,* 29, 2437-2447).

Polynucleic Acid Molecule Synthesis

[0210] In some embodiments, a polynucleic acid molecule described herein is constructed using chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a polynucleic acid molecule is chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the polynucleic acid molecule and target nucleic acids. Exemplary methods include those described in: U.S. Patent Nos. 5,142,047; 5,185,444; 5,889,136; 6,008,400; and 6,111,086; PCT Publication No. WO2009099942; or European Publication No. 1579015. Additional exemplary methods include those described in: Griffey et al., "2'-O-aminopropyl ribonucleotides: a zwitterionic modification that enhances the exonuclease resistance and biological activity of antisense oligonucleotides," J. *Med. Chem.* **39**(26):5100-5109 (1997)); Obika, et al. "Synthesis of 2′-O,4′-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3, -endo sugar puckering". *Tetrahedron Letters* **38** (50): 8735 (1997); Koizumi, M. "ENA oligonucleotides as therapeutics". *Current opinion in molecular therapeutics* **8** (2): 144–149 (2006); and Abramova et al., "Novel oligonucleotide analogues based on morpholino nucleoside subunits-antisense technologies: new chemical possibilities," Indian Journal of Chemistry 48B:1721-1726 (2009). Alternatively, the polynucleic acid molecule is produced biologically using an expression vector into which a polynucleic acid molecule has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted polynucleic acid molecule will be of an antisense orientation to a target polynucleic acid molecule of interest).

[0211] In some embodiments, a polynucleic acid molecule is synthesized via a tandem synthesis methodology, wherein both strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate fragments or strands that hybridize and permit purification of the duplex.

[0212] In some instances, a polynucleic acid molecule is also assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the molecule.

[0213] Additional modification methods for incorporating, for example, sugar, base and phosphate modifications include: Eckstein et al., International Publication PCT No. WO 92/07065; Perrault et al. *Nature,* 1990, 344, 565-568; Pieken et al. *Science,* 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.,* 1992, 17, 334-339; Usman et al. International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman et al., 1995, *J. Biol. Chem.,* 270, 25702; Beigelman et al.,

International PCT publication No. WO 97/26270; Beigelman et al., U.S. Pat. No. 5,716,824; Usman et al., U.S. Pat. No. 5,627,053; Woolf et al., International PCT Publication No. WO 98/13526; Thompson et al., U.S. Ser. No. 60/082,404 which was filed on Apr. 20, 1998; Karpeisky et al., 1998, *Tetrahedron Lett.,* 39, 1131; Earnshaw and Gait, 1998, *Biopolymers* (*Nucleic Acid Sciences*), 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.,* 67, 99-134; and Burlina et al., 1997, *Bioorg. Med. Chem.,* 5, 1999-2010. Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis.

[0214] In some instances, while chemical modification of the polynucleic acid molecule internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5′-methylphosphonate linkages improves stability, excessive modifications sometimes cause toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages in some cases is minimized. In such cases, the reduction in the concentration of these linkages lowers toxicity, increases efficacy and higher specificity of these molecules.

Nucleic Acid-Polypeptide Conjugate

[0215] In some embodiments, a polynucleic acid molecule is further conjugated to a polypeptide A for delivery to a site of interest. In some cases, a polynucleic acid molecule is conjugated to a polypeptide A and optionally a polymeric moiety.

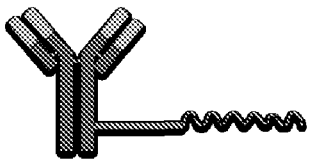
[0216] In some instances, at least one polypeptide A is conjugated to at least one B. In some instances, the at least one polypeptide A is conjugated to the at least one B to form an A-B conjugate. In some embodiments, at least one A is conjugated to the 5’ terminus of B, the 3’ terminus of B, an internal site on B, or in any combinations thereof. In some instances, the at least one polypeptide A is conjugated to at least two B. In some instances, the at least one polypeptide A is conjugated to at least 2, 3, 4, 5, 6, 7, 8, or more B.

[0217] In some embodiments, at least one polypeptide A is conjugated at one terminus of at least one B while at least one C is conjugated at the opposite terminus of the at least one B to form an A-B-C conjugate. In some instances, at least one polypeptide A is conjugated at one terminus of the at least one B while at least one of C is conjugated at an internal site on the at least one B. In some instances, at least one polypeptide A is conjugated directly to the at least one C. In some instances, the at least one B is conjugated indirectly to the at least one polypeptide A via the at least one C to form an A-C-B conjugate.

[0218] In some instances, at least one B and/or at least one C, and optionally at least one D are conjugated to at least one polypeptide A. In some instances, the at least one B is conjugated at a terminus (e.g., a 5’ terminus or a 3’ terminus) to the at least one polypeptide A or are conjugated via an internal site to the at least one polypeptide A. In some cases, the at least one C is conjugated either directly to the at least one polypeptide A or indirectly via the at least one B. If indirectly via the at least one B, the at least one C is conjugated either at the same terminus as the at least one polypeptide A on B, at opposing terminus from the at least one polypeptide A, or independently at an internal site. In some instances, at

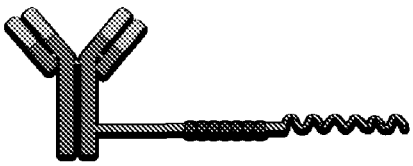
least one additional polypeptide A is further conjugated to the at least one polypeptide A, to B, or to C. In additional instances, the at least one D is optionally conjugated either directly or indirectly to the at least one polypeptide A, to the at least one B, or to the at least one C. If directly to the at least one polypeptide A, the at least one D is also optionally conjugated to the at least one B to form an A-D-B conjugate or is optionally conjugated to the at least one B and the at least one C to form an A-D-B-C conjugate. In some instances, the at least one D is directly conjugated to the at least one polypeptide A and indirectly to the at least one B and the at least one C to form a D-A-B-C conjugate. If indirectly to the at least one polypeptide A, the at least one D is also optionally conjugated to the at least one B to form an A-B-D conjugate or is optionally conjugated to the at least one B and the at least one C to form an A-B-D-C conjugate. In some instances, at least one additional D is further conjugated to the at least one polypeptide A, to B, or to C.

[0219] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



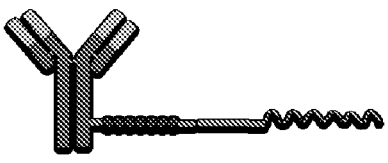
illustrated: .

[0220] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



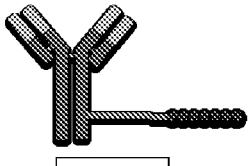
illustrated: .

[0221] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as

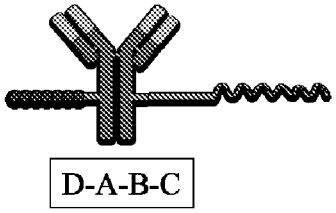


illustrated: .

[0222] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as



illustrated: .

[0223] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated: 

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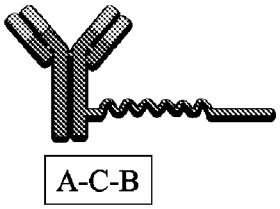
[0224] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated: 

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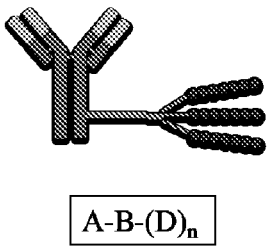
[0225] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated: 

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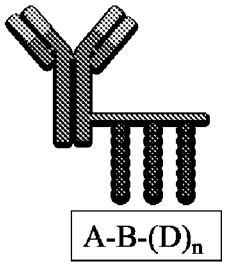
[0226] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated: 

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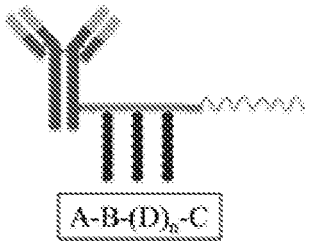
[0227] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated: 

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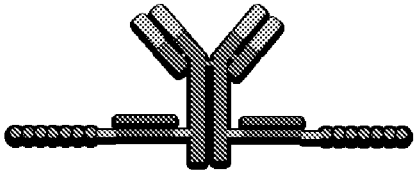
[0228] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated: 
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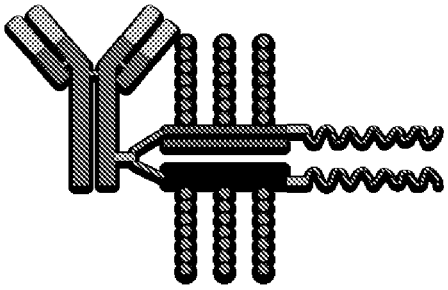
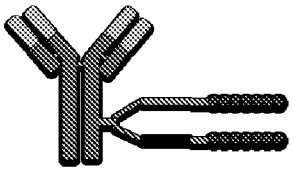
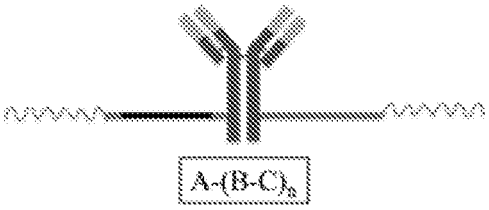
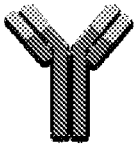
[0229] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated:  .

[0230] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated:  .

[0231] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated:. 

[0232] The  as illustrated above is for representation purposes only and encompasses a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof.

Binding Moiety

[0233] In some embodiments, the binding moiety A is a polypeptide. In some instances, the polypeptide is an antibody or its fragment thereof. In some cases, the fragment is a binding fragment. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab_2, $F(ab)'_3$ fragments, single-chain variable fragment (scFv), bis-scFv, $(scFv)_2$, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or biding fragment thereof, or a chemically modified derivative thereof.

[0234] In some instances, A is an antibody or binding fragment thereof. In some instances, A is a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab_2, $F(ab)'_3$ fragments, single-chain variable fragment (scFv), bis-scFv, $(scFv)_2$, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or biding fragment thereof, or a chemically modified derivative thereof. In some instances, A is a humanized antibody or binding fragment thereof. In some instances, A is a murine antibody or binding fragment thereof. In some instances, A is a chimeric antibody or binding fragment thereof. In some instances, A is a monoclonal antibody or binding fragment thereof. In some instances, A is a monovalent Fab'. In some instances, A is a diavalent Fab_2. In some instances, A is a single-chain variable fragment (scFv).

[0235] In some embodiments, the binding moiety A is a bispecific antibody or binding fragment thereof. In some instances, the bispecific antibody is a trifunctional antibody or a bispecific mini-antibody. In some cases, the bispecific antibody is a trifunctional antibody. In some instances, the trifunctional antibody is a full length monoclonal antibody comprising binding sites for two different antigens.

[0236] In some cases, the bispecific antibody is a bispecific mini-antibody. In some instances, the bispecific mini-antibody comprises divalent Fab_2, $F(ab)'_3$ fragments, bis-scFv, $(scFv)_2$, diabody, minibody, triabody, tetrabody or a bi-specific T-cell engager (BiTE). In some embodiments, the bi-specific T-cell engager is a fusion protein that contains two single-chain variable fragments (scFvs) in which the two scFvs target epitopes of two different antigens.

[0237] In some embodiments, the binding moiety A is a bispecific mini-antibody. In some instances, A is a bispecific Fab_2. In some instances, A is a bispecific $F(ab)'_3$ fragment. In some cases, A is a bispecific bis-scFv. In some cases, A is a bispecific $(scFv)_2$. In some embodiments, A is a bispecific diabody. In some embodiments, A is a bispecific minibody. In some embodiments, A is a bispecific triabody. In other embodiments, A is a bispecific tetrabody. In other embodiments, A is a bi-specific T-cell engager (BiTE).

[0238] In some embodiments, the binding moiety A is a trispecific antibody. In some instances, the trispecific antibody comprises $F(ab)'_3$ fragments or a triabody. In some instances, A is a trispecific $F(ab)'_3$ fragment. In some cases, A is a triabody. In some embodiments, A is a trispecific antibody as described in Dimas, *et al.*, "Development of a trispecific antibody designed to simultaneously and efficiently target three different antigens on tumor cells," *Mol. Pharmaceutics*, **12**(9): 3490-3501 (2015).

[0239] In some embodiments, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein on a muscle cell. Exemplary cell surface proteins recognized by an antibody or binding fragment thereof include, but are not limited to, Sca-1, CD34, Myo-D, myogenin, MRF4, NCAM, CD43, and CD95 (Fas).

[0240] In some instances, the cell surface protein comprises clusters of differentiation (CD) cell surface markers. Exemplary CD cell surface markers include, but are not limited to, CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), and the like.

[0241] In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a CD cell surface marker. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), or a combination thereof.

[0242] In some embodiments, the binding moiety A is an anti-myosin antibody, an anti-transferrin antibody, and an antibody that recognizes Muscle-Specific kinase (MuSK).

[0243] In some instances, the binding moiety A is an anti-myosin antibody. In some cases, the anti-myosin antibody is a humanized antibody. In other cases, the anti-myosin antibody is a chimeric antibody. In additional cases, the anti-myosin antibody is a monovalent, a divalent, or a multi-valent antibody.

[0244] In some instances, the binding moiety A is an anti-transferrin (anti-CD71) antibody. In some cases, the anti-transferrin antibody is a humanized antibody. In other cases, the anti-transferrin antibody is a chimeric antibody. In additional cases, the anti-transferrin antibody is a monovalent, a divalent, or a multi-valent antibody. In some embodiments, exemplary anti-transferrin antibodies include MAB5746 from R&D Systems, AHP858 from Bio-Rad Laboratories, A80-128A from Bethyl Laboratories, Inc., and T2027 from MilliporeSigma.

[0245] In some instances, the binding moiety A is an antibody that recognizes MuSK. In some cases, the anti-MuSK antibody is a humanized antibody. In other cases, the anti-MuSK antibody is a chimeric antibody. In additional cases, the anti-MuSK antibody is a monovalent, a divalent, or a multi-valent antibody.

[0246] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) non-specifically. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue or a cysteine residue, in a non-site specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue in a non-site specific manner. In some cases, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a cysteine residue in a non-site specific manner.

[0247] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) in a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue, a cysteine residue, at the 5'-terminus, at the 3'-terminus, an unnatural amino acid, or an enzyme-modified or enzyme-catalyzed residue, via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a cysteine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 5'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 3'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an unnatural amino acid via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an enzyme-modified or enzyme-catalyzed residue via a site-specific manner.

[0248] In some embodiments, one or more polynucleic acid molecule (B) is conjugated to a binding moiety A. In some instances, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 1 polynucleic acid molecule is conjugated to one binding moiety A. In some instances, about 2 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 3 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 4 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 5 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 6 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 7 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 8 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 9 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 10 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 11 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 12 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 13 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 14 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 15 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 16 polynucleic acid molecules are

conjugated to one binding moiety A. In some cases, the one or more polynucleic acid molecules are the same. In other cases, the one or more polynucleic acid molecules are different.

[0249] In some embodiments, the number of polynucleic acid molecule (B) conjugated to a binding moiety A forms a ratio. In some instances, the ratio is referred to as a DAR (drug-to-antibody) ratio, in which the drug as referred to herein is the polynucleic acid molecule (B). In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12 or greater.

[0250] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 13. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 14. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 15. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 16.

[0251] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 12.

[0252] In some instances, a conjugate comprising polynucleic acid molecule (B) and binding moiety A has improved activity as compared to a conjugate comprising polynucleic acid molecule (B) without a binding moiety A. In some instances, improved activity results in enhanced biologically relevant functions, e.g., improved stability, affinity, binding, functional activity, and efficacy in treatment or prevention of a disease state. In some instancs, the disease state is a result of one or more mutated exons of a gene. In some instances, the conjugate comprising polynucleic acid molecule (B) and binding moiety A results in increased exon skipping of the one or more mutated exons as compared to the conjugate comprising polynucleic acid molecule (B) without a binding moiety A. In some instances, exon skipping is increased by at least or about 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% in the conjugate comprising polynucleic acid molecule (B) and binding moiety A as compared to the conjugate comprising polynucleic acid molecule (B) without a binding moiety A.

[0253] In some embodiments, an antibody or its binding fragment is further modified using conventional techniques known in the art, for example, by using amino acid deletion, insertion, substitution, addition, and/or by recombination and/or any other modification (e.g. posttranslational and chemical modifications, such as glycosylation and phosphorylation) known in the art either alone or in combination. In some instances, the modification further comprises a modification for modulating interaction with Fc receptors. In some instances, the one or more modifications include those described in, for example, International Publication No. WO97/34631, which discloses amino acid residues involved in the interaction between the Fc domain and the FcRn receptor. Methods for introducing such modifications in the nucleic acid sequence underlying the amino acid sequence of an antibody or its binding fragment is well known to the person skilled in the art.

[0254] In some instances, an antibody binding fragment further encompasses its derivatives and includes polypeptide sequences containing at least one CDR.

[0255] In some instances, the term "single-chain" as used herein means that the first and second domains of a bi-specific single chain construct are covalently linked, preferably in the form of a co-linear amino acid sequence encodable by a single nucleic acid molecule.

[0256] In some instances, a bispecific single chain antibody construct relates to a construct comprising two antibody derived binding domains. In such embodiments, bi-specific single chain antibody construct is tandem bi-scFv or diabody. In some instances, a scFv contains a VH and VL domain connected by a

linker peptide. In some instances, linkers are of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities.

[0257] In some embodiments, binding to or interacting with as used herein defines a binding/interaction of at least two antigen-interaction-sites with each other. In some instances, antigen-interaction-site defines a motif of a polypeptide that shows the capacity of specific interaction with a specific antigen or a specific group of antigens. In some cases, the binding/interaction is also understood to define a specific recognition. In such cases, specific recognition refers to that the antibody or its binding fragment is capable of specifically interacting with and/or binding to at least two amino acids of each of a target molecule. For example, specific recognition relates to the specificity of the antibody molecule, or to its ability to discriminate between the specific regions of a target molecule. In additional instances, the specific interaction of the antigen-interaction-site with its specific antigen results in an initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc. In further embodiments, the binding is exemplified by the specificity of a "key-lock-principle". Thus in some instances, specific motifs in the amino acid sequence of the antigen-interaction-site and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. In such cases, the specific interaction of the antigen-interaction-site with its specific antigen results as well in a simple binding of the site to the antigen.

[0258] In some instances, specific interaction further refers to a reduced cross-reactivity of the antibody or its binding fragment or a reduced off-target effect. For example, the antibody or its binding fragment that bind to the polypeptide/protein of interest but do not or do not essentially bind to any of the other polypeptides are considered as specific for the polypeptide/protein of interest. Examples for the specific interaction of an antigen-interaction-site with a specific antigen comprise the specificity of a ligand for its receptor, for example, the interaction of an antigenic determinant (epitope) with the antigenic binding site of an antibody.

Additional Binding Moieties

[0259] In some embodiments, the binding moiety is a plasma protein. In some instances, the plasma protein comprises albumin. In some instances, the binding moiety A is albumin. In some instances, albumin is conjugated by one or more of a conjugation chemistry described herein to a polynucleic acid molecule. In some instances, albumin is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, albumin is conjugated by lysine conjugation to a polynucleic acid molecule.

[0260] In some instances, the binding moiety is a steroid. Exemplary steroids include cholesterol, phospholipids, di-and triacylglycerols, fatty acids, hydrocarbons that are saturated, unsaturated, comprise substitutions, or combinations thereof. In some instances, the steroid is cholesterol. In some instances, the binding moiety is cholesterol. In some instances, cholesterol is conjugated by one or more of a

conjugation chemistry described herein to a polynucleic acid molecule. In some instances, cholesterol is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, cholesterol is conjugated by lysine conjugation to a polynucleic acid molecule.

[0261] In some instances, the binding moiety is a polymer, including but not limited to polynucleic acid molecule aptamers that bind to specific surface markers on cells. In this instance the binding moiety is a polynucleic acid that does not hybridize to a target gene or mRNA, but instead is capable of selectively binding to a cell surface marker similarly to an antibody binding to its specific epitope of a cell surface marker.

[0262] In some cases, the binding moiety is a peptide. In some cases, the peptide comprises between about 1 and about 3 kDa. In some cases, the peptide comprises between about 1.2 and about 2.8 kDa, about 1.5 and about 2.5 kDa, or about 1.5 and about 2 kDa. In some instances, the peptide is a bicyclic peptide. In some cases, the bicyclic peptide is a constrained bicyclic peptide. In some instances, the binding moiety is a bicyclic peptide (e.g., bicycles from Bicycle Therapeutics).

[0263] In additional cases, the binding moiety is a small molecule. In some instances, the small molecule is an antibody-recruiting small molecule. In some cases, the antibody-recruiting small molecule comprises a target-binding terminus and an antibody-binding terminus, in which the target-binding terminus is capable of recognizing and interacting with a cell surface receptor. For example, in some instances, the target-binding terminus comprising a glutamate urea compound enables interaction with PSMA, thereby, enhances an antibody interaction with a cell that expresses PSMA. In some instances, a binding moiety is a small molecule described in Zhang et al., "A remote arene-binding site on prostate specific membrane antigen revealed by antibody-recruiting small molecules," J Am Chem Soc. 132(36): 12711-12716 (2010); or McEnaney, et al., "Antibody-recruiting molecules: an emerging paradigm for engaging immune function in treating human disease," ACS Chem Biol. 7(7): 1139-1151 (2012).

Conjugation Chemistry

[0264] In some embodiments, a polynucleic acid molecule B is conjugated to a binding moiety. In some instances, the binding moiety comprises amino acids, peptides, polypeptides, proteins, antibodies, antigens, toxins, hormones, lipids, nucleotides, nucleosides, sugars, carbohydrates, polymers such as polyethylene glycol and polypropylene glycol, as well as analogs or derivatives of all of these classes of substances. Additional examples of binding moiety also include steroids, such as cholesterol, phospholipids, di-and triacylglycerols, fatty acids, hydrocarbons (e.g., saturated, unsaturated, or contains substitutions), enzyme substrates, biotin, digoxigenin, and polysaccharides. In some instances, the binding moiety is an antibody or binding fragment thereof. In some instances, the polynucleic acid molecule is further conjugated to a polymer, and optionally an endosomolytic moiety.

[0265] In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety by a chemical ligation process. In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a native ligation. In some instances, the conjugation is as described in: Dawson, et al.

"Synthesis of proteins by native chemical ligation," *Science* **1994**, *266*, 776–779; Dawson, et al. "Modulation of Reactivity in Native Chemical Ligation through the Use of Thiol Additives," *J. Am. Chem. Soc*. **1997**, 119, 4325–4329; Hackeng, et al. "Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology.," *Proc. Natl. Acad. Sci. USA* **1999**, 96, 10068–10073; or Wu, et al. "Building complex glycopeptides: Development of a cysteine-free native chemical ligation protocol," *Angew. Chem. Int. Ed.* **2006**, *45*, 4116–4125. In some instances, the conjugation is as described in U.S. Patent No. 8,936,910. In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety either site-specifically or non-specifically via native ligation chemistry.

[0266] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing a "traceless" coupling technology (Philochem). In some instances, the "traceless" coupling technology utilizes an N-terminal 1,2-aminothiol group on the binding moiety which is then conjugate with a polynucleic acid molecule containing an aldehyde group. (*see* Casi *et al.,* "Site-specific traceless coupling of potent cytotoxic drugs to recombinant antibodies for pharmacodelivery," *JACS* **134**(13): 5887-5892 (2012))

[0267] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an unnatural amino acid incorporated into the binding moiety. In some instances, the unnatural amino acid comprises *p*-acetylphenylalanine (pAcPhe). In some instances, the keto group of pAcPhe is selectively coupled to an alkoxy-amine derivatived conjugating moiety to form an oxime bond. (*see* Axup *et al.,* "Synthesis of site-specific antibody-drug conjugates using unnatural amino acids," *PNAS* **109**(40): 16101-16106 (2012)).

[0268] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an enzyme-catalyzed process. In some instances, the site-directed method utilizes SMARTag™ technology (Redwood). In some instances, the SMARTag™ technology comprises generation of a formylglycine (FGly) residue from cysteine by formylglycine-generating enzyme (FGE) through an oxidation process under the presence of an aldehyde tag and the subsequent conjugation of FGly to an alkylhydraine-functionalized polynucleic acid molecule via hydrazino-Pictet-Spengler (HIPS) ligation. (*see* Wu *et al*., "Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag," *PNAS* **106**(9): 3000-3005 (2009); Agarwal, *et al*., "A Pictet-Spengler ligation for protein chemical modification," *PNAS* **110**(1): 46-51 (2013))

[0269] In some instances, the enzyme-catalyzed process comprises microbial transglutaminase (mTG). In some cases, the polynucleic acid molecule is conjugated to the binding moiety utilizing a microbial transglutaminze catalyzed process. In some instances, mTG catalyzes the formation of a covalent bond between the amide side chain of a glutamine within the recognition sequence and a primary amine of a functionalized polynucleic acid molecule. In some instances, mTG is produced from *Streptomyces mobarensis*. (*see* Strop *et al.,* "Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates," *Chemistry and Biology* **20**(2) 161-167 (2013))

[0270] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in PCT Publication No. WO2014/140317, which utilizes a sequence-specific transpeptidase.

[0271] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in U.S. Patent Publication Nos. 2015/0105539 and 2015/0105540.

Production of Antibodies or Binding Fragments Thereof

[0272] In some embodiments, polypeptides described herein (e.g., antibodies and its binding fragments) are produced using any method known in the art to be useful for the synthesis of polypeptides (e.g., antibodies), in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[0273] In some instances, an antibody or its binding fragment thereof is expressed recombinantly, and the nucleic acid encoding the antibody or its binding fragment is assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0274] Alternatively, a nucleic acid molecule encoding an antibody is optionally generated from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3′ and 5′ ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[0275] In some instances, an antibody or its binding is optionally generated by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody is optionally obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

[0276] In some embodiments, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity are used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

[0277] In some embodiments, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) are adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* are also optionally used (Skerra et al., 1988, *Science* 242:1038-1041).

[0278] In some embodiments, an expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody is transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

[0279] In some embodiments, a variety of host-expression vector systems is utilized to express an antibody or its binding fragment described herein. Such host-expression systems represent vehicles by which the coding sequences of the antibody is produced and subsequently purified, but also represent cells that are, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or its binding fragment in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an antibody or its binding fragment coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing an antibody or its binding fragment coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an antibody or its binding fragment coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an antibody or its binding fragment coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0280] For long-term, high-yield production of recombinant proteins, stable expression is preferred. In some instances, cell lines that stably express an antibody are optionally engineered. Rather than using expression vectors that contain viral origins of replication, host cells are transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are then allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn are cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody or its binding fragments.

[0281] In some instances, a number of selection systems are used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes are employed in tk−, hgprt− or aprt− cells, respectively. Also, antimetabolite resistance are used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215) and hygro, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1).

[0282] In some instances, the expression levels of an antibody are increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell Biol.* 3:257).

[0283] In some instances, any method known in the art for purification or analysis of an antibody or antibody conjugates is used, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Exemplary chromatography methods included, but are not limited to, strong anion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, and fast protein liquid chromatography.

Polymer Conjugating Moiety

[0284] In some embodiments, a polymer moiety C is further conjugated to a polynucleic acid molecule described herein, a binding moiety described herein, or in combinations thereof. In some instances, a polymer moiety C is conjugated a polynucleic acid molecule. In some cases, a polymer moiety C is conjugated to a binding moiety. In other cases, a polymer moiety C is conjugated to a

polynucleic acid molecule-binding moiety molecule. In additional cases, a polymer moiety C is conjugated, as illustrated *supra*.

[0285] In some instances, the polymer moiety C is a natural or synthetic polymer, consisting of long chains of branched or unbranched monomers, and/or cross-linked network of monomers in two or three dimensions. In some instances, the polymer moiety C includes a polysaccharide, lignin, rubber, or polyalkylen oxide (e.g., polyethylene glycol). In some instances, the at least one polymer moiety C includes, but is not limited to, alpha-, omega-dihydroxylpolyethyleneglycol, biodegradable lactone-based polymer, e.g. polyacrylic acid, polylactide acid (PLA), poly(glycolic acid) (PGA), polypropylene, polystyrene, polyolefin, polyamide, polycyanoacrylate, polyimide, polyethylenterephthalat (PET, PETG), polyethylene terephthalate (PETE), polytetramethylene glycol (PTG), or polyurethane as well as mixtures thereof. As used herein, a mixture refers to the use of different polymers within the same compound as well as in reference to block copolymers. In some cases, block copolymers are polymers wherein at least one section of a polymer is build up from monomers of another polymer. In some instances, the polymer moiety C comprises polyalkylene oxide. In some instances, the polymer moiety C comprises PEG. In some instances, the polymer moiety C comprises polyethylene imide (PEI) or hydroxy ethyl starch (HES).

[0286] In some instances, C is a PEG moiety. In some instances, the PEG moiety is conjugated at the 5' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 3' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated at the 3' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 5' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated to an internal site of the polynucleic acid molecule. In some instances, the PEG moiety, the binding moiety, or a combination thereof, are conjugated to an internal site of the polynucleic acid molecule. In some instances, the conjugation is a direct conjugation. In some instances, the conjugation is via native ligation.

[0287] In some embodiments, the polyalkylene oxide (e.g., PEG) is a polydispers or monodispers compound. In some instances, polydispers material comprises disperse distribution of different molecular weight of the material, characterized by mean weight (weight average) size and dispersity. In some instances, the monodisperse PEG comprises one size of molecules. In some embodiments, C is poly- or monodispersed polyalkylene oxide (e.g., PEG) and the indicated molecular weight represents an average of the molecular weight of the polyalkylene oxide, e.g., PEG, molecules.

[0288] In some embodiments, the molecular weight of the polyalkylene oxide (e.g., PEG) is about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da.

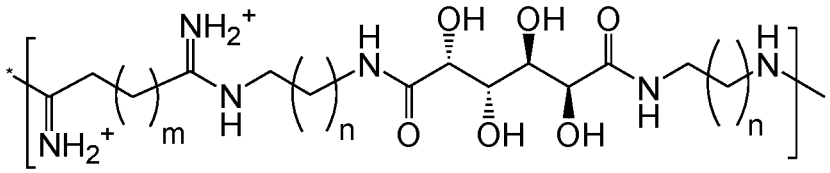
[0289] In some embodiments, C is polyalkylene oxide (e.g., PEG) and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750,

4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some embodiments, C is PEG and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some instances, the molecular weight of C is about 200 Da. In some instances, the molecular weight of C is about 300 Da. In some instances, the molecular weight of C is about 400 Da. In some instances, the molecular weight of C is about 500 Da. In some instances, the molecular weight of C is about 600 Da. In some instances, the molecular weight of C is about 700 Da. In some instances, the molecular weight of C is about 800 Da. In some instances, the molecular weight of C is about 900 Da. In some instances, the molecular weight of C is about 1000 Da. In some instances, the molecular weight of C is about 1100 Da. In some instances, the molecular weight of C is about 1200 Da. In some instances, the molecular weight of C is about 1300 Da. In some instances, the molecular weight of C is about 1400 Da. In some instances, the molecular weight of C is about 1450 Da. In some instances, the molecular weight of C is about 1500 Da. In some instances, the molecular weight of C is about 1600 Da. In some instances, the molecular weight of C is about 1700 Da. In some instances, the molecular weight of C is about 1800 Da. In some instances, the molecular weight of C is about 1900 Da. In some instances, the molecular weight of C is about 2000 Da. In some instances, the molecular weight of C is about 2100 Da. In some instances, the molecular weight of C is about 2200 Da. In some instances, the molecular weight of C is about 2300 Da. In some instances, the molecular weight of C is about 2400 Da. In some instances, the molecular weight of C is about 2500 Da. In some instances, the molecular weight of C is about 2600 Da. In some instances, the molecular weight of C is about 2700 Da. In some instances, the molecular weight of C is about 2800 Da. In some instances, the molecular weight of C is about 2900 Da. In some instances, the molecular weight of C is about 3000 Da. In some instances, the molecular weight of C is about 3250 Da. In some instances, the molecular weight of C is about 3350 Da. In some instances, the molecular weight of C is about 3500 Da. In some instances, the molecular weight of C is about 3750 Da. In some instances, the molecular weight of C is about 4000 Da. In some instances, the molecular weight of C is about 4250 Da. In some instances, the molecular weight of C is about 4500 Da. In some instances, the molecular weight of C is about 4600 Da. In some instances, the molecular weight of C is about 4750 Da. In some instances, the molecular weight of C is about 5000 Da. In some instances, the molecular weight of C is about 5500 Da. In some instances, the molecular weight of C is about 6000 Da. In some instances, the molecular weight of C is about 6500 Da. In some instances, the molecular weight of C is about 7000 Da. In some instances, the molecular weight of C is about 7500 Da. In some instances, the molecular weight of C is about 8000 Da. In some instances, the molecular weight of C is about 10,000 Da. In some instances, the molecular weight of C is about 12,000 Da. In some instances, the molecular weight of C is about 20,000 Da. In some instances, the molecular weight of C is about 35,000 Da. In some instances, the molecular weight of C is about 40,000 Da. In some instances, the molecular weight of C is about 50,000 Da. In

some instances, the molecular weight of C is about 60,000 Da. In some instances, the molecular weight of C is about 100,000 Da.

[0290] In some embodiments, the polyalkylene oxide (e.g., PEG) is a discrete PEG, in which the discrete PEG is a polymeric PEG comprising more than one repeating ethylene oxide units. In some instances, a discrete PEG (dPEG) comprises from 2 to 60, from 2 to 50, or from 2 to 48 repeating ethylene oxide units. In some instances, a dPEG comprises about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 35, 40, 42, 48, 50 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 2 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 3 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 4 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 5 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 6 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 7 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 8 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 9 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 10 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 11 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 12 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 13 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 14 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 15 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 16 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 17 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 18 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 19 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 20 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 22 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 24 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 26 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 28 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 30 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 35 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 40 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 42 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 48 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 50 or more repeating ethylene oxide units. In some cases, a dPEG is synthesized as a single molecular weight compound from pure (e.g., about 95%, 98%, 99%, or 99.5%) staring material in a step-wise fashion. In some cases, a dPEG has a specific molecular weight, rather than an average molecular weight. In some cases, a dPEG described herein is a dPEG from Quanta Biodesign, LMD.

[0291] In some embodiments, the polymer moiety C comprises a cationic mucic acid-based polymer (cMAP). In some instances, cMAP comprises one or more subunit of at least one repeating subunit, and the subunit structure is represented as Formula (V):



Formula V

[0292] wherein m is independently at each occurrence 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, preferably 4-6 or 5; and n is independently at each occurrence 1, 2, 3, 4, or 5. In some embodiments, m and n are, for example, about 10.

[0293] In some instances, cMAP is further conjugated to a PEG moiety, generating a cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some instances, the PEG moiety is in a range of from about 500 Da to about 50,000 Da. In some instances, the PEG moiety is in a range of from about 500 Da to about 1000 Da, greater than 1000 Da to about 5000 Da, greater than 5000 Da to about 10,000 Da, greater than 10,000 to about 25,000 Da, greater than 25,000 Da to about 50,000 Da, or any combination of two or more of these ranges.

[0294] In some instances, the polymer moiety C is cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some cases, the polymer moiety C is cMAP-PEG copolymer. In other cases, the polymer moiety C is an mPEG-cMAP-PEGm triblock polymer. In additional cases, the polymer moiety C is a cMAP-PEG-cMAP triblock polymer.

[0295] In some embodiments, the polymer moiety C is conjugated to the polynucleic acid molecule, the binding moiety, and optionally to the endosomolytic moiety as illustrated *supra*.

Endosomolytic Moiety

[0296] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, further comprises an additional conjugating moiety. In some instances, the additional conjugating moiety is an endosomolytic moiety. In some cases, the endosomolytic moiety is a cellular compartmental release component, such as a compound capable of releasing from any of the cellular compartments known in the art, such as the endosome, lysosome, endoplasmic reticulum (ER), golgi apparatus, microtubule, peroxisome, or other vesicular bodies with the cell. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide, an endosomolytic polymer, an endosomolytic lipid, or an endosomolytic small molecule. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide. In other cases, the endosomolytic moiety comprises an endosomolytic polymer.

Endosomolytic Polypeptides

[0297] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic polypeptide. In some embodiments, a molecule of Formula (V): A-$(X^1$-B$)_n$ or Formula (II): A-X^1-(B-X^2-C$)_n$ is further conjugated with an endosomolytic polypeptide. In some cases, the endosomolytic polypeptide is a pH-dependent membrane active peptide. In some cases, the endosomolytic polypeptide is an amphipathic polypeptide. In additional cases, the endosomolytic polypeptide is a peptidomimetic. In some instances, the endosomolytic polypeptide comprises INF,

melittin, meucin, or their respective derivatives thereof. In some instances, the endosomolytic polypeptide comprises INF or its derivatives thereof. In other cases, the endosomolytic polypeptide comprises melittin or its derivatives thereof. In additional cases, the endosomolytic polypeptide comprises meucin or its derivatives thereof.

[0298] In some instances, INF7 is a 24 residue polypeptide those sequence comprises CGIFGEIEELIEEGLENLIDWGNA (SEQ ID NO: 1), or GLFEAIEGFIENGWEGMIDGWYGC (SEQ ID NO: 2). In some instances, INF7 or its derivatives comprise a sequence of: GLFEAIEGFIENGWEGMIWDYGSGSCG (SEQ ID NO: 3), GLFEAIEGFIENGWEGMIDG WYG-(PEG)6-NH2 (SEQ ID NO: 4), or GLFEAIEGFIENGWEGMIWDYG-SGSC-K(GalNAc)2 (SEQ ID NO: 5).

[0299] In some cases, melittin is a 26 residue polypeptide those sequence comprises CLIGAILKVLATGLPTLISWIKNKRKQ (SEQ ID NO: 6), or GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO: 7). In some instances, melittin comprises a polypeptide sequence as described in U.S. Patent No. 8,501,930.

[0300] In some instances, meucin is an antimicrobial peptide (AMP) derived from the venom gland of the scorpion Mesobuthus eupeus. In some instances, meucin comprises of meucin-13 those sequence comprises IFGAIAGLLKNIF-NH_2 (SEQ ID NO: 8) and meucin-18 those sequence comprises FFGHLFKLATKIIPSLFQ (SEQ ID NO: 9).

[0301] In some instances, the endosomolytic polypeptide comprises a polypeptide in which its sequence is at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% sequence identity to INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof. In some instances, the endosomolytic moiety comprises INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof.

[0302] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1-5. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2-5. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2-5. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2-5.

[0303] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 6 or 7. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6.

In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7. In some cases, the endosomolytic moiety comprises SEQ ID NO: 6. In some cases, the endosomolytic moiety comprises SEQ ID NO: 7. In some cases, the endosomolytic moiety consists of SEQ ID NO: 6. In some cases, the endosomolytic moiety consists of SEQ ID NO: 7.

[0304] In some instances, the endosomolytic moiety is meucin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 8 or 9. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9. In some cases, the endosomolytic moiety comprises SEQ ID NO: 8. In some cases, the endosomolytic moiety comprises SEQ ID NO: 9. In some cases, the endosomolytic moiety consists of SEQ ID NO: 8. In some cases, the endosomolytic moiety consists of SEQ ID NO: 9.

[0305] In some instances, the endosomolytic moiety comprises a sequence as illustrated in **Table 1** below.

Name	Origin	Amino Acid Sequence	SEQ ID NO:	Type
Pep-1	NLS from Simian Virus 40 large antigen and Reverse transcriptase of HIV	KETWWETWWTEWSQPKKKRKV	10	Primary amphipathic
pVEC	VE-cadherin	LLIILRRRRIRKQAHAHSK	11	Primary amphipathic
VT5	Synthetic peptide	DPKGDPKGVTVTVTVTVTGKGDPKPD	12	β-sheet amphipathic
C105Y	1-antitrypsin	CSIPPEVKFNKPFVYLI	13	-
Transportan	Galanin and mastoparan	GWTLNSAGYLLGKINLKALAALAKKIL	14	Primary amphipathic
TP10	Galanin and mastoparan	AGYLLGKINLKALAALAKKIL	15	Primary amphipathic
MPG	A hydrofobic domain from the fusion sequence of HIV gp41 and NLS of SV40 T antigen	GALFLGFLGAAGSTMGA	16	β-sheet amphipathic
gH625	Glycoprotein gH of HSV type I	HGLASTLTRWAHYNALIRAF	17	Secondary amphipathic α-helical
CADY	PPTG1 peptide	GLWRALWRLLRSLWRLLWRA	18	Secondary amphipathic α-helical
GALA	Synthetic peptide	WEAALAEALAEALAEHLAEALAEALEALAA	19	Secondary amphipathic α-helical
INF	Influenza HA2 fusion	GLFEAIEGFIENGWEGMIDGW	20	Secondary

	peptide	YGC		amphipathic α-helical/ pH-dependent membrane active peptide
HA2E5-TAT	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDGW YG	21	Secondary amphipathic α-helical/ pH-dependent membrane active peptide
HA2-penetratin	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDGR QIKIWFQNRRMKW KK-amide	22	pH-dependent membrane active peptide
HA-K4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDG-SSKKKK	23	pH-dependent membrane active peptide
HA2E4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFEAIAGFIENGWEGMIDGG GYC	24	pH-dependent membrane active peptide
H5WYG	HA2 analogue	GLFHAIAHFIHGGWH GLIHGWYG	25	pH-dependent membrane active peptide
GALA-INF3-(PEG)6-NH	INF3 fusion peptide	GLFEAIEGFIENGWEGLAEALA EALEALAA-(PEG)6-NH2	26	pH-dependent membrane active peptide
CM18-TAT11	Cecropin-A-Melittin$_{2-12}$ (CM_{18}) fusion peptide	KWKLFKKIGAVLKVLTTG-YGRKKRRQRRR	27	pH-dependent membrane active peptide

[0306] In some cases, the endosomolytic moiety comprises a Bak BH3 polypeptide which induces apoptosis through antagonization of suppressor targets such as Bcl-2 and/or Bcl-x_L. In some instances, the endosomolytic moiety comprises a Bak BH3 polypeptide described in Albarran, *et al.,* "Efficient intracellular delivery of a pro-apoptotic peptide with a pH-responsive carrier," *Reactive & Functional Polymers* **71**: 261-265 (2011).

[0307] In some instances, the endosomolytic moiety comprises a polypeptide (e.g., a cell-penetrating polypeptide) as described in PCT Publication Nos. WO2013/166155 or WO2015/069587.

Endosomolytic Polymers

[0308] In some embodiments, a molecule of Formula (V): A-$(X^1$-B$)_n$ or Formula (VI): A-X^1-(B-X^2-C$)_n$ is further conjugated with an endosomolytic polymer. As used herein, an endosomolytic polymer comprises a linear, a branched network, a star, a comb, or a ladder type of polymer. In some instances, an endosomolytic polymer is a homopolymer or a copolymer comprising two ro more different types of monomers. In some cases, an endosomolytic polymer is a polycation polymer. In other cases, an endosomolytic polymer is a polyanion polymer.

[0309] In some instances, a polycation polymer comprises monomer units that are charge positive, charge neutral, or charge negative, with a net charge being positive. In other cases, a polycation polymer

comprises a non-polymeric molecule that contains two or more positive charges. Exemplary cationic polymers include, but are not limited to, poly(L-lysine) (PLL), poly(L-arginine) (PLA), polyethyleneimine (PEI), poly[α-(4-aminobutyl)-L-glycolic acid] (PAGA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), or N,N-Diethylaminoethyl Methacrylate (DEAEMA).

[0310] In some cases, a polyanion polymer comprises monomer units that are charge positive, charge neutral, or charge negative, with a net charge being negative. In other cases, a polyanion polymer comprises a non-polymeric molecule that contains two or more negative charges. Exemplary anionic polymers include p(alkylacrylates) (e.g., poly(propyl acrylic acid) (PPAA)) or poly(N-isopropylacrylamide) (NIPAM). Additional examples include PP75, a L-phenylalanine-poly(L-lysine isophthalamide) polymer described in Khormaee, *et al.*, "Edosomolytic anionic polymer for the cytoplasmic delivery of siRNAs in localized in vivo applications," *Advanced Functional Materials* **23**: 565-574 (2013).

[0311] In some embodiments, an endosomolytic polymer described herein is a pH-responsive endosomolytic polymer. A pH-responsive polymer comprises a polymer that increases in size (swell) or collapses depending on the pH of the environment. Polyacrylic acid and chitosan are examples of pH-responsive polymers.

[0312] In some instances, an endosomolytic moiety described herein is a membrane-disruptive polymer. In some cases, the membrane-disruptive polymer comprises a cationic polymer, a neutral or hydrophobic polymer, or an anionic polymer. In some instances, the membrane-disruptive polymer is a hydrophilic polymer.

[0313] In some instances, an endosomolytic moiety described herein is a pH-responsive membrane-disruptive polymer. Exemplary pH-responsive membrane-disruptive polymers include p(alkylacrylic acids), poly(N-isopropylacrylamide) (NIPAM) copolymers, succinylated p(glycidols), and p(β-malic acid) polymers.

[0314] In some instances, p(alkylacrylic acids) include poly(propylacrylic acid) (polyPAA), poly(methacrylic acid) (PMAA), poly(ethylacrylic acid) (PEAA), and poly(propyl acrylic acid) (PPAA). In some instances, a p(alkylacrylic acid) include a p(alkylacrylic acid) described in Jones, *et al.*, *Biochemistry Journal* **372**: 65-75 (2003).

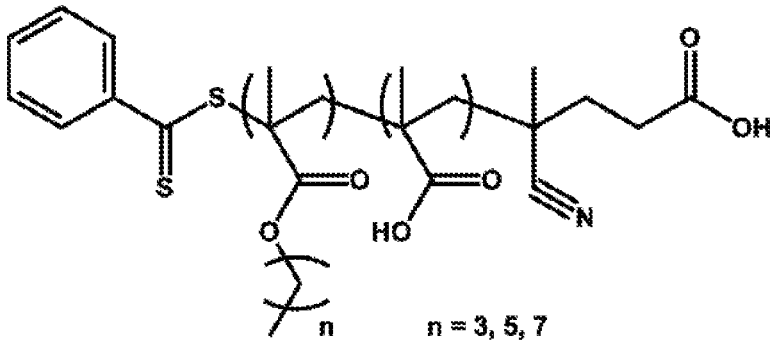
[0315] In some embodiments, a pH-responsive membrane-disruptive polymer comprises p(butyl acrylate-co-methacrylic acid). (*see* Bulmus, *et al.*, *Journal of Controlled Release* **93**: 105-120 (2003); and Yessine, *et al.*, *Biochimica et Biophysica Acta* **1613**: 28-38 (2003))

[0316] In some embodiments, a pH-responsive membrane-disruptive polymer comprises p(styrene-alt-maleic anhydride). (*see* Henry, *et al.*, *Biomacromolecules* **7**: 2407-2414 (2006))

[0317] In some embodiments, a pH-responsive membrane-disruptive polymer comprises pyridyldisulfide acrylate (PDSA) polymers such as poly(MAA-*co*-PDSA), poly(EAA-*co*-PDSA), poly(PAA-*co*-PDSA), poly(MAA-*co*-BA-*co*-PDSA), poly(EAA-*co*-BA-*co*-PDSA), or poly(PAA-*co*-BA-*co*-PDSA) polymers. (*see* El-Sayed, *et al.*, "Rational design of composition and activity correlations for pH-responsive and glutathione-reactive polymer therapeutics," *Journal of Controlled Release* **104**: 417-

427 (2005); or Flanary *et al.*, "Antigen delivery with poly(propylacrylic acid) conjugation enhanced MHC-1 presentation and T-cell activation," *Bioconjugate Chem.* **20**: 241-248 (2009))

[0318] In some embodiments, a pH-responsive membrane-disruptive polymer comprises a lytic polymer comprising the base structure of:



[0319] In some instances, an endosomolytic moiety described herein is further conjugated to an additional conjugate, e.g., a polymer (e.g., PEG), or a modified polymer (e.g., cholesterol-modified polymer).

[0320] In some instances, the additional conjugate comprises a detergent (e.g., Triton X-100). In some instances, an endosomolytic moiety described herein comprises a polymer (e.g., a poly(amidoamine)) conjugated with a detergent (e.g., Triton X-100). In some instances, an endosomolytic moiety described herein comprises poly(amidoamine)-Triton X-100 conjugate (Duncan, *et al.*, "A polymer-Triton X-100 conjugate capable of pH-dependent red blood cell lysis: a model system illustrating the possibility of drug delivery within acidic intracellular compartments," *Journal of Drug Targeting* **2**: 341-347 (1994)).

Endosomolytic Lipids

[0321] In some embodiments, the endosomolytic moiety is a lipid (e.g., a fusogenic lipid). In some embodiments, a molecule of Formula (V): A-$(X^1$-B$)_n$ or Formula (VI): A-X^1-(B-X^2-C$)_n$ is further conjugated with an endosomolytic lipid (e.g., fusogenic lipid). Exemplary fusogenic lipids include 1,2-dileoyl-sn-3-phosphoethanolamine (DOPE), phosphatidylethanolamine (POPE), palmitoyloleoylphosphatidylcholine (POPC), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (Di-Lin), N-methyl(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)methanamine (DLin-k-DMA) and N-methyl-2-(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)ethanamine (XTC).

[0322] In some instances, an endosomolytic moiety is a lipid (e.g., a fusogenic lipid) described in PCT Publication No. WO09/126,933.

Endosomolytic Small Molecules

[0323] In some embodiments, the endosomolytic moiety is a small molecule. In some embodiments, a molecule of Formula (I): A-$(X^1$-B$)_n$ or Formula (II): A-X^1-(B-X^2-C$)_n$ is further conjugated with an endosomolytic small molecule. Exemplary small molecules suitable as endosomolytic moieties include, but are not limited to, quinine, chloroquine, hydroxychloroquines, amodiaquins (carnoquines),

amopyroquines, primaquines, mefloquines, nivaquines, halofantrines, quinone imines, or a combination thereof. In some instances, quinoline endosomolytic moieties include, but are not limited to, 7-chloro-4-(4-diethylamino-1-methylbutyl-amino)quinoline (chloroquine); 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutyl-amino)quinoline (hydroxychloroquine); 7-fluoro-4-(4-diethylamino-1-methylbutyl-amino)quinoline; 4-(4-diethylamino-1-methylbutylamino) quinoline; 7-hydroxy-4-(4-diethyl-amino-1-methylbutylamino)quinoline; 7-chloro-4-(4-diethylamino-1-butylamino)quinoline (desmethylchloroquine); 7-fluoro-4-(4-diethylamino-1-butylamino)quinoline); 4-(4-diethyl-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-butylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-butylamino) quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(4-ethyl-(2-hydroxy-ethyl)-amino-1-methylbutylamino-)quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; hydroxychloroquine phosphate; 7-chloro-4-(4-ethyl-(2-hydroxyethyl-1)-amino-1-butylamino)quinoline (desmethylhydroxychloroquine); 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino) quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 8-[(4-aminopentyl)amino-6-methoxydihydrochloride quinoline; 1-acetyl-1,2,3,4-tetrahydroquinoline; 8-[(4-aminopentyl)amino]-6-methoxyquinoline dihydrochloride; 1-butyryl-1,2,3,4-tetrahydroquinoline; 3-chloro-4-(4-hydroxy-alpha,alpha′-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethyl-amino)-1-methylbutyl-amino]-6-methoxyquinoline; 3-fluoro-4-(4-hydroxy-alpha,alpha′-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 4-(4-hydroxy-alpha,alpha′-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline; 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 3,4-dihydro-1-(2H)-quinolinecarboxyaldehyde; 1,1′-pentamethylene diquinoleinium diiodide; 8-quinolinol sulfate and amino, aldehyde, carboxylic, hydroxyl, halogen, keto, sulfhydryl and vinyl derivatives or analogs thereof. In some instances, an endosomolytic moiety is a small molecule described in Naisbitt et al (1997, J Pharmacol Exp Therapy 280:884-893) and in U.S. Patent No. 5,736,557.

In some embodiments, the endosomolytic moiety is nigericin or a conjugate thereof, e.g., such as a folate-nigericin ester conjugate, a folate-nigericin amide conjugate, or a folate-nigericin carbamate

conjugate. In some instances, the endosomolytic moiety is nigericin described in Rangasamy, *et. al.*, "New mechanism for release of endosomal contents: osmotic lysis via nigericin-mediated K+/H+ exchange," *Bioconjugate Chem.* **29**:1047-1059 (2018).

Linkers

[0324] In some embodiments, a linker described herein is a cleavable linker or a non-cleavable linker. In some instances, the linker is a cleavable linker. In other instances, the linker is a non-cleavable linker.

[0325] In some cases, the linker is a non-polymeric linker. A non-polymeric linker refers to a linker that does not contain a repeating unit of monomers generated by a polymerization process. Exemplary non-polymeric linkers include, but are not limited to, C_1-C_6 alkyl group (e.g., a C_5, C_4, C_3, C_2, or C_1 alkyl group), homobifunctional cross linkers, heterobifunctional cross linkers, peptide linkers, traceless linkers, self-immolative linkers, maleimide-based linkers, or combinations thereof. In some cases, the non-polymeric linker comprises a C_1-C_6 alkyl group (e.g., a C_5, C_4, C_3, C_2, or C_1 alkyl group), a homobifunctional cross linker, a heterobifunctional cross linker, a peptide linker, a traceless linker, a self-immolative linker, a maleimide-based linker, or a combination thereof. In additional cases, the non-polymeric linker does not comprise more than two of the same type of linkers, e.g., more than two homobifunctional cross linkers, or more than two peptide linkers. In further cases, the non-polymeric linker optionally comprises one or more reactive functional groups.

[0326] In some instances, the non-polymeric linker does not encompass a polymer that is described above. In some instances, the non-polymeric linker does not encompass a polymer encompassed by the polymer moiety C. In some cases, the non-polymeric linker does not encompass a polyalkylene oxide (e.g., PEG). In some cases, the non-polymeric linker does not encompass a PEG.

[0327] In some instances, the linker comprises a homobifunctional linker. Exemplary homobifunctional linkers include, but are not limited to, Lomant's reagent dithiobis (succinimidylpropionate) DSP, 3′3′-dithiobis(sulfosuccinimidyl proprionate (DTSSP), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo DST), ethylene glycobis(succinimidylsuccinate) (EGS), disuccinimidyl glutarate (DSG), N,N′-disuccinimidyl carbonate (DSC), dimethyl adipimidate (DMA), dimethyl pimelimidate (DMP), dimethyl suberimidate (DMS), dimethyl-3,3′-dithiobispropionimidate (DTBP), 1,4-di-3′-(2′-pyridyldithio)propionamido)butane (DPDPB), bismaleimidohexane (BMH), aryl halide-containing compound (DFDNB), such as e.g. 1,5-difluoro-2,4-dinitrobenzene or 1,3-difluoro-4,6-dinitrobenzene, 4,4′-difluoro-3,3′-dinitrophenylsulfone (DFDNPS), bis-[β-(4-azidosalicylamido)ethyl]disulfide (BASED), formaldehyde, glutaraldehyde, 1,4-butanediol diglycidyl ether, adipic acid dihydrazide, carbohydrazide, o-toluidine, 3,3′-dimethylbenzidine, benzidine, α,α′-p-diaminodiphenyl, diiodo-p-xylene sulfonic acid, N,N′-ethylene-bis(iodoacetamide), or N,N′-hexamethylene-bis(iodoacetamide).

[0328] In some embodiments, the linker comprises a heterobifunctional linker. Exemplary heterobifunctional linker include, but are not limited to, amine-reactive and sulfhydryl cross-linkers such as N-succinimidyl 3-(2-pyridyldithio)propionate (sPDP), long-chain N-succinimidyl 3-(2-

pyridyldithio)propionate (LC-sPDP), water-soluble-long-chain N-succinimidyl 3-(2-pyridyldithio) propionate (sulfo-LC-sPDP), succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)toluene (sMPT), sulfosuccinimidyl-6-[α-methyl-α-(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-sMPT), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBs), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBs), N-succinimidyl(4-iodoacteyl)aminobenzoate (sIAB), sulfosuccinimidyl(4-iodoacteyl)aminobenzoate (sulfo-sIAB), succinimidyl-4-(p-maleimidophenyl)butyrate (sMPB), sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate (sulfo-sMPB), N-(γ-maleimidobutyryloxy)succinimide ester (GMBs), N-(γ-maleimidobutyryloxy)sulfosuccinimide ester (sulfo-GMBs), succinimidyl 6-((iodoacetyl)amino)hexanoate (sIAX), succinimidyl 6-[6-(((iodoacetyl)amino)hexanoyl)amino]hexanoate (sIAXX), succinimidyl 4-(((iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIAC), succinimidyl 6-((((4-iodoacetyl)amino)methyl)cyclohexane-1-carbonyl)amino) hexanoate (sIACX), p-nitrophenyl iodoacetate (NPIA), carbonyl-reactive and sulfhydryl-reactive cross-linkers such as 4-(4-N-maleimidophenyl)butyric acid hydrazide (MPBH), 4-(N-maleimidomethyl)cyclohexane-1-carboxyl-hydrazide-8 (M_2C_2H), 3-(2-pyridyldithio)propionyl hydrazide (PDPH), amine-reactive and photoreactive cross-linkers such as N-hydroxysuccinimidyl-4-azidosalicylic acid (NHs-AsA), N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (sulfo-NHs-AsA), sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (sulfo-NHs-LC-AsA), sulfosuccinimidyl-2-(ρ-azidosalicylamido)ethyl-1,3′-dithiopropionate (sAsD), N-hydroxysuccinimidyl-4-azidobenzoate (HsAB), N-hydroxysulfosuccinimidyl-4-azidobenzoate (sulfo-HsAB), N-succinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate (sANPAH), sulfosuccinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate (sulfo-sANPAH), N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOs), sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3′-dithiopropionate (sAND), N-succinimidyl-4(4-azidophenyl)1,3′-dithiopropionate (sADP), N-sulfosuccinimidyl(4-azidophenyl)-1,3′-dithiopropionate (sulfo-sADP), sulfosuccinimidyl 4-(ρ-azidophenyl)butyrate (sulfo-sAPB), sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide)ethyl-1,3′-dithiopropionate (sAED), sulfosuccinimidyl 7-azido-4-methylcoumain-3-acetate (sulfo-sAMCA), ρ-nitrophenyl diazopyruvate (ρNPDP), ρ-nitrophenyl-2-diazo-3,3,3-trifluoropropionate (PNP-DTP), sulfhydryl-reactive and photoreactive cross-linkers such as1-(ρ-Azidosalicylamido)-4-(iodoacetamido)butane (AsIB), N-[4-(ρ-azidosalicylamido)butyl]-3′-(2′-pyridyldithio)propionamide (APDP), benzophenone-4-iodoacetamide, benzophenone-4-maleimide carbonyl-reactive and photoreactive cross-linkers such as ρ-azidobenzoyl hydrazide (ABH), carboxylate-reactive and photoreactive cross-linkers such as 4-(ρ-azidosalicylamido)butylamine (AsBA), and arginine-reactive and photoreactive cross-linkers such as ρ-azidophenyl glyoxal (APG).

[0329] In some instances, the linker comprises a reactive functional group. In some cases, the reactive functional group comprises a nucleophilic group that is reactive to an electrophilic group present on a binding moiety. Exemplary electrophilic groups include carbonyl groups—such as aldehyde, ketone, carboxylic acid, ester, amide, enone, acyl halide or acid anhydride. In some embodiments, the reactive

functional group is aldehyde. Exemplary nucleophilic groups include hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0330] In some embodiments, the linker comprises a maleimide goup. In some instances, the maleimide group is also referred to as a maleimide spacer. In some instances, the maleimide group further encompasses a caproic acid, forming maleimidocaproyl (mc). In some cases, the linker comprises maleimidocaproyl (mc). In some cases, the linker is maleimidocaproyl (mc). In other instances, the maleimide group comprises a maleimidomethyl group, such as succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC) or sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC) described above.

[0331] In some embodiments, the maleimide group is a self-stablizing maleimide. In some instances, the self-stablizing maleimide utilizes diaminopropionic acid (DPR) to incorporate a basic amino group adjacent to the maleimide to provide intramolecular catalysis of tiosuccinimide ring hydrolysis, thereby eliminating maleimide from undergoing an elimination reaction through a retro-Michael reaction. In some instances, the self-stabilizing maleimide is a maleimide group described in Lyon, *et al.*, "Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates," *Nat. Biotechnol.* **32**(10):1059-1062 (2014). In some instances, the linker comprises a self-stablizing maleimide. In some instances, the linker is a self-stablizing maleimide.

[0332] In some embodiments, the linker comprises a peptide moiety. In some instances, the peptide moiety comprises at least 2, 3, 4, 5, or 6 more amino acid residues. In some instances, the peptide moiety comprises at most 2, 3, 4, 5, 6, 7, or 8 amino acid residues. In some instances, the peptide moiety comprises about 2, about 3, about 4, about 5, or about 6 amino acid residues. In some instances, the peptide moiety is a cleavable peptide moiety (e.g., either enzymatically or chemically). In some instances, the peptide moiety is a non-cleavable peptide moiety. In some instances, the peptide moiety comprises Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly, Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu, or Gly-Phe-Leu-Gly. In some instances, the linker comprises a peptide moiety such as: Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly, Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu, or Gly-Phe-Leu-Gly. In some cases, the linker comprises Val-Cit. In some cases, the linker is Val-Cit.

[0333] In some embodiments, the linker comprises a benzoic acid group, or its derivatives thereof. In some instances, the benzoic acid group or its derivatives thereof comprise paraaminobenzoic acid (PABA). In some instances, the benzoic acid group or its derivatives thereof comprise gamma-aminobutyric acid (GABA).

[0334] In some embodiments, the linker comprises one or more of a maleimide group, a peptide moiety, and/or a benzoic acid group, in any combination. In some embodiments, the linker comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In some instances, the maleimide group is maleimidocaproyl (mc). In some instances, the peptide group is val-cit. In some instances, the benzoic acid group is PABA. In some instances, the linker comprises a mc-val-cit group.

In some cases, the linker comprises a val-cit-PABA group. In additional cases, the linker comprises a mc-val-cit-PABA group.

[0335] In some embodiments, the linker is a self-immolative linker or a self-elimination linker. In some cases, the linker is a self-immolative linker. In other cases, the linker is a self-elimination linker (e.g., a cyclization self-elimination linker). In some instances, the linker comprises a linker described in U.S. Patent No. 9,089,614 or PCT Publication No. WO2015038426.

[0336] In some embodiments, the linker is a dendritic type linker. In some instances, the dendritic type linker comprises a branching, multifunctional linker moiety. In some instances, the dendritic type linker is used to increase the molar ratio of polynucleotide B to the binding moiety A. In some instances, the dendritic type linker comprises PAMAM dendrimers.

[0337] In some embodiments, the linker is a traceless linker or a linker in which after cleavage does not leave behind a linker moiety (e.g., an atom or a linker group) to a binding moiety A, a polynucleotide B, a polymer C, or an endosomolytic moiety D. Exemplary traceless linkers include, but are not limited to, germanium linkers, silicium linkers, sulfur linkers, selenium linkers, nitrogen linkers, phosphorus linkers, boron linkers, chromium linkers, or phenylhydrazide linker. In some cases, the linker is a traceless aryl-triazene linker as described in Hejesen, *et al.*, "A traceless aryl-triazene linker for DNA-directed chemistry," *Org Biomol Chem* **11**(15): 2493-2497 (2013). In some instances, the linker is a traceless linker described in Blaney, *et al.*, "Traceless solid-phase organic synthesis," *Chem. Rev.* **102**: 2607-2024 (2002). In some instances, a linker is a traceless linker as described in U.S. Patent No. 6,821,783.

[0338] In some instances, the linker is a linker described in U.S. Patent Nos. 6,884,869; 7,498,298; 8,288,352; 8,609,105; or 8,697,688; U.S. Patent Publication Nos. 2014/0127239; 2013/028919; 2014/286970; 2013/0309256; 2015/037360; or 2014/0294851; or PCT Publication Nos. WO2015057699; WO2014080251; WO2014197854; WO2014145090; or WO2014177042.

[0339] In some embodiments, X, Y, and L are independently a bond or a linker. In some instances, X, Y, and L are independently a bond. In some cases, X, Y, and L are independently a linker.

[0340] In some instances, X is a bond or a linker. In some instances, X is a bond. In some instances, X is a linker. In some instances, the linker is a C_1-C_6 alkyl group. In some cases, X is a C_1-C_6 alkyl group, such as for example, a C_5, C_4, C_3, C_2, or C_1 alkyl group. In some cases, the C_1-C_6 alkyl group is an unsubstituted C_1-C_6 alkyl group. As used in the context of a linker, and in particular in the context of X, alkyl means a saturated straight or branched hydrocarbon radical containing up to six carbon atoms. In some instances, X is a non-polymeric linker. In some instances, X includes a homobifunctional linker or a heterobifunctional linker described *supra*. In some cases, X includes a heterobifunctional linker. In some cases, X includes sMCC. In other instances, X includes a heterobifunctional linker optionally conjugated to a C_1-C_6 alkyl group. In other instances, X includes sMCC optionally conjugated to a C_1-C_6 alkyl group. In additional instances, X does not include a homobifunctional linker or a heterobifunctional linker described *supra*.

[0341] In some instances, Y is a bond or a linker. In some instances, Y is a bond. In other cases, Y is a linker. In some embodiments, Y is a C_1-C_6 alkyl group. In some instances, Y is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, Y is a homobifunctional linker described *supra*. In some instances, Y is a heterobifunctional linker described *supra*. In some instances, Y comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, Y comprises a peptide moiety, such as Val-Cit. In some instances, Y comprises a benzoic acid group, such as PABA. In additional instances, Y comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, Y comprises a mc group. In additional instances, Y comprises a mc-val-cit group. In additional instances, Y comprises a val-cit-PABA group. In additional instances, Y comprises a mc-val-cit-PABA group.

[0342] In some instances, L is a bond or a linker. In some cases, L is a bond. In other cases, L is a linker. In some embodiments, L is a C_1-C_6 alkyl group. In some instances, L is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, L is a homobifunctional linker described *supra*. In some instances, L is a heterobifunctional linker described *supra*. In some instances, L comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, L comprises a peptide moiety, such as Val-Cit. In some instances, L comprises a benzoic acid group, such as PABA. In additional instances, L comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, L comprises a mc group. In additional instances, L comprises a mc-val-cit group. In additional instances, L comprises a val-cit-PABA group. In additional instances, L comprises a mc-val-cit-PABA group.

[0343] In some embodiments, X^1 and X^2 are each independently a bond or a non-polymeric linker. In some instances, X^1 and X^2 are each independently a bond. In some cases, X^1 and X^2 are each independently a non-polymeric linker.

[0344] In some instances, X^1 is a bond or a non-polymeric linker. In some instances, X^1 is a bond. In some instances, X^1 is a non-polymeric linker. In some instances, the linker is a C_1-C_6 alkyl group. In some cases, X^1 is a C_1-C_6 alkyl group, such as for example, a C_5, C_4, C_3, C_2, or C_1 alkyl group. In some cases, the C_1-C_6 alkyl group is an unsubstituted C_1-C_6 alkyl group. As used in the context of a linker, and in particular in the context of X^1, alkyl means a saturated straight or branched hydrocarbon radical containing up to six carbon atoms. In some instances, X^1 includes a homobifunctional linker or a heterobifunctional linker described *supra*. In some cases, X^1 includes a heterobifunctional linker. In some cases, X^1 includes sMCC. In other instances, X^1 includes a heterobifunctional linker optionally conjugated to a C_1-C_6 alkyl group. In other instances, X^1 includes sMCC optionally conjugated to a C_1-C_6 alkyl group. In additional instances, X^1 does not include a homobifunctional linker or a heterobifunctional linker described *supra*.

[0345] In some instances, X^2 is a bond or a linker. In some instances, X^2 is a bond. In other cases, X^2 is a linker. In additional cases, X^2 is a non-polymeric linker. In some embodiments, X^2 is a C_1-C_6 alkyl group. In some instances, X^2 is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, X^2 is a homobifunctional linker described *supra*. In some instances, X^2 is a

heterobifunctional linker described *supra*. In some instances, X^2 comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, X^2 comprises a peptide moiety, such as Val-Cit. In some instances, X^2 comprises a benzoic acid group, such as PABA. In additional instances, X^2 comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, X^2 comprises a mc group. In additional instances, X^2 comprises a mc-val-cit group. In additional instances, X^2 comprises a val-cit-PABA group. In additional instances, X^2 comprises a mc-val-cit-PABA group.

Pharmaceutical Formulation

[0346] In some embodiments, the pharmaceutical formulations described herein are administered to a subject by multiple administration routes, including but not limited to, parenteral (e.g., intravenous, subcutaneous, intramuscular), oral, intranasal, buccal, rectal, or transdermal administration routes. In some instances, the pharmaceutical composition describe herein is formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular, intra-arterial, intraperitoneal, intrathecal, intracerebral, intracerebroventricular, or intracranial) administration. In other instances, the pharmaceutical composition describe herein is formulated for oral administration. In still other instances, the pharmaceutical composition describe herein is formulated for intranasal administration.

[0347] In some embodiments, the pharmaceutical formulations include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations (e.g., nanoparticle formulations), and mixed immediate and controlled release formulations.

[0348] In some instances, the pharmaceutical formulation includes multiparticulate formulations. In some instances, the pharmaceutical formulation includes nanoparticle formulations. In some instances, nanoparticles comprise cMAP, cyclodextrin, or lipids. In some cases, nanoparticles comprise solid lipid nanoparticles, polymeric nanoparticles, self-emulsifying nanoparticles, liposomes, microemulsions, or micellar solutions. Additional exemplary nanoparticles include, but are not limited to, paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes and quantum dots. In some instances, a nanoparticle is a metal nanoparticle, e.g., a nanoparticle of scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, yttrium, zirconium, niobium, molybdenum, ruthenium, rhodium, palladium, silver, cadmium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, gadolinium, aluminum, gallium, indium, tin, thallium, lead, bismuth, magnesium, calcium, strontium, barium, lithium, sodium, potassium, boron, silicon, phosphorus, germanium, arsenic, antimony, and combinations, alloys or oxides thereof.

[0349] In some instances, a nanoparticle includes a core or a core and a shell, as in a core-shell nanoparticle.

[0350] In some instances, a nanoparticle is further coated with molecules for attachment of functional elements (e.g., with one or more of a polynucleic acid molecule or binding moiety described herein). In some instances, a coating comprises chondroitin sulfate, dextran sulfate, carboxymethyl dextran, alginic acid, pectin, carragheenan, fucoidan, agaropectin, porphyran, karaya gum, gellan gum, xanthan gum, hyaluronic acids, glucosamine, galactosamine, chitin (or chitosan), polyglutamic acid, polyaspartic acid, lysozyme, cytochrome C, ribonuclease, trypsinogen, chymotrypsinogen, α-chymotrypsin, polylysine, polyarginine, histone, protamine, ovalbumin or dextrin or cyclodextrin. In some instances, a nanoparticle comprises a graphene-coated nanoparticle.

[0351] In some cases, a nanoparticle has at least one dimension of less than about 500nm, 400nm, 300nm, 200nm, or 100nm.

[0352] In some instances, the nanoparticle formulation comprises paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes or quantum dots. In some instances, a polynucleic acid molecule or a binding moiety described herein is conjugated either directly or indirectly to the nanoparticle. In some instances, at least 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more polynucleic acid molecules or binding moieties described herein are conjugated either directly or indirectly to a nanoparticle.

[0353] In some embodiments, the pharmaceutical formulation comprise a delivery vector, e.g., a recombinant vector, the delivery of the polynucleic acid molecule into cells. In some instances, the recombinant vector is DNA plasmid. In other instances, the recombinant vector is a viral vector. Exemplary viral vectors include vectors derived from adeno-associated virus, retrovirus, adenovirus, or alphavirus. In some instances, the recombinant vectors capable of expressing the polynucleic acid molecules provide stable expression in target cells. In additional instances, viral vectors are used that provide for transient expression of polynucleic acid molecules.

[0354] In some embodiments, the pharmaceutical formulations include a carrier or carrier materials selected on the basis of compatibility with the composition disclosed herein, and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. Pharmaceutically compatible carrier materials include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrollidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphotidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980;

and *Pharmaceutical Dosage Fo*rms *and* Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999).

[0355] In some instances, the pharmaceutical formulations further include pH adjusting agents or buffering agents which include acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[0356] In some instances, the pharmaceutical formulation includes one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[0357] In some instances, the pharmaceutical formulations further include diluent which are used to stabilize compounds because they provide a more stable environment. Salts dissolved in buffered solutions (which also provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain instances, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds include e.g., lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel®; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac® (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner's sugar; monobasic calcium sulfate monohydrate, calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[0358] In some cases, the pharmaceutical formulations include disintegration agents or disintegrants to facilitate the breakup or disintegration of a substance. The term "disintegrate" include both the dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel®, or sodium starch glycolate such as Promogel® or Explotab®, a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel®, Avicel® PH101, Avicel® PH102, Avicel® PH105, Elcema® P100, Emcocel®, Vivacel®, Ming Tia®, and Solka-Floc®, methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol®), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum® HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural

sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[0359] In some instances, the pharmaceutical formulations include filling agents such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[0360] Lubricants and glidants are also optionally included in the pharmaceutical formulations described herein for preventing, reducing or inhibiting adhesion or friction of materials. Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumerate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex®), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as Carbowax™, sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid™, Cab-O-Sil®, a starch such as corn starch, silicone oil, a surfactant, and the like.

[0361] Plasticizers include compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, e.g., polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. Plasticizers also function as dispersing agents or wetting agents.

[0362] Solubilizers include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium doccusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcutol, propylene glycol, and dimethyl isosorbide and the like.

[0363] Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[0364] Suspending agents include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol has a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[0365] Surfactants include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic® (BASF), and the like. Additional surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10, octoxynol 40. Sometimes, surfactants is included to enhance physical stability or for other purposes.

[0366] Viscosity enhancing agents include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[0367] Wetting agents include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium doccusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

Therapeutic Regimens

[0368] In some embodiments, the pharmaceutical compositions described herein are administered for therapeutic applications. In some embodiments, the pharmaceutical composition is administered once per day, twice per day, three times per day or more. The pharmaceutical composition is administered daily, every day, every alternate day, five days a week, once a week, every other week, two weeks per month, three weeks per month, once a month, twice a month, three times per month, or more. The pharmaceutical composition is administered for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 2 years, 3 years, or more.

[0369] In some embodiments, one or more pharmaceutical compositions are administered simultaneously, sequentially, or at an interval period of time. In some embodiments, one or more pharmaceutical compositions are administered simultaneously. In some cases, one or more pharmaceutical compositions are administered sequentially. In additional cases, one or more pharmaceutical compositions are administered at an interval period of time (e.g., the first administration of a first pharmaceutical composition is on day one followed by an interval of at least 1, 2, 3, 4, 5, or more days prior to the administration of at least a second pharmaceutical composition).

[0370] In some embodiments, two or more different pharmaceutical compositions are coadministered. In some instances, the two or more different pharmaceutical compositions are coadministered simultaneously. In some cases, the two or more different pharmaceutical compositions are coadministered sequentially without a gap of time between administrations. In other cases, the two or more different pharmaceutical compositions are coadministered sequentially with a gap of about 0.5 hour, 1 hour, 2 hour, 3 hour, 12 hours, 1 day, 2 days, or more between administrations.

[0371] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the composition is given continuously; alternatively, the dose of the composition being administered is temporarily reduced or temporarily suspended for a certain length of time (i.e., a "drug holiday"). In some instances, the length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday is from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0372] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained.

[0373] In some embodiments, the amount of a given agent that correspond to such an amount varies depending upon factors such as the particular compound, the severity of the disease, the identity (e.g., weight) of the subject or host in need of treatment, but nevertheless is routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, and the subject or host being treated. In some instances, the desired dose is conveniently presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

[0374] The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages is altered depending on a number of variables, not limited to the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

[0375] In some embodiments, toxicity and therapeutic efficacy of such therapeutic regimens are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it is expressed as the ratio between LD50 and ED50. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with minimal toxicity. The dosage varies within this range depending upon the dosage form employed and the route of administration utilized.

Kits/Article of Manufacture

[0376] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more of the compositions and methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[0377] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[0378] For example, the container(s) include target nucleic acid molecule described herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[0379] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[0380] In one embodiment, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

[0381] In certain embodiments, the pharmaceutical compositions are presented in a pack or dispenser device which contains one or more unit dosage forms containing a compound provided herein. The pack, for example, contains metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is also accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, is the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. In one embodiment, compositions containing a compound provided herein formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Certain Terminology

[0382] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. In this application, the use of

the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[0383] As used herein, ranges and amounts can be expressed as "about" a particular value or range. About also includes the exact amount. Hence "about 5 μL" means "about 5 μL" and also "5 μL." Generally, the term "about" includes an amount that would be expected to be within experimental error.

[0384] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0385] As used herein, the terms "individual(s)", "subject(s)" and "patient(s)" mean any mammal. In some embodiments, the mammal is a human. In some embodiments, the mammal is a non-human. None of the terms require or are limited to situations characterized by the supervision (e.g. constant or intermittent) of a health care worker (e.g. a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly or a hospice worker).

EXAMPLES

[0386] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

[0387] **Example 1. Antisense oligonucleotide sequences and synthesis**

[0388] Phosphorodiamidate morpholino oligomers (PMO), phosphorothioate antisense oligonucleotides (PS ASO), and antisense oligonucleotides (ASOs) were synthesized.

[0389] The PMO sequence was 5'GGCCAAACCTCGGCTTACCTGAAAT3' Primary amine (SEQ ID NO: 28) and can be seen in **Fig. 1** with end nucleotides expanded. The PMO contains a C3-NH_2 conjugation handle at the 3' end of the molecule for conjugation. PMOs were fully assembled on solid phase using standard solid phase synthesis protocols and purified over HPLC.

[0390] The PS ASO sequence was Amine-C6-GGCCAAACCUCGGCUUACCU (SEQ ID NO: 29) and can be seen in **Figs. 2A-2B** with end nucleotides expanded. The structure of the PS ASO comprised a phosphate backbone that was 100% phosphorothioate linkages and all the ribose sugars contained a 2' 2'OMe modification. The PS ASO also contained a C6-NH_2 conjugation handle at the 5' end of the molecule for conjugation. The PS ASOs were fully assembled on the solid phase using standard solid phase phosphoramidite chemistry and purified over HPLC.

[0391] ASOs were fully assembled on the solid phase using standard solid phase phosphoramidite chemistry and purified over HPLC. ASOs contained a C6-NH_2 conjugation handle at the 5' end of the molecule for conjugation.

[0392] **Example 2. Detection of *DMD* exon skipping**

[0393] *Methods for Determining DMD Exon 23 Skipping in Differentiated C1C12 Cells*

[0394] Mouse myoblast C2C12 cells were plated at 50,000-100,000/well in 24-well plates in 0.5 mL 10% FBS RPMI 1640 media and incubated at 37 ºC with 5% CO_2 overnight. On the second day, cells were switched to differentiation media (2% horse serum RPMI 1640 and 1 μM insulin) and incubated for 3-5 days. Following incubation, samples were added and incubated for 24 hours. After the sample treatment, 1 mL of fresh media (with no compounds) was changed every day for 2 more days. At 72 hours after the start of treatments, cells were harvested. RNAs were isolated using InviTrap RNA Cell HTS 96 Kit (B-Bridge International #7061300400) and reverse transcribed using High Capacity cDNA Reverse transcription Kit (ThermoFisher #4368813). PCR reactions were performed using DreamTaqTM PCR Mastermix (ThermoFisher #K1072). The primary PCR used primers in exon 20 (Ex20F 5'-CAGAATTCTGCCAATTGCTGAG) (SEQ ID NO: 30) and exon 26 (Ex26R 5'-TTCTTCAGCTTGTGTCATCC) (SEQ ID NO: 31) to amplify both skipped and unskipped molecules using the protocol in **Table 2.**

Table 2. PCR Protocol

Hot Start	95 °C for 2 minutes
Denaturation	95 °C for 0.5 minute
Annealing of primers	50°C for 0.5 minute
Primer extension	72°C for 1 minute
Final extension	72°C for 5 minutes
Number of Cycles	10

[0395] For the nested PCR, primary PCR reactions were diluted with water 100X, and 5 μl was used for nested PCR reaction (50 μl total reaction volume). Nested PCR used primers in exon 20 (Ex20F2: 5'-ACCCAGTCTACCACCCTATC) (SEQ ID NO: 32) and exon 25 (Ex25R: 5'-CTCTTTATCTTCTGCCCACCTT) (SEQ ID NO: 33) to amplify both skipped and unskipped molecules using the protocol in **Table 3.**

Table 3. Nested PCR Protocol

Hot Start	95 °C for 2 minutes
Denaturation	95 °C for 0.5 minute
Annealing of primers	50°C for 0.5 minute
Primer extension	72°C for 1 minute
Final extension	72°C for 5 minutes
Number of Cycles	35

[0396] PCR reactions were analyzed using 4% TAE agarose gels. The wild-type (WT) *DMD* product had an expected size of 788 base pairs and the skipped *DMD* Δ23 of 575 base pairs.

[0397] *Animals*

[0398] All animal studies were conducted following protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) at Explora BioLabs, which adhere to the regulations outlined in the USDA Animal Welfare Act as well as the "Guide for the Care and Use of Laboratory Animals" (National Research Council publication, 8th Ed., revised in 2011). All mice were obtained from either Charles River Laboratories or Harlan Laboratories.

[0399] *In vivo mouse model*

[0400] WT CD-1 mice (4-6 weeks old) were dosed via intravenous (iv) injection with the indicated antisense conjugates (ASCs) and doses. The "naked" PMO or ASO were dosed via intramuscular injection at the indicated doses. After 4, 7, or 14 days, heart and gastrocnemius muscle tissues were harvested and snap-frozen in liquid nitrogen. RNAs were isolated with Trizol and RNeasy Plus 96 Kit (Qiagen, #74192) and reversed transcribed using High Capacity cDNA Reverse transcription Kit (ThermoFisher #4368813). Nested PCR reactions were performed as described. PCR reactions were analyzed in 4% TAE agarose gels which were quantitated by densitometry.

[0401] To confirm exon 23 skipping in treated mice, DNA fragments were isolated from the 4% agarose gels and sequenced.

[0402] To quantitatively determine the skipped *DMD* mRNA copy number, qPCR primer/probe sets were designed to quantify skipped and WT *DMD* mRNA (**Fig. 3**). qPCR quantification standards were designed and produced via PCR using designed PCR primers as seen in **Table 4**. For the qPCR standard for WT and DMD, following PCR a 733 base pair fragment was isolated from the agarose gel. For qPCR standard for skipped DMA, the nested primers were used.

[0403] The amplification efficiency of the qPCR primer/probes were determined to be within 10% of expected efficiency. qPCR reactions were performed in QuantStudio 7 and TaqmanTM PCR Universal Mastermix II (ThermoFisher #4440041) according to manufacturer's instructions.

Table 4.

	SEQ ID NO	**Primer/Probe**	**Sequence**
DMD Δ-23, for Ex23 skipping	34	Forward Primer	5' GCGCTATCAGGAGACAATGAG
	35	Reverse Primer	5' GTTTTTATGTGATTCTGTAATTTCCC
	36	Probe	5' CTCTCTGTACCTTATCTTAGTGTT
DMD Ex22-23, for WT DMD only	37	Forward Primer	5' TGGAGGAGAGACTCGGGAAA
	38	Reverse Primer	5' TTGAAGCCATTTTGTTGCTCTTT
	39	Probe	5' ACAGGCTCTGCAAAGT
DMD Ex20-21, for All DMD	40	Forward Primer	5' AACAGATGACAACTACTGCCGAAA
	41	Reverse Primer	5' TTGGCTCTGATAGGGTGGTAGAC
	42	Probe	5' CTTGTTGAAAACCC
qPCR standard for WT and all DMD	43	Forward Primer	5' TGAGGGTGTTAATGCTGAAAGTA
	44	Reverse Primer	5' CACCAACTGGGAGGAAAGTT

[0404] **Example 3: Conjugate Synthesis**

[0405] *Analytical and Purification Methods*

[0406] Analytical and purification methods were performed according to **Tables 5-11.**

Table 5. Size exclusion chromatography (SEC) methods

Size Exclusion Chromatography (SEC) Method	Column	Mobile Phase	Flow Rate
method 1	TOSOH Biosciences, TSKgelG3000SW XL, 7.8 X 300 mm, 5 μM	150 mM phosphate buffer	1.0 mL/minute for 20 minutes
method 2	TOSOH Biosciences, TSKgelG3000SW, 21.5 X 600 mm, 5 μM	PBS pH 7.4	1.0 mL/minute for 180 minutes

Table 6. Hydrophobic interaction chromatography (HIC) method 1

Column	Solvent	Gradient		
		Column Volume	%A	%B
GE, HiScreen Butyl HP, 4.7 mL	**Solvent A**: 50 mM phosphate buffer, 0.8M Ammonium Sulfate, pH 7.0 **Solvent B**: 80% 50 mM phosphate buffer, 20% IPA, pH 7.0 **Flow Rate**: 1.0 mL/minute	1.00 30 5	95 0 0	5 100 100

Table 7. Hydrophobic interaction chromatography (HIC) method 2

Column	Solvent	Gradient		
		Time	%A	%B
Thermo Scientific, MAbPac HIC-20, 4.6 mm ID X 10 cm, 5 um	**Solvent A**: 100 mM phosphate buffer, 1.8 M Ammonium Sulfate, pH 7.0 **Solvent B**: 80% 100 mM phosphate buffer, 20% IPA, pH 7.0 **Flow Rate**: 0.7 mL/minute	0.00 2.00 22.00 25.00 26.00 30.00	100 100 0 0 100 100	0 0 100 100 0 0

Table 8. Hydrophobic interaction chromatography (HIC) method 3

Column	Solvent	Gradient		
		Column Volume	%A	%B
GE, HiScreen Butyl HP, 4.7 mL	**Solvent A**: 50 mM phosphate buffer, 0.8 M Ammonium Sulfate, pH 7.0 **Solvent B**: 80% 50 mM phosphate buffer, 20% IPA, pH 7.0 **Flow Rate**: 1.0 mL/minute	1 25 1 2	100 0 0 0	0 80 100 100

Table 9. Hydrophobic interaction chromatography (HIC) method 4

Column	Solvent	Gradient		
		Time	%A	%B
Thermo Scientific, MAbPac HIC-20, 4.6 mm ID X 10 cm, 5 um	**Solvent A**: 100 mM phosphate buffer, 1.8 M Ammonium Sulfate, pH 7.0 **Solvent B**: 80% 100 mM phosphate buffer, 20% IPA, pH 7.0 **Flow Rate**: 0.5 mL/minute	0.00 5.00 20.00 25.00 26.00 30.00	100 100 0 0 100 100	0 0 100 100 0 0

Table 10. Strong anion exchange chromatography (SAX) method 1

Column	Solvent	Gradient		
		Column Volume	%A	%B
Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um	**Solvent A**: 20 mM TRIS buffer, pH 8.0; **Solvent B**: 20 mM TRIS, 1.5 M NaCl, pH 8.0 **Flow Rate**: 6.0 mL/minute	0.5 0.5 17 0.5 0.5	100 80 20 0 0	0 20 80 100 100

Table 11. Strong anion exchange chromatography (SAX) method 2

Column	Solvent	Gradient		
		Time	%A	%B
Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm	**Solvent A**: 80% 10 mM TRIS pH 8, 20% ethanol **Solvent B**: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl **Flow Rate**: 0.75 mL/minute	0.0 3.00 17.00 21.00 22.00 25.00	90 90 0 0 90 90	10 10 100 100 10 10

[0407] *Anti-transferrin receptor antibody*

[0408] Anti-mouse transferrin receptor antibody or anti-CD71 mAb that was used was a rat IgG2a subclass monoclonal antibody that binds mouse CD71 or mouse transferrin receptor 1 (mTfR1). The antibody was produced by BioXcell and it is commercially available (Catalog # BE0175).

[0409] **Anti-CD71 antibody morpholino antisense oligonucleotide conjugate (anti-CD71 mAb-PMO)**

[0410] *Anti-CD71 mAb-PMO conjugation*

[0411] Anti-CD71 antibody (10 mg/mL) in borate buffer (25 mM sodium tetraborate, 25 mM NaCl, 1 mM Diethylene triamine pentaacetic acid, pH 8.0) was reduced by adding 4 equivalents of tris(2-carboxyethyl)phosphine (TCEP) in water and incubating at 37 °C for 4 hours. 4(N-Maleimidomethyl)cyclohexanecarboxylic acid N-hydroxysuccinimide ester (SMCC) was coupled to the primary amine on the 3' end of the phosphorodiamidate morpholino oligomer (PMO) by incubating the PMO (50 mg/mL) in DMSO with 10 equivalents of SMCC (10 mg/mL) in DMSO for one hour. Unconjugated SMCC was removed by ultrafiltration using Amicon Ultra-15 centrifugal filter units with a MWCO of 3 kDa. The PMO-SMCC was washed three times with acetate buffer (10 mM sodium acetate, pH 6.0) and used immediately. The reduced antibody was mixed with 2.25 equivalents of PMO-SMCC and incubated overnight at 4 °C. The pH of the reaction mixture was then reduced to 7.5, and 8 equivalents of N-Ethylmaleimide was added to the mixture at room temperature for 30 minutes to quench unreacted cysteines. Analysis of the reaction mixture by hydrophobic interaction chromatography (HIC) method 2 showed antibody-PMO conjugates along with unreacted antibody and PMO (**Fig. 4**). **Fig. 4** shows a chromatogram of anti-CD71 mAb-PMO reaction mixture produced with HIC method 2 showing free antibody peak (1), free PMO (2), DAR 1 (3), DAR 2 (4), DAR 3 (5), DAR > 3 (6). "DAR" refers to a drug-to-antibody ratio. The number in parentheses refers to the peak in the chromatogram.

[0412] *Purification*

[0413] The reaction mixture was purified with an AKTA Explorer FPLC using HIC method 1. Fractions containing conjugates with a drug to antibody ratio of one (DAR 1) and two (DAR 2) were combined and concentrated with Amicon Ultra-15 centrifugal filter units with a MWCO of 50 kDa separately from conjugates with a DAR greater than 2. Concentrated conjugates were buffer exchanged with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units prior to analysis.

[0414] *Analysis of the purified conjugate*

[0415] The isolated conjugates were characterized by size exclusion chromatography (SEC) and HIC. SEC method 1 was used to confirm the absence of high molecular weight aggregates and unconjugated PMOs (**Figs. 5A-5C**). **Fig. 5A** shows a chromatogram of anti-CD71 mAb produced using SEC method 1. **Fig. 5B** shows a chromatogram of anti-CD71 mAb-PMO DAR 1,2 produced using SEC method 1. **Fig. 5C** shows a chromatogram of anti-CD71 mAb-PMO DAR greater than 2 produced using SEC method 1. "DAR" refers to a drug-to-antibody ratio.

[0416] The purity of the conjugate was assessed by analytical HPLC using HIC method 2 (**Figs. 6A-6C**). **Fig. 6A** shows a chromatogram of anti-CD71 mAb produced using HIC method 2. **Fig. 6B** shows a chromatogram of purified anti-CD71 mAb-PMO DAR 1,2 conjugate produced using HIC method 2. **Fig. 6C** shows a chromatogram of purified anti-CD71 mAb-PMO DAR >2 conjugate produced using HIC method 2. The 260/280nm UV absorbance ratio of each sample was compared to a standard curve of known ratios of PMO and antibody to confirm DAR. The DAR 1,2 sample had an average DAR of ~1.6 while the DAR greater than 2 sample had an average DAR of ~3.7. "DAR" refers to a drug-to-antibody ratio.

[0417] **Anti-CD71 Fab morpholino antisense oligonucleotide conjugate (anti-CD71 Fab-PMO)**

[0418] *Antibody digestion with pepsin*

[0419] Anti-CD71 antibody (5 mg/mL) in 20 mM acetate buffer (pH 4.0) was incubated with immobilized pepsin for 3 hours at 37 °C. The resin was removed and the reaction mixture was washed with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units with a MWCO of 30 kDa. The retentate was collected and purified using size exclusion chromatography (SEC) method 2 to isolate the F(ab')2 fragment.

[0420] *Anti-CD71 (Fab)-PMO conjugation*

[0421] The F(ab')2 fragment (15 mg/mL) in borate buffer (pH 8.0) was reduced by adding 10 equivalents of TCEP in water and incubating at 37 °C for 2 hours. SMCC was added to the primary amine on the 3' end of the PMO by incubating the PMO (50 mg/mL) in DMSO with 10 equivalents of SMCC (10 mg/mL) in DMSO for 1 hour. Unconjugated SMCC was removed by ultrafiltration using Amicon Ultra-15 centrifugal filter units with a MWCO of 3 kDa. The PMO-SMCC was washed three times with acetate buffer (pH 6.0) and used immediately. The reduced F(ab') fragment (Fab) was buffer exchanged into borate buffer (pH 8.0) using Amicon Ultra-15 Centrifugal Filter Units with a MWCO of 10 kDa, and 1.75 equivalents of PMO-SMCC was added and incubated overnight at 4 °C. The pH of the reaction mixture was then reduced to 7.5, and 6 equivalents of N-Ethylmaleimide was added to the mixture at room temperature for 30 minutes to quench unreacted cysteines. Analysis of the reaction

mixture by hydrophobic interaction chromatography (HIC) method 3 showed anti-CD71 (Fab)-PMO conjugates along with unreacted Fab (**Fig. 7A**). **Fig. 7A** shows a chromatogram of FPLC purification of anti-CD71 Fab-PMO using HIC method 3.

[0422] *Purification*

[0423] The reaction mixture was purified with an AKTA Explorer FPLC using HIC method 3. Fractions containing conjugates with a DAR of one, two and three were combined and concentrated separately. Concentrated conjugates were buffer exchanged with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units with a MWCO of 10 kDa prior to analysis.

[0424] *Analysis of the purified conjugate*

[0425] The isolated conjugates were characterized by SEC, and HIC. SEC method 1 was used to confirm the absence of high molecular weight aggregates and unconjugated PMO. *See **Figs. 7B-7E**.* **Fig. 7B** shows a chromatogram of anti-CD71 Fab produced using SEC method 1. **Fig. 7C** shows a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using SEC method 1. **Fig. 7D** shows a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using SEC method 1. **Fig. 7E** shows a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using SEC method 1. The purity of the conjugate was assessed by analytical HPLC using HIC method 4. *See **Figs. 7F-7I.*** **Fig. 7F** shows a chromatogram of anti-CD71 Fab produced using HIC method 4. **Fig. 7G** shows a chromatogram of anti-CD71 Fab-PMO DAR 1 conjugate produced using HIC method 4. **Fig. 7H** shows a chromatogram of anti-CD71 Fab-PMO DAR 2 conjugate produced using HIC method 4. **Fig. 7I** shows a chromatogram of anti-CD71 Fab-PMO DAR 3 conjugate produced using HIC method 4. "DAR" refers to drug-to-antibody ratio. The 260/280nm UV absorbance ratio of each sample was compared to a standard curve of known ratios of PMO and Fab to confirm DAR.

[0426] Anti-CD71 antibody phosphorothioate antisense oligonucleotide conjugate (anti-CD71 mAb-PS ASO)

[0427] *Anti-CD71 mAb-PS ASO*

[0428] Anti-CD71 antibody (10 mg/mL) in borate buffer (pH 8.0) was reduced by adding 4 equivalents of TCEP in water and incubating at 37°C for 4 hours. 4(N-Maleimidomethyl)cyclohexanecarboxylic acid N-hydroxysuccinimide ester (SMCC) was added to the primary amine on the 5' end of the PS-ASO by incubating the PS ASO (50 mg/mL) in 1:1 mixture of 250 mM PB (pH 7.5) and DMSO with 10 equivalents of SMCC (10 mg/mL) in DMSO for 1 hour. Unconjugated SMCC was removed by ultrafiltration using Amicon Ultra-15 centrifugal filter units with a MWCO of 3 kDa. The PS ASO-SMCC was washed three times with acetate buffer (pH 6.0) and used immediately. The reduced antibody was mixed with 1.7 equivalents of PS ASO-SMCC and incubated overnight at 4°C. The pH of the reaction mixture was then reduced to 7.4, and 8 equivalents of N-Ethylmaleimide was added to the mixture at room temperature for 30 minutes to quench unreacted cysteines. Analysis of the reaction mixture by strong anion exchange chromatography (SAX) method 2 showed antibody-PS ASO conjugates along with unreacted antibody and ASO (**Fig. 8A**). **Fig. 8A** shows a chromatogram of anti-CD71 mAb-PS ASO reaction mixture produced with SAX method 2 showing

free antibody peak (1), free PS ASO (5), DAR 1 (2), DAR 2 (3), DAR > 2 (4). "DAR" refers to a drug-to-antibody ratio. The number in parentheses refers to the peak.

[0429] *Purification*

[0430] The reaction mixture was purified with an AKTA Explorer FPLC using SAX method 1. Fractions containing conjugates with a drug-to-antibody ratio (DAR) of one, two and three were combined and concentrated separately and buffer exchanged with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units with a MWCO of 50 kDa prior to analysis.

[0431] *Analysis of the purified conjugate*

[0432] The isolated conjugates were characterized by size exclusion chromatography (SEC) and SAX. Size exclusion chromatography method 1 was used to confirm the absence of high molecular weight aggregates and unconjugated ASO. *See* ***Figs. 8B-8E.*** **Fig. 8B** shows a chromatogram of anti-CD71 mAb produced using SEC method 1. **Fig. 8C** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SEC method 1. **Fig. 8D** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SEC method 1. **Fig. 8E** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SEC method 1. The purity of the conjugate was assessed by analytical HPLC using SAX method 2. *See* ***Figs. 8F-8H.*** **Fig. 8F** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 1 conjugate produced using SAX method 2. **Fig. 8G** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 2 conjugate produced using SAX method 2. **Fig. 8H** shows a chromatogram of anti-CD71 mAb-PS ASO DAR 3 conjugate produced using SAX method 2. The 260/280nm UV absorbance ratio of each sample was compared to a standard curve of known ratios of ASO and antibody to confirm drug-to-antibody ratio (DAR).

[0433] **Example 4: *In vitro* activity of anti-CD71 mAb-PMO conjugate**

[0434] The anti-CD71 mAb-PMO conjugate was made and characterized as described in Example 3. The conjugate was assessed for its ability to mediate exon skipping in vitro in differentiated C2C12 cells using nested PCR using methods similar to Example 2. Briefly, the potency of "naked" morpholino ASO ("PMO") was compared to an anti-CD71 mAb-PMO conjugate at multiple concentrations with the relevant vehicle controls. Controls included vehicle ("Veh"), scramble morpholino at 50 uM ("Scr50"), and no antibody ("Neg-Ab"). The concentrations of PMO used included 50 uM, 1 uM, and 0.02 uM. The concentrations of anti-CD71 mAB-PMO DAR 1,2 used included 200 nM, 20 nM, and 2 nM. "DAR" refers to drug-to-antibody ratio.

[0435] Following cDNA synthesis, two rounds of PCR amplification (primary and nested PCR) were used to detect exon-skipping. PCR reactions were analyzed in a 4% TAE agarose gel (**Fig. 9**).

[0436] Referring to **Fig. 9**, anti-CD71 mAb-PMO conjugate produced measurable exon 23 skipping in differentiated C2C12 cells and lower concentrations than the "naked" PMO control. The wild-type product had an expected size of 788 base pairs and the skipped DMD Δ23 of 575 base pairs.

[0437] A second experiment included an anti-CD71 Fab-PMO conjugate and a PMO targeted with an anti-EGFR ("Z-PMO") as a negative control (**Fig. 10**). The concentrations of PMO used included 10 uM and 2 uM. The concentrations of anti-CD71 mAb-PMO used included 0.2 uM and 0.04 uM. Anti-CD71

mAb-PMO had a DAR of 2. Z-PMO was used at a concentration of 0.2 uM and had a DAR of 2. Concentrations of anti-CD71 Fab-PMO included 0.6 uM and 0.12 uM. DAR of 1, 2, and 3 for anti-CD71 mAb-PMO at 0.6 uM and 0.12 uM were assayed.

[0438] Referring to **Fig. 10**, Receptor mediated uptake utilizing the transferrin receptor, the anti-CD71 mAb-PMO, and anti-CD71 Fab-PMO conjugates resulted in measurable exon 23 skipping in C2C12 cells and lower concentrations than the "naked" PMO control. There was no measurable exon 23 skipping from the Z-PMO at the concentration tested, which produced skipping from the anti-CD71 conjugates.

[0439] **Example 5. *In vitro* activity of anti-CD71-ASO mAb PS conjugate**

[0440] The anti-CD71 mAb-PS ASO conjugate was made and characterized as described in Example 3. The conjugate was assessed for its ability to mediate exon skipping in vitro in differentiated C2C12 cells using nested PCR using similar methods as described in Example 2. Briefly, the potency of "naked" phosphorothioate ASO (PS ASO) was compared to an anti-CD71 mAb-PS ASO conjugate at multiple concentrations, with the relevant vehicle control. Two rounds of of PCR amplification (primary and nested PCR) were performed following cDNA synthesis to detect exon-skipping. PCR reactions were analyzed in a 4% TAE agarose gel (**Fig. 11**). **Fig. 11** shows an agarose gel of PMO, ASO, conjugated anti-CD71 mAb-ASO of DAR1 ("ASC-DAR1"), conjugated anti-CD71 mAb-ASO of DAR2 ("ASC-DAR2"), and conjugated anti-CD71 mAb-ASO of DAR3 ("ASC-DAR3"). "PMO" and "ASO" refers to free PMO and ASO, unconjugated to antibody. "Veh" refers to vehicle only. The concentrations tested included 0.2, 1, and 5 micromolar (μM).

[0441] Referring to **Fig. 11**, the anti-CD71 mAb-PS ASO conjugate produced measurable exon 23 skipping in differentiated C2C12 cells and lower concentrations than the "naked" PS ASO control. The wild-type product had an expected size of 788 base pairs and the skipped DMD Δ23 of 575 base pairs.

[0442] **Example 6: *In vivo* activity of anti-CD71 mAb-PMO conjugate**

[0443] The anti-CD71 mAb-PMO conjugate was made and characterized as described in Example 3. The conjugate anti-CD71 mAb-PMO DAR1,2 anti-CD71 and mAb-PMO DAR>2 were assessed for its ability to mediate exon skipping *in vivo* in wild-type CD-1 mice using similar methods as described in Example 2. "DAR" refers to drug-to-antibody ratio.

[0444] Mice were dosed via intravenous (iv) injection with the mAb, vehicle control, and antisense conjugates (ASCs) at the doses as provided in **Table 12**. "DAR" refers to drug-to-antibody ratio. The "naked" PMO was dosed via intramuscular injection into the gastrocnemius muscle at the doses provided in **Table 12**. After 4, 7, or 14 days, heart and gastrocnemius muscle tissues were harvested and snap-frozen in liquid nitrogen. RNAs were isolated, reversed transcribed and a nested PCR reactions were performed. PCR reactions were analyzed in 4% TAE agarose gels which were then quantitated by densitometry.

Table 12. *In vivo* study design

Group	Test Article	N	mAb dose (mg/kg)	PMO Dose (mg/kg)	PMO: mAb Ratio	Harvest Time

					(mol/mol)	(h)
1	anti-CD71 mAb-PMO, DAR1,2	3	50	4.8	1.6	96
2	anti-CD71 mAb-PMO, DAR1,2	3	50	4.8	1.6	168
3	anti-CD71 mAb-PMO, DAR1,2	3	50	4.8	1.6	336
4	anti-CD71 mAb-PMO, DAR>2	3	50	10.5	3.7	96
5	anti-CD71 mAb-PMO, DAR>2	3	50	10.5	3.7	168
6	anti-CD71 mAb-PMO, DAR>2	3	50	10.5	3.7	336
7	anti-CD71 mAb	3	50			96
8	anti-CD71 mAb	3	50			168
9	anti-CD71 mAb	3	50			336
10	PMO	3	40 ug/inj.			96
11	PMO	3	40 ug/inj.			168
12	PMO	3	40 ug/inj.			336
13	Vehicle	3				96
14	Vehicle	3				168
15	Vehicle	3				336

[0445] **Fig. 12A** shows a gel electrophoresis of gastrocnemius muscle samples from mice administered anti-CD71 mAb-PMO DAR 1,2, anti-CD71 mAb-PMO DAR>2, anti-CD71 mAb, PMO, and vehicle for 4, 7, or 14 days. The wild-type product had an expected size of 788 base pairs and the skipped DMD Δ23 of 575 base pairs. Anti-CD71 mAb-PMO DAR 1,2 and anti-CD71 mAb-PMO DAR>2 produced measurable exon 23 skipping in gastrocnemius muscle and lower concentrations than the "naked" PMO control. The intensity of the bands on the gel (**FIG. 12A**) was quantitated by densitometry as seen in **Fig. 12B**. **Fig. 12C** shows the quantification of *in vivo* exon skipping in wild-type mice gastrocnemius muscle using Taqman qPCR.

[0446] **Fig. 13A** shows a gel electrophoresis of heart samples from mice administered anti-CD71 mAb-PMO DAR 1,2, anti-CD71 mAb-PMO DAR>2, anti-CD71 mAb, PMO, and vehicle for 4, 7, or 14 days. The wild-type product had an expected size of 788 base pairs and the skipped DMD Δ23 of 575 base pairs. The intensity of the bands on the gel (**FIG. 13A**) was quantitated by densitometry as seen in **Fig. 13B**. Similar results as with the gastrocnemius muscle samples were obtained. Anti-CD71 mAb-PMO DAR 1,2 and anti-CD71 mAb-PMO DAR>2 produced measurable exon 23 skipping in gastrocnemius muscle and lower concentrations than the "naked" PMO control.

[0447] DNA fragments were then isolated from the 4% agarose gels and sequenced. The sequencing data confirmed the correct sequence in the skipped and wild-type products as seen in **Fig. 14**.

[0448] **Example 7. Sequences**

[0449] **Table 13** illustrates exemplary target sequences to induce insertion, deletion, duplications, or alteration in the *DMD* gene using compositions and methods as described herein. **Table 14** illustrates exemplary nucleotide sequences to induce an insertion, deletion, duplication, or alteration in the *DMD* gene using compositions and methods as described herein. **Table 15** and **Table 16** illustrate exemplary target sequences in several genes for inducing an insertion, deletion, duplications, or alteration in the gene. **Table 17** illustrates exemplary sequences, including sequences in the *DMD* gene to induce an

insertion, deletion, duplication, or alteration in the gene using compositions and methods as described herein.

Table 13.

Target Exon	Antisense Sequence	SEQ ID NO.
19	5' GCCUGAGCUGAUCUGCUGGCAUCUUGCAGUU 3'	45
19 or 20	5'GCAGAAUUCGAUCCACCGGCUGUUCAAGCCUGAGC UGAUCUGCUCGCAUCUUGCAGU3'	46
20	5' CAGCAGUAGUUGUCAUCUGCUC 3'	47
21	5' CACAAAGUCUGCAUCCAGGAACAUGGGUC 3'	48
22	5' CUGCAAUUCCCCGAGUCUCUGC 3'	49
51	5' CUCAUACCUUCUGCUUGAUGAUC 3'	50
52	5' UCCAACUGGGGACGCCUCUGUUCCAAAUCC 3'	51

Table 14.

Gene	Target Location	Nucleotide Sequence (5'-3')	SEQ ID NO.
DMD	H8A(−06+18)	GAUAGGUGGUAUCAACAUCUGUAA	52
DMD	H8A(−03+18)	GAUAGGUGGUAUCAACAUCUG	53
DMD	H8A(−07+18)	GAUAGGUGGUAUCAACAUCUGUAAG	54
DMD	H8A(−06+14)	GGUGGUAUCAACAUCUGUAA	55
DMD	H8A(−10+10)	GUAUCAACAUCUGUAAGCAC	56
DMD	H7A(+45+67)	UGCAUGUUCCAGUCGUUGUGUGG	57
DMD	H7A(+02+26)	CACUAUUCCAGUCAAAUAGGUCUGG	58
DMD	H7D(+15−10)	AUUUACCAACCUUCAGGAUCGAGUA	59
DMD	H7A(−18+03)	GGCCUAAAACACAUACACAUA	60
DMD	C6A(−10+10)	CAUUUUUGACCUACAUGUGG	61
DMD	C6A(−14+06)	UUUGACCUACAUGUGGAAAG	62
DMD	C6A(−14+12)	UACAUUUUUGACCUACAUGUGGAAAG	63
DMD	C6A(−13+09)	AUUUUUGACCUACAUGGGAAAG	64
DMD	CH6A(+69+91)	UACGAGUUGAUUGUCGGACCCAG	65
DMD	C6D(+12−13)	GUGGUCUCCUUACCUAUGACUGUGG	66
DMD	C6D(+06−11)	GGUCUCCUUACCUAUGA	67
DMD	H6D(+04−21)	UGUCUCAGUAAUCUUCUUACCUAU	68
DMD	H6D(+18−04)	UCUUACCUAUGACUAUGGAUGAGA	69
DMD	H4A(+13+32)	GCAUGAACUCUUGUGGAUCC	70
DMD	H4D(+04−16)	CCAGGGUACUACUUACAUUA	71
DMD	H4D(−24−44)	AUCGUGUGUCACAGCAUCCAG	72
DMD	H4A(+11+40)	UGUUCAGGGCAUGAACUCUUGUGGAUCCUU	73
DMD	H3A(+30+60)	UAGGAGGCGCCUCCCAUCCUGUAGGUCACUG	74
DMD	H3A(+35+65)	AGGUCUAGGAGGCGCCUCCCAUCCUGUAGGU	75
DMD	H3A(+30+54)	GCGCCUCCCAUCCUGUAGGUCACUG	76
DMD	H3D(+46−21)	CUUCGAGGAGGUCUAGGAGGCGCCUC	77
DMD	H3A(+30+50)	CUCCCAUCCUGUAGGUCACUG	78
DMD	H3D(+19−03)	UACCAGUUUUUGCCCUGUCAGG	79
DMD	H3A(−06+20)	UCAAUAUGCUGCUUCCCAAACUGAAA	80
DMD	H3A(+37+61)	CUAGGAGGCGCCUCCCAUCCUGUAG	81

DMD	H5A(+20+50)	UUAUGAUUUCCAUCUACGAUGUCAGUACUUC	82
DMD	H5D(+25−05)	CUUACCUGCCAGUGGAGGAUUAUAUUCCAAA	83
DMD	H5D(+10−15)	CAUCAGGAUUCUUACCUGCCAGUGG	84
DMD	H5A(+10+34)	CGAUGUCAGUACUUCCAAUAUUCAC	85
DMD	H5D(−04−21)	ACCAUUCAUCAGGAUUCU	86
DMD	H5D(+16−02)	ACCUGCCAGUGGAGGAUU	87
DMD	H5A(−07+20)	CCAAUAUUCACUAAAUCAACCUGUUAA	88
DMD	H5D(+18−12)	CAGGAUUGUUACCUGCCAGUGGAGGAUUAU	89
DMD	H5A(+05+35)	ACGAUGUCAGUACUUCCAAUAUUCACUAAAU	90
DMD	H5A(+15+45)	AUUUCCAUCUACGAUGUCAGUACUUCCAAUA	91
DMD	H10A(−05+16)	CAGGAGCUUCCAAAUGCUGCA	92
DMD	H10A(−05+24)	CUUGUCUUCAGGAGCUUCCAAAUGCUGCA	93
DMD	H10A(+98+119)	UCCUCAGCAGAAAGAAGCCACG	94
DMD	H10A(+130+149)	UUAGAAAUCUCUCCUUGUGC	95
DMD	H10A(−33−14)	UAAAUUGGGUGUUACACAAU	96
DMD	H11D(+26+49)	CCCUGAGGCAUUCCCAUCUUGAAU	97
DMD	H11D(+11−09)	AGGACUUACUUGCUUUGUUU	98
DMD	H11A(+118+140)	CUUGAAUUUAGGAGAUUCAUCUG	99
DMD	H11A(+75+97)	CAUCUUCUGAUAAUUUUCCUGUU	100
DMD	H12A(+52+75)	UCUUCUGUUUUUGUUAGCCAGUCA	101
DMD	H12A(−10+10)	UCUAUGUAAACUGAAAAUUU	102
DMD	H12A(+11+30)	UUCUGGAGAUCCAUUAAAAC	103
DMD	H13A(+77+100)	CAGCAGUUGCGUGAUCUCCACUAG	104
DMD	H13A(+55+75)	UUCAUCAACUACCACCACCAU	105
DMD	H13D(+06−19)	CUAAGCAAAAUAAUCUGACCUUAAG	106
DMD	H14A(+37+64)	CUUGUAAAAGAACCCAGCGGUCUUCUGU	107
DMD	H14A(+14+35)	CAUCUACAGAUGUUUGCCCAUC	108
DMD	H14A(+51+73)	GAAGGAUGUCUUGUAAAAGAACC	109
DMD	H14D(−02+18)	ACCUGUUCUUCAGUAAGACG	110
DMD	H14D(+14−10)	CAUGACACACCUGUUCUUCAGUAA	111
DMD	H14A(+61+80)	CAUUUGAGAAGGAUGUCUUG	112
DMD	H14A(−12+12)	AUCUCCCAAUACCUGGAGAAGAGA	113
DMD	H15A(−12+19)	GCCAUGCACUAAAAAGGCACUGCAAGACAUU	114
DMD	H15A(+48+71)	UCUUUAAAGCCAGUUGUGUGAAUC	115
DMD	H15A(+08+28)	UUUCUGAAAGCCAUGCACUAA	116
DMD	H15D(+17−08)	GUACAUACGGCCAGUUUUUGAAGAC	117
DMD	H16A(−12+19)	CUAGAUCCGCUUUUAAAACCUGUUAAAACAA	118
DMD	H16A(−06+25)	UCUUUUCUAGAUCCGCUUUUAAAACCUGUUA	119
DMD	H16A(−06+19)	CUAGAUCCGCUUUUAAAACCUGUUA	120
DMD	H16A(+87+109)	CCGUCUUCUGGGUCACUGACUUA	121
DMD	H16A(−07+19)	CUAGAUCCGCUUUUAAAACCUGUUAA	122
DMD	H16A(−07+13)	CCGCUUUUAAAACCUGUUAA	123
DMD	H16A(+12+37)	UGGAUUGCUUUUUCUUUUCUAGAUCC	124
DMD	H16A(+92+116)	CAUGCUUCCGUCUUCUGGGUCACUG	125
DMD	H16A(+45+67)	GAUCUUGUUUGAGUGAAUACAGU	126
DMD	H16A(+105+126)	GUUAUCCAGCCAUGCUUCCGUC	127

DMD	H16D(+05−20)	UGAUAAUUGGUAUCACUAACCUGUG	128
DMD	H16D(+12−11)	GUAUCACUAACCUGUGCUGUAC	129
DMD	H19A(+35+53)	CUGCUGGCAUCUUGCAGUU	130
DMD	H19A(+35+65)	GCCUGAGCUGAUCUGCUGGCAUCUUGCAGUU	131
DMD	H20A(+44+71)	CUGGCAGAAUUCGAUCCACCGGCUGUUC	132
DMD	H20A(+147+168)	CAGCAGUAGUUGUCAUCUGCUC	133
DMD	H20A(+185+203)	UGAUGGGGUGGUGGGUUGG	134
DMD	H20A(−08+17)	AUCUGCAUUAACACCCUCUAGAAAG	135
DMD	H20A(+30+53)	CCGGCUGUUCAGUUGUUCUGAGGC	136
DMD	H20A(−11+17)	AUCUGCAUUAACACCCUCUAGAAAGAAA	137
DMD	H20D(+08−20)	GAAGGAGAAGAGAUUCUUACCUUACAAA	138
DMD	H20A(+44+63)	AUUCGAUCCACCGGCUGUUC	139
DMD	H20A(+149+168	CAGCAGUAGUUGUCAUCUGC	140
DMD	H21A(−06+16)	GCCGGUUGACUUCAUCCUGUGC	141
DMD	H21A(+85+106)	CUGCAUCCAGGAACAUGGGUCC	142
DMD	H21A(+85+108)	GUCUGCAUCCAGGAACAUGGGUC	143
DMD	H21A(+08+31)	GUUGAAGAUCUGAUAGCCGGUUGA	144
DMD	H21D(+18−07)	UACUUACUGUCUGUAGCUCUUUCU	145
DMD	H22A(+22+45)	CACUCAUGGUCUCCUGAUAGCGCA	146
DMD	H22A(+125+106)	CUGCAAUUCCCCGAGUCUCUGC	147
DMD	H22A(+47+69)	ACUGCUGGACCCAUGUCCUGAUG	148
DMD	H22A(+80+101)	CUAAGUUGAGGUAUGGAGAGU	149
DMD	H22D(+13−11)	UAUUCACAGACCUGCAAUUCCCC	150
DMD	H23A(+34+59)	ACAGUGGUGCUGAGAUAGUAUAGGCC	151
DMD	H23A(+18+39)	UAGGCCACUUUGUUGCUCUUGC	152
DMD	H23A(+72+90)	UUCAGAGGGCGCUUUCUUC	153
DMD	H24A(+48+70)	GGGCAGGCCAUUCCUCCUUCAGA	154
DMD	H24A(−02+22)	UCUUCAGGGUUUGUAUGUGAUUCU	155
DMD	H25A(+9+36)	CUGGGCUGAAUUGUCUGAAUAUCACUG	156
DMD	H25A(+131+156)	CUGUUGGCACAUGUGAUCCCACUGAG	157
DMD	H25D(+16−08)	GUCUAUACCUGUUGGCACAUGUGA	158
DMD	H26A(+132+156)	UGCUUUCUGUAAUUCAUCUGGAGUU	159
DMD	H26A(−07+19)	CCUCCUUUCUGGCAUAGACCUUCCAC	160
DMD	H26A(+68+92)	UGUGUCAUCCAUUCGUGCAUCUCUG	161
DMD	H27A(+82+106)	UUAAGGCCUCUUGUGCUACAGGUGG	162
DMD	H27A(−4+19)	GGGGCUCUUCUUUAGCUCUCUGA	163
DMD	H27D(+19−03)	GACUUCCAAAGUCUUGCAUUUC	164
DMD	H28A(−05+19)	GCCAACAUGCCCAAACUUCCUAAG	165
DMD	H28A(+99+124)	CAGAGAUUUCCUCAGCUCCGCCAGGA	166
DMD	H28D(+16−05)	CUUACAUCUAGCACCUCAGAG	167
DMD	H29A(+57+81)	UCCGCCAUCUGUUAGGGUCUGUGCC	168
DMD	H29A(+18+42)	AUUUGGGUUAUCCUCUGAAUGUCGC	169
DMD	H29D(+17−05)	CAUACCUCUUCAUGUAGUUCCC	170
DMD	H30A(+122+147)	CAUUUGAGCUGCGUCCACCUUGUCUG	171
DMD	H30A(+25+50)	UCCUGGGCAGACUGGAUGCUCUGUUC	172
DMD	H30D(+19−04)	UUGCCUGGGCUUCCUGAGGCAUU	173

DMD	H31D(+06−18)	UUCUGAAAUAACAUAUACCUGUGC	174
DMD	H31D(+03−22)	UAGUUUCUGAAAUAACAUAUACCUG	175
DMD	H31A(+05+25)	GACUUGUCAAAUCAGAUUGGA	176
DMD	H31D(+04−20)	GUUUCUGAAAUAACAUAUACCUGU	177
DMD	H32D(+04−16)	CACCAGAAAUACAUACCACA	178
DMD	H32A(+151+170)	CAAUGAUUUAGCUGUGACUG	179
DMD	H32A(+10+32)	CGAAACUUCAUGGAGACAUCUUG	180
DMD	H32A(+49+73)	CUUGUAGACGCUGCUCAAAAUUGGC	181
DMD	H33D(+09−11)	CAUGCACACACCUUUGCUCC	182
DMD	H33A(+53+76)	UCUGUACAAUCUGACGUCCAGUCU	183
DMD	H33A(+30+56)	GUCUUUAUCACCAUUUCCACUUCAGAC	184
DMD	H33A(+64+88)	CCGUCUGCUUUUUCUGUACAAUCUG	185
DMD	H34A(+83+104)	UCCAUAUCUGUAGCUGCCAGCC	186
DMD	H34A(+143+165)	CCAGGCAACUUCAGAAUCCAAAU	187
DMD	H34A(−20+10)	UUUCUGUUACCUGAAAAGAAUUAUAAUGAA	188
DMD	H34A(+46+70)	CAUUCAUUUCCUUUCGCAUCUUACG	189
DMD	H34A(+95+120)	UGAUCUCUUUGUCAAUUCCAUAUCUG	190
DMD	H34D(+10−20)	UUCAGUGAUAUAGGUUUUACCUUUCCCCAG	191
DMD	H34A(+72+96)	CUG UAG CUG CCA GCC AUU CUG UCA AG	192
DMD	H35A(+141+161)	UCU UCU GCU CGG GAG GUG ACA	193
DMD	H35A(+116+135)	CCA GUU ACU AUU CAG AAG AC	194
DMD	H35A(+24+43)	UCU UCA GGU GCA CCU UCU GU	195
DMD	H36A(+26+50)	UGUGAUGUGGUCCACAUUCUGGUCA	196
DMD	H36A(−02+18)	CCAUGUGUUUCUGGUAUUCC	197
DMD	H37A(+26+50)	CGUGUAGAGUCCACCUUUGGGCGUA	198
DMD	H37A(+82+105)	UACUAAUUUCCUGCAGUGGUCACC	199
DMD	H37A(+134+157)	UUCUGUGUGAAAUGGCUGCAAAUC	200
DMD	H38A(−01+19)	CCUUCAAAGGAAUGGAGGCC	201
DMD	H38A(+59+83)	UGCUGAAUUUCAGCCUCCAGUGGUU	202
DMD	H38A(+88+112)	UGAAGUCUUCCUCUUUCAGAUUCAC	203
DMD	H39A(+62+85)	CUGGCUUUCUCUCAUCUGUGAUUC	204
DMD	H39A(+39+58)	GUUGUAAGUUGUCUCCUCUU	205
DMD	H39A(+102+121)	UUGUCUGUAACAGCUGCUGU	206
DMD	H39D(+10−10)	GCUCUAAUACCUUGAGAGCA	207
DMD	H40A(−05+17)	CUUUGAGACCUCAAAUCCUGUU	208
DMD	H40A(+129+153)	CUUUAUUUUCCUUUCAUCUCUGGGC	209
DMD	H42A(−04+23)	AUCGUUUCUUCACGGACAGUGUGCUGG	210
DMD	H42A(+86+109)	GGGCUUGUGAGACAUGAGUGAUUU	211
DMD	H42D(+19−02)	ACCUUCAGAGGACUCCUCUUGC	212
DMD	H43D(+10−15)	UAUGUGUUACCUACCCUUGUCGGUC	213
DMD	H43A(+101+120)	GGAGAGAGCUUCCUGUAGCU	214
DMD	H43A(+78+100)	UCACCCUUUCCACAGGCGUUGCA	215
DMD	H44A(+85+104)	UUUGUGUCUUUCUGAGAAAC	216
DMD	H44D(+10−10)	AAAGACUUACCUUAAGAUAC	217
DMD	H44A(−06+14)	AUCUGUCAAAUCGCCUGCAG	218
DMD	H46D(+16−04)	UUACCUUGACUUGCUCAAGC	219

DMD	H46A(+90+109)	UCCAGGUUCAAGUGGGAUAC	220
DMD	H47A(+76+100)	GCUCUUCUGGGCUUAUGGGAGCACU	221
DMD	H47D(+25−02)	ACCUUUAUCCACUGGAGAUUUGUCUGC	222
DMD	H47A(−9+12)	UUCCACCAGUAACUGAAACAG	223
DMD	H50A(+02+30)	CCACUCAGAGCUCAGAUCUUCUAACUUCC	224
DMD	H50A(+07+33)	CUUCCACUCAGAGCUCAGAUCUUCUAA	225
DMD	H50D(+07−18)	GGGAUCCAGUAUACUUACAGGCUCC	226
DMD	H51A(−01+25)	ACCAGAGUAACAGUCUGAGUAGGAGC	227
DMD	H51D(+16−07)	CUCAUACCUUCUGCUUGAUGAUC	228
DMD	H51A(+111+134)	UUCUGUCCAAGCCCGGUUGAAAUC	229
DMD	H51A(+61+90)	ACAUCAAGGAAGAUGGCAUUUCUAGUUUGG	230
DMD	H51A(+66+90)	ACAUCAAGGAAGAUGGCAUUUCUAG	231
DMD	H51A(+66+95)	CUCCAACAUCAAGGAAGAUGGCAUUUCUAG	232
DMD	H51D(+08−17)	AUCAUUUUUUCUCAUACCUUCUGCU	233
DMD	H51A/D(+08−17)	AUCAUUUUUUCUCAUACCUUCUGCUAG	234
DMD	&(−15+)	GAGCUAAAA	235
DMD	H51A(+175+195)	CACCCACCAUCACCCUCUGUG	236
DMD	H51A(+199+220)	AUCAUCUCGUUGAUAUCCUCAA	237
DMD	H52A(−07+14)	UCCUGCAUUGUUGCCUGUAAG	238
DMD	H52A(+12+41)	UCCAACUGGGGACGCCUCUGUUCCAAAUCC	239
DMD	H52A(+17+37)	ACUGGGGACGCCUCUGUUCCA	240
DMD	H52A(+93+112)	CCGUAAUGAUUGUUCUAGCC	241
DMD	H52D(+05−15)	UGUUAAAAAACUUACUUCGA	242
DMD	H53A(+45+69)	CAUUCAACUGUUGCCUCCGGUUCUG	243
DMD	H53A(+39+62)	CUGUUGCCUCCGGUUCUGAAGGUG	244
DMD	H53A(+39+69)	CAUUCAACUGUUGCCUCCGGUUCUGAAGGUG	245
DMD	H53D(+14−07)	UACUAACCUUGGUUUCUGUGA	246
DMD	H53A(+23+47)	CUGAAGGUGUUCUUGUACUUCAUCC	247
DMD	H53A(+150+176)	UGUAUAGGGACCCUCCUUCCAUGACUC	248
DMD	H53D(+20−05)	CUAACCUUGGUUUCUGUGAUUUUCU	249
DMD	H53D(+09−18)	GGUAUCUUUGAUACUAACCUUGGUUUC	250
DMD	H53A(−12+10)	AUUCUUUCAACUAGAAUAAAAG	251
DMD	H53A(−07+18)	GAUUCUGAAUUCUUUCAACUAGAAU	252
DMD	H53A(+07+26)	AUCCCACUGAUUCUGAAUUC	253
DMD	H53A(+124+145)	UUGGCUCUGGCCUGUCCUAAGA	254
DMD	H46A(+86+115)	CUCUUUUCCAGGUUCAAGUGGGAUACUAGC	255
DMD	H46A(+107+137)	CAAGCUUUUCUUUUAGUUGCUGCUCUUUUCC	256
DMD	H46A(−10+20)	UAUUCUUUUGUUCUUCUAGCCUGGAGAAAG	257
DMD	H46A(+50+77)	CUGCUUCCUCCAACCAUAAAACAAAUUC	258
DMD	H45A(−06+20)	CCAAUGCCAUCCUGGAGUUCCUGUAA	259
DMD	H45A(+91+110)	UCCUGUAGAAUACUGGCAUC	260
DMD	H45A(+125+151)	UGCAGACCUCCUGCCACCGCAGAUUCA	261
DMD	H45D(+16−04)	CUACCUCUUUUUUCUGUCUG	262
DMD	H45A(+71+90)	UGUUUUUGAGGAUUGCUGAA	263

* The first letter designates the species (e.g. H: human, M: murine, C: canine). "#" designates target *DMD* exon number. "A/D" indicates acceptor or donor splice site at the beginning and end of the exon,

respectively. (x y) represents the annealing coordinates where "-" or "+" indicate intronic or exonic sequences respectively.

Table 15.

Gene	Nucleotide Sequence (5' - 3')	SEQ ID NO.
Bcl-x	TGGTTCTTACCCAGCCGCCG	264
β-globin 623	GTTATTCTTTAGAATGGTGC	265
β-globin 654	TGCTATTACCTTAACCCAGA	266
c-myc	CTGTGCTTACCGGGTTTTCCACCTCCC	267
c-myc	ATCGTCGTGACTGTCTGTTGGAGGG	268
c-myc	GCTCACGTTGAGGGGCATCG	269
c-myc	ACGTTGAGGGGCATCGTCGC	270
c-myc	GGGGCAUCGUCGUGACUGU/CUGUUGGAGGG	271
c-myc	CGUCGUGACUGUCUGUUGGAGG	272
c-myc	CGTCGTGACTGTCTGTTGGAGG	273
c-myc	GGCAUCGUCGCGGGAGGCUGCUGGAGCG	274
c-myc	CCGCGACAUAGGACGGAGAGCAGAGCCC	275
c-myc	ACTGTGAGGGCGATCGCTGC	276
c-myc	ACGATGAGTGGCATAGTCGC	277
c-myc	GGCATCGTCGCGGGAGGCTG	278
c-myc	GGGCATCGTCGCGGGAGGCT	279
c-myc	GGGGCATCGTCGCGGGAGGC	280
c-myc	AGGGGCATCGTCGCGGGAGG	281
c-myc	GAGGGGCATCGTCGCGGGAG	282
c-myc	TGAGGGGCATCGTCGCGGGA	283
c-myc	TTGAGGGGCATCGTCGCGGG	284
c-myc	GTTGAGGGGCATCGTCGCGG	285
c-myc	CGTTGAGGGGCATCGTCGCG	286
c-myc	ACGTTGAGGGGCATCGTCGC	287
c-myc	AACGTTGAGGGGCATCGTCG	288
c-myc	TAACGTTGAGGGGCATCGTC	289
c-myc	CTAACGTTGAGGGGCATCGT	290
c-myc	GCTAACGTTGAGGGGCATCG	291
c-myc	AGCTAACGTTGAGGGGCATC	292
c-myc	AAGCTAACGTTGAGGGGCAT	293
c-myc	GAAGCTAACGTTGAGGGGCA	294
BCL-2 (rat)	CTCCGCAATGCTGAAAGGTG	295
PCNA-1 (rat)	GGCGUGCCUCAAACAUGGUGGCGG	296

Table 16.

Gene	Target Location	Nucleotide Sequence (5'-3')	SEQ ID NO.
Rat c-myc	2553-79	CTGTGCTTACCGGGTTTTCCACCTCCC	297
Rat c-myc	4140-64	ATCGTCGTGACTGTCTGTTGGAGGG	298
Rat c-myc	4161-80	GCTCACGTTGAGGGGCATCG	299
Rat CYP3A2	1155-74	GGTCACTCACCGGTAGAGAA	300

Rat CYP3A2	1526-45	GGGTTCCAAGTCTATAAAGG	301
Human androgen receptor exon 2	31-44	TGTGTCTTTTCCAG	302
Human androgen receptor exon 2	45-67	TTTGGAGACTGCCAGGGACCATG	303
Human androgen receptor exon 2	48-67	CATGGTCCCTGGCAGTCTCC	304
Human androgen receptor exon 2	45-80	TCAATGGGCAAAACATGGTCCCTGGCAGTCTCCAAA	305
Human androgen receptor exon 3	28-43	TTTGTGTTCTCCCAG	306
Human androgen receptor exon 3	44-66	GGAAACAGAAGTACCTGTGCGCC	307
Human androgen receptor exon 3	49-66	GGCGCACAGGTACTTCTG	308
Human androgen receptor exon 3	44-79	AATCATTTCTGCTGGCGCACAGGTACTTCTGTTTCC	309
Human HCG-β subunit	1321-38	CCCCTGCAGCACGCGGGT	310
Human HCG-β subunit	1321-57	GAGGCAGGGCCGGCAGGACCCCCTGCAGCACGCGGGT	311
Human c-myc	4506-25	GGCATCGTCGCGGGAGGCTG	312
Human c-myc	4507-26	GGGCATCGTCGCGGGAGGCT	313
Human c-myc	4508-27	GGGGCATCGTCGCGGGAGGC	314
Human c-myc	4509-28	AGGGGCATCGTCGCGGGAGG	315
Human c-myc	4510-29	GAGGGGCATCGTCGCGGGAG	316
Human c-myc	4511-30	TGAGGGGCATCGTCGCGGGA	317
Human c-myc	4512-31	TTGAGGGGCATCGTCGCGGG	318
Human c-myc	4513-32	GTTGAGGGGCATCGTCGCGG	319
Human c-myc	4514-33	CGTTGAGGGGCATCGTCGCG	320
Human c-myc	4515-34	ACGTTGAGGGGCATCGTCGC	321
Human c-myc	4516-35	AACGTTGAGGGGCATCGTCG	322
Human c-myc	4517-36	TAACGTTGAGGGGCATCGTC	323
Human c-myc	4518-37	CTAACGTTGAGGGGCATCGT	324
Human c-myc	4519-38	GCTAACGTTGAGGGGCATCG	325
Human c-myc	4520-39	AGCTAACGTTGAGGGGCATC	326
Human c-myc	4521-40	AAGCTAACGTTGAGGGGCAT	327
Human c-myc	4522-41	GAAGCTAACGTTGAGGGGCA	328
Human c-myc	6656-75	TCCTCATCTTCTTGTTCCTC	329
Human c-myc	6656-91	AACAACATCGATTTCTTCCTCATCTTCTTGTTCCTC	330
Human p53	11691-708	CCCGGAAGGCAGTCTGGC	331
Human p53	11689-724	TCCTCCATGGCAGTGACCCGGAAGGCAGTCTGGCTG	332
Human abl (ds of bcr-abl fusion point)	376-94	CTACTGGCCGCTGAAGGGC	333
Human abl (ds of bcr-abl fusion point)	374-409	GCTCAAAGTCAGATGCTACTGGCCGCTGAAGGGCTT	334
HW-1 rev	5517-43	TCGTCGGTCTCTCCGCTTCTTCTTGCC	335
HW-1 rev	7885-7904	CTCTGGTGGTGGGTAAGGGT	336
HW-1 rev	7885-7921	CGGGTCTGTCGGGTTCCCTCTGGTGGTGGGTAAGGGT	337

Rat c-myc	4140-69	GGGGCAUCGUCGUGACUGUCUGUUGGAGGG	338
Rat c-myc	4141-62	CGUCGUGACUGUCUGUUGGAGG	339
Rat c-myc	4141-62	CGTCGTGACTGTCTGTTGGAGG	340
Human c-myc	4498-4505	GGCAUCGUCGCGGGAGGCUG/CUGGAGCG	341
Rat c-myc	4364-91	CCGCGACAUAGGACGGAGAGCAGAGCCC	342

Table 17.

Target	Nucleotide Sequence (5' - 3')	SEQ ID NO.
Hu.DMD.Exon44.25.001	CTGCAGGTAAAAGCATATGGATCAA	343
Hu.DMD.Exon44.25.002	ATCGCCTGCAGGTAAAAGCATATGG	344
Hu.DMD.Exon44.25.003	GTCAAATCGCCTGCAGGTAAAAGCA	345
Hu.DMD.Exon44.25.004	GATCTGTCAAATCGCCTGCAGGTAA	346
Hu.DMD.Exon44.25.005	CAACAGATCTGTCAAATCGCCTGCA	347
Hu.DMD.Exon44.25.006	TTTCTCAACAGATCTGTCAAATCGC	348
Hu.DMD.Exon44.25.007	CCATTTCTCAACAGATCTGTCAAAT	349
Hu.DMD.Exon44.25.008	ATAATGAAAACGCCGCCATTTCTCA	350
Hu.DMD.Exon44.25.009	AAATATCTTTATATCATAATGAAAA	351
Hu.DMD.Exon44.25.010	TGTTAGCCACTGATTAAATATCTTT	352
Hu.DMD.Exon44.25.011	AAACTGTTCAGCTTCTGTTAGCCAC	353
Hu.DMD.Exon44.25.012	TTGTGTCTTTCTGAGAAACTGTTCA	354
Hu.DMD.Exon44.25.013	CCAATTCTCAGGAATTTGTGTCTTT	355
Hu.DMD.Exon44.25.014	GTATTTAGCATGTTCCCAATTCTCA	356
Hu.DMD.Exon44.25.015	CTTAAGATACCATTTGTATTTAGCA	357
Hu.DMD.Exon44.25.016	CTTACCTTAAGATACCATTTGTATT	358
Hu.DMD.Exon44.25.017	AAAGACTTACCTTAAGATACCATTT	359
Hu.DMD.Exon44.25.018	AAATCAAAGACTTACCTTAAGATAC	360
Hu.DMD.Exon44.25.019	AAAACAAATCAAAGACTTACCTTAA	361
Hu.DMD.Exon44.25.020	TCGAAAAAACAAATCAAAGACTTAC	362
Hu.DMD.Exon45.25.001	CTGTAAGATACCAAAAAGGCAAAAC	363
Hu.DMD.Exon45.25.002	CCTGTAAGATACCAAAAAGGCAAAA	364
Hu.DMD.Exon45.25.002.2	AGTTCCTGTAAGATACCAAAAAGGC	365
Hu.DMD.Exon45.25.003	GAGTTCCTGTAAGATACCAAAAAGG	366
Hu.DMD.Exon45.25.003.2	CCTGGAGTTCCTGTAAGATACCAAA	367
Hu.DMD.Exon45.25.004	TCCTGGAGTTCCTGTAAGATACCAA	368
Hu.DMD.Exon45.25.004.2	GCCATCCTGGAGTTCCTGTAAGATA	369
Hu.DMD.Exon45.25.005	TGCCATCCTGGAGTTCCTGTAAGAT	370
Hu.DMD.Exon45.25.005.2	CCAATGCCATCCTGGAGTTCCTGTA	371
Hu.DMD.Exon45.25.006	CCCAATGCCATCCTGGAGTTCCTGT	372
Hu.DMD.Exon45.25.006.2	GCTGCCCAATGCCATCCTGGAGTTC	373
Hu.DMD.Exon45.25.007	CGCTGCCCAATGCCATCCTGGAGTT	374
Hu.DMD.Exon45.25.008	AACAGTTTGCCGCTGCCCAATGCCA	375
Hu.DMD.Exon45.25.008.2	CTGACAACAGTTTGCCGCTGCCCAA	376
Hu.DMD.Exon45.25.009	GTTGCATTCAATGTTCTGACAACAG	377
Hu.DMD.Exon45.25.010	GCTGAATTATTTCTTCCCCAGTTGC	378
Hu.DMD.Exon45.25.010.2	ATTATTTCTTCCCCAGTTGCATTCA	379

Hu.DMD.Exon45.25.011	GGCATCTGTTTTTGAGGATTGCTGA	380
Hu.DMD.Exon45.25.011.2	TTTGAGGATTGCTGAATTATTTCTT	381
Hu.DMD.Exon45.25.012	AATTTTTCCTGTAGAATACTGGCAT	382
Hu.DMD.Exon45.25.012.2	ATACTGGCATCTGTTTTTGAGGATT	383
Hu.DMD.Exon45.25.013	ACCGCAGATTCAGGCTTCCCAATTT	384
Hu.DMD.Exon45.25.013.2	AATTTTTCCTGTAGAATACTGGCAT	385
Hu.DMD.Exon45.25.014	CTGTTTGCAGACCTCCTGCCACCGC	386
Hu.DMD.Exon45.25.014.2	AGATTCAGGCTTCCCAATTTTTCCT	387
Hu.DMD.Exon45.25.015	CTCTTTTTTCTGTCTGACAGCTGTT	388
Hu.DMD.Exon45.25.015.2	ACCTCCTGCCACCGCAGATTCAGGC	389
Hu.DMD.Exon45.25.016	CCTACCTCTTTTTTCTGTCTGACAG	390
Hu.DMD.Exon45.25.016.2	GACAGCTGTTTGCAGACCTCCTGCC	391
Hu.DMD.Exon45.25.017	GTCGCCCTACCTCTTTTTTCTGTCT	392
Hu.DMD.Exon45.25.018	GATCTGTCGCCCTACCTCTTTTTTC	393
Hu.DMD.Exon45.25.019	TATTAGATCTGTCGCCCTACCTCTT	394
Hu.DMD.Exon45.25.020	ATTCCTATTAGATCTGTCGCCCTAC	395
Hu.DMD.Exon45.20.001	AGATACCAAAAAGGCAAAAC	396
Hu.DMD.Exon45.20.002	AAGATACCAAAAAGGCAAAA	397
Hu.DMD.Exon45.20.003	CCTGTAAGATACCAAAAAGG	398
Hu.DMD.Exon45.20.004	GAGTTCCTGTAAGATACCAA	399
Hu.DMD.Exon45.20.005	TCCTGGAGTTCCTGTAAGAT	400
Hu.DMD.Exon45.20.006	TGCCATCCTGGAGTTCCTGT	401
Hu.DMD.Exon45.20.007	CCCAATGCCATCCTGGAGTT	402
Hu.DMD.Exon45.20.008	CGCTGCCCAATGCCATCCTG	403
Hu.DMD.Exon45.20.009	CTGACAACAGTTTGCCGCTG	404
Hu.DMD.Exon45.20.010	GTTGCATTCAATGTTCTGAC	405
Hu.DMD.Exon45.20.011	ATTATTTCTTCCCCAGTTGC	406
Hu.DMD.Exon45.20.012	TTTGAGGATTGCTGAATTAT	407
Hu.DMD.Exon45.20.013	ATACTGGCATCTGTTTTTGA	408
Hu.DMD.Exon45.20.014	AATTTTTCCTGTAGAATACT	409
Hu.DMD.Exon45.20.015	AGATTCAGGCTTCCCAATTT	410
Hu.DMD.Exon45.20.016	ACCTCCTGCCACCGCAGATT	411
Hu.DMD.Exon45.20.017	GACAGCTGTTTGCAGACCTC	412
Hu.DMD.Exon45.20.018	CTCTTTTTTCTGTCTGACAG	413
Hu.DMD.Exon45.20.019	CCTACCTCTTTTTTCTGTCT	414
Hu.DMD.Exon45.20.020	GTCGCCCTACCTCTTTTTTC	415
Hu.DMD.Exon45.20.021	GATCTGTCGCCCTACCTCTT	416
Hu.DMD.Exon45.20.022	TATTAGATCTGTCGCCCTAC	417
Hu.DMD.Exon45.20.023	ATTCCTATTAGATCTGTCGC	418
Hu.DMD.Exon46.25.001	GGGGGATTTGAGAAAATAAAATTAC	419
Hu.DMD.Exon46.25.002	ATTTGAGAAAATAAAATTACCTTGA	420
Hu.DMD.Exon46.25.002.2	CTAGCCTGGAGAAAGAAGAATAAAA	421
Hu.DMD.Exon46.25.003	AGAAAATAAAATTACCTTGACTTGC	422
Hu.DMD.Exon46.25.003.2	TTCTTCTAGCCTGGAGAAAGAAGAA	423
Hu.DMD.Exon46.25.004	ATAAAATTACCTTGACTTGCTCAAG	424
Hu.DMD.Exon46.25.004.2	TTTTGTTCTTCTAGCCTGGAGAAAG	425

Hu.DMD.Exon46.25.005	ATTACCTTGACTTGCTCAAGCTTTT	426
Hu.DMD.Exon46.25.005.2	TATTCTTTTGTTCTTCTAGCCTGGA	427
Hu.DMD.Exon46.25.006	CTTGACTTGCTCAAGCTTTTCTTTT	428
Hu.DMD.Exon46.25.006.2	CAAGATATTCTTTTGTTCTTCTAGC	429
Hu.DMD.Exon46.25.007	CTTTTAGTTGCTGCTCTTTTCCAGG	430
Hu.DMD.Exon46.25.008	CCAGGTTCAAGTGGGATACTAGCAA	431
Hu.DMD.Exon46.25.008.2	ATCTCTTTGAAATTCTGACAAGATA	432
Hu.DMD.Exon46.25.009	AGCAATGTTATCTGCTTCCTCCAAC	433
Hu.DMD.Exon46.25.009.2	AACAAATTCATTTAAATCTCTTTGA	434
Hu.DMD.Exon46.25.010	CCAACCATAAAACAAATTCATTTAA	435
Hu.DMD.Exon46.25.010.2	TTCCTCCAACCATAAAACAAATTCA	436
Hu.DMD.Exon46.25.011	TTTAAATCTCTTTGAAATTCTGACA	437
Hu.DMD.Exon46.25.012	TGACAAGATATTCTTTTGTTCTTCT	438
Hu.DMD.Exon46.25.012.2	TTCAAGTGGGATACTAGCAATGTTA	439
Hu.DMD.Exon46.25.013	AGATATTCTTTTGTTCTTCTAGCCT	440
Hu.DMD.Exon46.25.013.2	CTGCTCTTTTCCAGGTTCAAGTGGG	441
Hu.DMD.Exon46.25.014	TTCTTTTGTTCTTCTAGCCTGGAGA	442
Hu.DMD.Exon46.25.014.2	CTTTTCTTTTAGTTGCTGCTCTTTT	443
Hu.DMD.Exon46.25.015	TTGTTCTTCTAGCCTGGAGAAAGAA	444
Hu.DMD.Exon46.25.016	CTTCTAGCCTGGAGAAAGAAGAATA	445
Hu.DMD.Exon46.25.017	AGCCTGGAGAAAGAAGAATAAAATT	446
Hu.DMD.Exon46.25.018	CTGGAGAAAGAAGAATAAAATTGTT	447
Hu.DMD.Exon46.20.001	GAAAGAAGAATAAAATTGTT	448
Hu.DMD.Exon46.20.002	GGAGAAAGAAGAATAAAATT	449
Hu.DMD.Exon46.20.003	AGCCTGGAGAAAGAAGAATA	450
Hu.DMD.Exon46.20.004	CTTCTAGCCTGGAGAAAGAA	451
Hu.DMD.Exon46.20.005	TTGTTCTTCTAGCCTGGAGA	452
Hu.DMD.Exon46.20.006	TTCTTTTGTTCTTCTAGCCT	453
Hu.DMD.Exon46.20.007	TGACAAGATATTCTTTTGTT	454
Hu.DMD.Exon46.20.008	ATCTCTTTGAAATTCTGACA	455
Hu.DMD.Exon46.20.009	AACAAATTCATTTAAATCTC	456
Hu.DMD.Exon46.20.010	TTCCTCCAACCATAAAACAA	457
Hu.DMD.Exon46.20.011	AGCAATGTTATCTGCTTCCT	458
Hu.DMD.Exon46.20.012	TTCAAGTGGGATACTAGCAA	459
Hu.DMD.Exon46.20.013	CTGCTCTTTTCCAGGTTCAA	460
Hu.DMD.Exon46.20.014	CTTTTCTTTTAGTTGCTGCT	461
Hu.DMD.Exon46.20.015	CTTGACTTGCTCAAGCTTTT	462
Hu.DMD.Exon46.20.016	ATTACCTTGACTTGCTCAAG	463
Hu.DMD.Exon46.20.017	ATAAAATTACCTTGACTTGC	464
Hu.DMD.Exon46.20.018	AGAAAATAAAATTACCTTGA	465
Hu.DMD.Exon46.20.019	ATTTGAGAAAATAAAATTAC	466
Hu.DMD.Exon46.20.020	GGGGGATTTGAGAAAATAAA	467
Hu.DMD.Exon47.25.001	CTGAAACAGACAAATGCAACAACGT	468
Hu.DMD.Exon47.25.002	AGTAACTGAAACAGACAAATGCAAC	469
Hu.DMD.Exon47.25.003	CCACCAGTAACTGAAACAGACAAAT	470
Hu.DMD.Exon47.25.004	CTCTTCCACCAGTAACTGAAACAGA	471

Hu.DMD.Exon47.25.005	GGCAACTCTTCCACCAGTAACTGAA	472
Hu.DMD.Exon47.25.006	GCAGGGGCAACTCTTCCACCAGTAA	473
Hu.DMD.Exon47.25.007	CTGGCGCAGGGGCAACTCTTCCACC	474
Hu.DMD.Exon47.25.008	TTTAATTGTTTGAGAATTCCCTGGC	475
Hu.DMD.Exon47.25.008.2	TTGTTTGAGAATTCCCTGGCGCAGG	476
Hu.DMD.Exon47.25.009	GCACGGGTCCTCCAGTTTCATTTAA	477
Hu.DMD.Exon47.25.009.2	TCCAGTTTCATTTAATTGTTTGAGA	478
Hu.DMD.Exon47.25.010	GCTTATGGGAGCACTTACAAGCACG	479
Hu.DMD.Exon47.25.010.2	TACAAGCACGGGTCCTCCAGTTTCA	480
Hu.DMD.Exon47.25.011	AGTTTATCTTGCTCTTCTGGGCTTA	481
Hu.DMD.Exon47.25.012	TCTGCTTGAGCTTATTTTCAAGTTT	482
Hu.DMD.Exon47.25.012.2	ATCTTGCTCTTCTGGGCTTATGGGA	483
Hu.DMD.Exon47.25.013	CTTTATCCACTGGAGATTTGTCTGC	484
Hu.DMD.Exon47.25.013.2	CTTATTTTCAAGTTTATCTTGCTCT	485
Hu.DMD.Exon47.25.014	CTAACCTTTATCCACTGGAGATTTG	486
Hu.DMD.Exon47.25.014.2	ATTTGTCTGCTTGAGCTTATTTTCA	487
Hu.DMD.Exon47.25.015	AATGTCTAACCTTTATCCACTGGAG	488
Hu.DMD.Exon47.25.016	TGGTTAATGTCTAACCTTTATCCAC	489
Hu.DMD.Exon47.25.017	AGAGATGGTTAATGTCTAACCTTTA	490
Hu.DMD.Exon47.25.018	ACGGAAGAGATGGTTAATGTCTAAC	491
Hu.DMD.Exon47.20.001	ACAGACAAATGCAACAACGT	492
Hu.DMD.Exon47.20.002	CTGAAACAGACAAATGCAAC	493
Hu.DMD.Exon47.20.003	AGTAACTGAAACAGACAAAT	494
Hu.DMD.Exon47.20.004	CCACCAGTAACTGAAACAGA	495
Hu.DMD.Exon47.20.005	CTCTTCCACCAGTAACTGAA	496
Hu.DMD.Exon47.20.006	GGCAACTCTTCCACCAGTAA	497
Hu.DMD.Exon47.20.007	CTGGCGCAGGGGCAACTCTT	498
Hu.DMD.Exon47.20.008	TTGTTTGAGAATTCCCTGGC	499
Hu.DMD.Exon47.20.009	TCCAGTTTCATTTAATTGTT	500
Hu.DMD.Exon47.20.010	TACAAGCACGGGTCCTCCAG	501
Hu.DMD.Exon47.20.011	GCTTATGGGAGCACTTACAA	502
Hu.DMD.Exon47.20.012	ATCTTGCTCTTCTGGGCTTA	503
Hu.DMD.Exon47.20.013	CTTATTTTCAAGTTTATCTT	504
Hu.DMD.Exon47.20.014	ATTTGTCTGCTTGAGCTTAT	505
Hu.DMD.Exon47.20.015	CTTTATCCACTGGAGATTTG	506
Hu.DMD.Exon47.20.016	CTAACCTTTATCCACTGGAG	507
Hu.DMD.Exon47.20.017	AATGTCTAACCTTTATCCAC	508
Hu.DMD.Exon47.20.018	TGGTTAATGTCTAACCTTTA	509
Hu.DMD.Exon47.20.019	AGAGATGGTTAATGTCTAAC	510
Hu.DMD.Exon47.20.020	ACGGAAGAGATGGTTAATGT	511
Hu.DMD.Exon48.25.001	CTGAAAGGAAAATACATTTTAAAAA	512
Hu.DMD.Exon48.25.002	CCTGAAAGGAAAATACATTTTAAAA	513
Hu.DMD.Exon48.25.002.2	GAAACCTGAAAGGAAAATACATTTT	514
Hu.DMD.Exon48.25.003	GGAAACCTGAAAGGAAAATACATTT	515
Hu.DMD.Exon48.25.003.2	CTCTGGAAACCTGAAAGGAAAATAC	516
Hu.DMD.Exon48.25.004	GCTCTGGAAACCTGAAAGGAAAATA	517

Hu.DMD.Exon48.25.004.2	TAAAGCTCTGGAAACCTGAAAGGAA	518
Hu.DMD.Exon48.25.005	GTAAAGCTCTGGAAACCTGAAAGGA	519
Hu.DMD.Exon48.25.005.2	TCAGGTAAAGCTCTGGAAACCTGAA	520
Hu.DMD.Exon48.25.006	CTCAGGTAAAGCTCTGGAAACCTGA	521
Hu.DMD.Exon48.25.006.2	GTTTCTCAGGTAAAGCTCTGGAAAC	522
Hu.DMD.Exon48.25.007	TGTTTCTCAGGTAAAGCTCTGGAAA	523
Hu.DMD.Exon48.25.007.2	AATTTCTCCTTGTTTCTCAGGTAAA	524
Hu.DMD.Exon48.25.008	TTTGAGCTTCAATTTCTCCTTGTTT	525
Hu.DMD.Exon48.25.008	TTTTATTTGAGCTTCAATTTCTCCT	526
Hu.DMD.Exon48.25.009	AAGCTGCCCAAGGTCTTTTATTTGA	527
Hu.DMD.Exon48.25.010	AGGTCTTCAAGCTTTTTTTCAAGCT	528
Hu.DMD.Exon48.25.010.2	TTCAAGCTTTTTTTCAAGCTGCCCA	529
Hu.DMD.Exon48.25.011	GATGATTTAACTGCTCTTCAAGGTC	530
Hu.DMD.Exon48.25.011.2	CTGCTCTTCAAGGTCTTCAAGCTTT	531
Hu.DMD.Exon48.25.012	AGGAGATAACCACAGCAGCAGATGA	532
Hu.DMD.Exon48.25.012.2	CAGCAGATGATTTAACTGCTCTTCA	533
Hu.DMD.Exon48.25.013	ATTTCCAACTGATTCCTAATAGGAG	534
Hu.DMD.Exon48.25.014	CTTGGTTTGGTTGGTTATAAATTTC	535
Hu.DMD.Exon48.25.014.2	CAACTGATTCCTAATAGGAGATAAC	536
Hu.DMD.Exon48.25.015	CTTAACGTCAAATGGTCCTTCTTGG	537
Hu.DMD.Exon48.25.015.2	TTGGTTATAAATTTCCAACTGATTC	538
Hu.DMD.Exon48.25.016	CCTACCTTAACGTCAAATGGTCCTT	539
Hu.DMD.Exon48.25.016.2	TCCTTCTTGGTTTGGTTGGTTATAA	540
Hu.DMD.Exon48.25.017	AGTTCCCTACCTTAACGTCAAATGG	541
Hu.DMD.Exon48.25.018	CAAAAAGTTCCCTACCTTAACGTCA	542
Hu.DMD.Exon48.25.019	TAAAGCAAAAAGTTCCCTACCTTAA	543
Hu.DMD.Exon48.25.020	ATATTTAAAGCAAAAAGTTCCCTAC	544
Hu.DMD.Exon48.20.001	AGGAAAATACATTTTAAAAA	545
Hu.DMD.Exon48.20.002	AAGGAAAATACATTTTAAAA	546
Hu.DMD.Exon48.20.003	CCTGAAAGGAAAATACATTT	547
Hu.DMD.Exon48.20.004	GGAAACCTGAAAGGAAAATA	548
Hu.DMD.Exon48.20.005	GCTCTGGAAACCTGAAAGGA	549
Hu.DMD.Exon48.20.006	GTAAAGCTCTGGAAACCTGA	550
Hu.DMD.Exon48.20.007	CTCAGGTAAAGCTCTGGAAA	551
Hu.DMD.Exon48.20.008	AATTTCTCCTTGTTTCTCAG	552
Hu.DMD.Exon48.20.009	TTTTATTTGAGCTTCAATTT	553
Hu.DMD.Exon48.20.010	AAGCTGCCCAAGGTCTTTTA	554
Hu.DMD.Exon48.20.011	TTCAAGCTTTTTTTCAAGCT	555
Hu.DMD.Exon48.20.012	CTGCTCTTCAAGGTCTTCAA	556
Hu.DMD.Exon48.20.013	CAGCAGATGATTTAACTGCT	557
Hu.DMD.Exon48.20.014	AGGAGATAACCACAGCAGCA	558
Hu.DMD.Exon48.20.015	CAACTGATTCCTAATAGGAG	559
Hu.DMD.Exon48.20.016	TTGGTTATAAATTTCCAACT	560
Hu.DMD.Exon48.20.017	TCCTTCTTGGTTTGGTTGGT	561
Hu.DMD.Exon48.20.018	CTTAACGTCAAATGGTCCTT	562
Hu.DMD.Exon48.20.019	CCTACCTTAACGTCAAATGG	563

Hu.DMD.Exon48.20.020	AGTTCCCTACCTTAACGTCA	564
Hu.DMD.Exon48.20.021	CAAAAAGTTCCCTACCTTAA	565
Hu.DMD.Exon48.20.022	TAAAGCAAAAAGTTCCCTAC	566
Hu.DMD.Exon48.20.023	ATATTTAAAGCAAAAAGTTC	567
Hu.DMD.Exon49.25.001	CTGGGGAAAAGAACCCATATAGTGC	568
Hu.DMD.Exon49.25.002	TCCTGGGGAAAAGAACCCATATAGT	569
Hu.DMD.Exon49.25.002.2	GTTTCCTGGGGAAAAGAACCCATAT	570
Hu.DMD.Exon49.25.003	CAGTTTCCTGGGGAAAAGAACCCAT	571
Hu.DMD.Exon49.25.003.2	TTTCAGTTTCCTGGGGAAAAGAACC	572
Hu.DMD.Exon49.25.004	TATTTCAGTTTCCTGGGGAAAAGAA	573
Hu.DMD.Exon49.25.004.2	TGCTATTTCAGTTTCCTGGGGAAAA	574
Hu.DMD.Exon49.25.005	ACTGCTATTTCAGTTTCCTGGGGAA	575
Hu.DMD.Exon49.25.005.2	TGAACTGCTATTTCAGTTTCCTGGG	576
Hu.DMD.Exon49.25.006	CTTGAACTGCTATTTCAGTTTCCTG	577
Hu.DMD.Exon49.25.006.2	TAGCTTGAACTGCTATTTCAGTTTC	578
Hu.DMD.Exon49.25.007	TTTAGCTTGAACTGCTATTTCAGTT	579
Hu.DMD.Exon49.25.008	TTCCACATCCGGTTGTTTAGCTTGA	580
Hu.DMD.Exon49.25.009	TGCCCTTTAGACAAAATCTCTTCCA	581
Hu.DMD.Exon49.25.009.2	TTTAGACAAAATCTCTTCCACATCC	582
Hu.DMD.Exon49.25.010	GTTTTTCCTTGTACAAATGCTGCCC	583
Hu.DMD.Exon49.25.010.2	GTACAAATGCTGCCCTTTAGACAAA	584
Hu.DMD.Exon49.25.011	CTTCACTGGCTGAGTGGCTGGTTTT	585
Hu.DMD.Exon49.25.011.2	GGCTGGTTTTTCCTTGTACAAATGC	586
Hu.DMD.Exon49.25.012	ATTACCTTCACTGGCTGAGTGGCTG	587
Hu.DMD.Exon49.25.013	GCTTCATTACCTTCACTGGCTGAGT	588
Hu.DMD.Exon49.25.014	AGGTTGCTTCATTACCTTCACTGGC	589
Hu.DMD.Exon49.25.015	GCTAGAGGTTGCTTCATTACCTTCA	590
Hu.DMD.Exon49.25.016	ATATTGCTAGAGGTTGCTTCATTAC	591
Hu.DMD.Exon49.20.001	GAAAAGAACCCATATAGTGC	592
Hu.DMD.Exon49.20.002	GGGAAAAGAACCCATATAGT	593
Hu.DMD.Exon49.20.003	TCCTGGGGAAAAGAACCCAT	594
Hu.DMD.Exon49.20.004	CAGTTTCCTGGGGAAAAGAA	595
Hu.DMD.Exon49.20.005	TATTTCAGTTTCCTGGGGAA	596
Hu.DMD.Exon49.20.006	ACTGCTATTTCAGTTTCCTG	597
Hu.DMD.Exon49.20.007	CTTGAACTGCTATTTCAGTT	598
Hu.DMD.Exon49.20.008	TTTAGCTTGAACTGCTATTT	599
Hu.DMD.Exon49.20.009	TTCCACATCCGGTTGTTTAG	600
Hu.DMD.Exon49.20.010	TTTAGACAAAATCTCTTCCA	601
Hu.DMD.Exon49.20.011	GTACAAATGCTGCCCTTTAG	602
Hu.DMD.Exon49.20.012	GGCTGGTTTTTCCTTGTACA	603
Hu.DMD.Exon49.20.013	CTTCACTGGCTGAGTGGCTG	604
Hu.DMD.Exon49.20.014	ATTACCTTCACTGGCTGAGT	605
Hu.DMD.Exon49.20.015	GCTTCATTACCTTCACTGGC	606
Hu.DMD.Exon49.20.016	AGGTTGCTTCATTACCTTCA	607
Hu.DMD.Exon49.20.017	GCTAGAGGTTGCTTCATTAC	608
Hu.DMD.Exon49.20.018	ATATTGCTAGAGGTTGCTTC	609

Hu.DMD.Exon50.25.001	CTTTAACAGAAAAGCATACACATTA	610
Hu.DMD.Exon50.25.002	TCCTCTTTAACAGAAAAGCATACAC	611
Hu.DMD.Exon50.25.002.2	TTCCTCTTTAACAGAAAAGCATACA	612
Hu.DMD.Exon50.25.003	TAACTTCCTCTTTAACAGAAAAGCA	613
Hu.DMD.Exon50.25.003.2	CTAACTTCCTCTTTAACAGAAAAGC	614
Hu.DMD.Exon50.25.004	TCTTCTAACTTCCTCTTTAACAGAA	615
Hu.DMD.Exon50.25.004.2	ATCTTCTAACTTCCTCTTTAACAGA	616
Hu.DMD.Exon50.25.005	TCAGATCTTCTAACTTCCTCTTTAA	617
Hu.DMD.Exon50.25.005.2	CTCAGATCTTCTAACTTCCTCTTTA	618
Hu.DMD.Exon50.25.006	AGAGCTCAGATCTTCTAACTTCCTC	619
Hu.DMD.Exon50.25.006.2 NG-08-0731	CAGAGCTCAGATCTTCTAACTTCCT	620
Hu.DMD.Exon50.25.007	CACTCAGAGCTCAGATCTTCTACT	621
Hu.DMD.Exon50.25.007.2	CCTTCCACTCAGAGCTCAGATCTTC	622
Hu.DMD.Exon50.25.008	GTAAACGGTTTACCGCCTTCCACTC	623
Hu.DMD.Exon50.25.009	CTTTGCCCTCAGCTCTTGAAGTAAA	624
Hu.DMD.Exon50.25.009.2	CCCTCAGCTCTTGAAGTAAACGGTT	625
Hu.DMD.Exon50.25.010	CCAGGAGCTAGGTCAGGCTGCTTTG	626
Hu.DMD.Exon50.25.010.2	GGTCAGGCTGCTTTGCCCTCAGCTC	627
Hu.DMD.Exon50.25.011	AGGCTCCAATAGTGGTCAGTCCAGG	628
Hu.DMD.Exon50.25.011.2	TCAGTCCAGGAGCTAGGTCAGGCTG	629
Hu.DMD.Exon50.25.012 AVI-5038	CTTACAGGCTCCAATAGTGGTCAGT	630
Hu.DMD.Exon50.25.013	GTATACTTACAGGCTCCAATAGTGG	631
Hu.DMD.Exon50.25.014	ATCCAGTATACTTACAGGCTCCAAT	632
Hu.DMD.Exon50.25.015 NG-08-0741	ATGGGATCCAGTATACTTACAGGCT	633
Hu.DMD.Exon50.25.016 NG-08-0742	AGAGAATGGGATCCAGTATACTTAC	634
Hu.DMD.Exon50.20.001	ACAGAAAAGCATACACATTA	635
Hu.DMD.Exon50.20.002	TTTAACAGAAAAGCATACAC	636
Hu.DMD.Exon50.20.003	TCCTCTTTAACAGAAAAGCA	637
Hu.DMD.Exon50.20.004	TAACTTCCTCTTTAACAGAA	638
Hu.DMD.Exon50.20.005	TCTTCTAACTTCCTCTTTAA	639
Hu.DMD.Exon50.20.006	TCAGATCTTCTAACTTCCTC	640
Hu.DMD.Exon50.20.007	CCTTCCACTCAGAGCTCAGA	641
Hu.DMD.Exon50.20.008	GTAAACGGTTTACCGCCTTC	642
Hu.DMD.Exon50.20.009	CCCTCAGCTCTTGAAGTAAA	643
Hu.DMD.Exon50.20.010	GGTCAGGCTGCTTTGCCCTC	644
Hu.DMD.Exon50.20.011	TCAGTCCAGGAGCTAGGTCA	645
Hu.DMD.Exon50.20.012	AGGCTCCAATAGTGGTCAGT	646
Hu.DMD.Exon50.20.013	CTTACAGGCTCCAATAGTGG	647
Hu.DMD.Exon50.20.014	GTATACTTACAGGCTCCAAT	648
Hu.DMD.Exon50.20.015	ATCCAGTATACTTACAGGCT	649
Hu.DMD.Exon50.20.016	ATGGGATCCAGTATACTTAC	650
Hu.DMD.Exon50.20.017	AGAGAATGGGATCCAGTATA	651

Hu.DMD.Exon51.25.001-44	CTAAAATATTTTGGGTTTTTGCAAAA	652
Hu.DMD.Exon51.25.002-45	GCTAAAATATTTTGGGTTTTTGCAAA	653
Hu.DMD.Exon51.25.002.2-46	TAGGAGCTAAAATATTTTGGGTTTTT	654
Hu.DMD.Exon51.25.003	AGTAGGAGCTAAAATATTTTGGGTT	655
Hu.DMD.Exon51.25.003.2	TGAGTAGGAGCTAAAATATTTTGGG	656
Hu.DMD.Exon51.25.004	CTGAGTAGGAGCTAAAATATTTTGGG	657
Hu.DMD.Exon51.25.004.2	CAGTCTGAGTAGGAGCTAAAATATT	658
Hu.DMD.Exon51.25.005	ACAGTCTGAGTAGGAGCTAAAATATT	659
Hu.DMD.Exon51.25.005.2	GAGTAACAGTCTGAGTAGGAGCTAAA	660
Hu.DMD.Exon51.25.006	CAGAGTAACAGTCTGAGTAGGAGCT	661
Hu.DMD.Exon51.25.006.2	CACCAGAGTAACAGTCTGAGTAGGAG	662
Hu.DMD.Exon51.25.007	GTCACCAGAGTAACAGTCTGAGTAG	663
Hu.DMD.Exon51.25.007.2	AACCACAGGTTGTGTCACCAGAGTAA	664
Hu.DMD.Exon51.25.008	GTTGTGTCACCAGAGTAACAGTCTG	665
Hu.DMD.Exon51.25.009	TGGCAGTTTCCTTAGTAACCACAGGT	666
Hu.DMD.Exon51.25.010	ATTTCTAGTTTGGAGATGGCAGTTTC	667
Hu.DMD.Exon51.25.010.2	GGAAGATGGCATTTCTAGTTTGGAG	668
Hu.DMD.Exon51.25.011	CATCAAGGAAGATGGCATTTCTAGTT	669
Hu.DMD.Exon51.25.011.2	GAGCAGGTACCTCCAACATCAAGGAA	670
Hu.DMD.Exon51.25.012	ATCTGCCAGAGCAGGTACCTCCAAC	671
Hu.DMD.Exon51.25.013	AAGTTCTGTCCAAGCCCGGTTGAAAT	672
Hu.DMD.Exon51.25.013.2	CGGTTGAAATCTGCCAGAGCAGGTAC	673
Hu.DMD.Exon51.25.014	GAGAAAGCCAGTCGGTAAGTTCTGTC	674
Hu.DMD.Exon51.25.014.2	GTCGGTAAGTTCTGTCCAAGCCCGG	675
Hu.DMD.Exon51.25.015	ATAACTTGATCAAGCAGAGAAAGCCA	676
Hu.DMD.Exon51.25.015.2	AAGCAGAGAAAGCCAGTCGGTAAGT	677
Hu.DMD.Exon51.25.016	CACCCTCTGTGATTTTATAACTTGAT	678
Hu.DMD.Exon51.25.017	CAAGGTCACCCACCATCACCCTCTGT	679
Hu.DMD.Exon51.25.017.2	CATCACCCTCTGTGATTTTATAACT	680
Hu.DMD.Exon51.25.018	CTTCTGCTTGATGATCATCTCGTTGA	681
Hu.DMD.Exon51.25.019	CCTTCTGCTTGATGATCATCTCGTTG	682
Hu.DMD.Exon51.25.019.2	ATCTCGTTGATATCCTCAAGGTCACC	683
Hu.DMD.Exon51.25.020	TCATACCTTCTGCTTGATGATCATCT	684
Hu.DMD.Exon51.25.020.2	TCATTTTTTCTCATACCTTCTGCTTG	685
Hu.DMD.Exon51.25.021	TTTTCTCATACCTTCTGCTTGATGAT	686
Hu.DMD.Exon51.25.022	TTTTATCATTTTTTCTCATACCTTCT	687
Hu.DMD.Exon51.25.023	CCAACTTTTATCATTTTTTCTCATAC	688
Hu.DMD.Exon51.20.001	ATATTTTGGGTTTTTGCAAA	689
Hu.DMD.Exon51.20.002	AAAATATTTTGGGTTTTTGC	690
Hu.DMD.Exon51.20.003	GAGCTAAAATATTTTGGGTT	691
Hu.DMD.Exon51.20.004	AGTAGGAGCTAAAATATTTT	692
Hu.DMD.Exon51.20.005	GTCTGAGTAGGAGCTAAAAT	693
Hu.DMD.Exon51.20.006	TAACAGTCTGAGTAGGAGCT	694
Hu.DMD.Exon51.20.007	CAGAGTAACAGTCTGAGTAG	695
Hu.DMD.Exon51.20.008	CACAGGTTGTGTCACCAGAG	696
Hu.DMD.Exon51.20.009	AGTTTCCTTAGTAACCACAG	697

Hu.DMD.Exon51.20.010	TAGTTTGGAGATGGCAGTTT	698
Hu.DMD.Exon51.20.011	GGAAGATGGCATTTCTAGTT	699
Hu.DMD.Exon51.20.012	TACCTCCAACATCAAGGAAG	700
Hu.DMD.Exon51.20.013	ATCTGCCAGAGCAGGTACCT	701
Hu.DMD.Exon51.20.014	CCAAGCCCGGTTGAAATCTG	702
Hu.DMD.Exon51.20.015	GTCGGTAAGTTCTGTCCAAG	703
Hu.DMD.Exon51.20.016	AAGCAGAGAAAGCCAGTCGG	704
Hu.DMD.Exon51.20.017	TTTTATAACTTGATCAAGCA	705
Hu.DMD.Exon51.20.018	CATCACCCTCTGTGATTTTA	706
Hu.DMD.Exon51.20.019	CTCAAGGTCACCCACCATCA	707
Hu.DMD.Exon51.20.020	CATCTCGTTGATATCCTCAA	708
Hu.DMD.Exon51.20.021	CTTCTGCTTGATGATCATCT	709
Hu.DMD.Exon51.20.022	CATACCTTCTGCTTGATGAT	710
Hu.DMD.Exon51.20.023	TTTCTCATACCTTCTGCTTG	711
Hu.DMD.Exon51.20.024	CATTTTTTCTCATACCTTCT	712
Hu.DMD.Exon51.20.025	TTTATCATTTTTTCTCATAC	713
Hu.DMD.Exon51.20.026	CAACTTTTATCATTTTTTCT	714
Hu.DMD.Exon52.25.001	CTGTAAGAACAAATATCCCTTAGTA	715
Hu.DMD.Exon52.25.002	TGCCTGTAAGAACAAATATCCCTTA	716
Hu.DMD.Exon52.25.002.2	GTTGCCTGTAAGAACAAATATCCCT	717
Hu.DMD.Exon52.25.003	ATTGTTGCCTGTAAGAACAAATATC	718
Hu.DMD.Exon52.25.003.2	GCATTGTTGCCTGTAAGAACAAATA	719
Hu.DMD.Exon52.25.004	CCTGCATTGTTGCCTGTAAGAACAA	720
Hu.DMD.Exon52.25.004.2	ATCCTGCATTGTTGCCTGTAAGAAC	721
Hu.DMD.Exon52.25.005	CAAATCCTGCATTGTTGCCTGTAAG	722
Hu.DMD.Exon52.25.005.2	TCCAAATCCTGCATTGTTGCCTGTA	723
Hu.DMD.Exon52.25.006	TGTTCCAAATCCTGCATTGTTGCCT	724
Hu.DMD.Exon52.25.006.2	TCTGTTCCAAATCCTGCATTGTTGC	725
Hu.DMD.Exon52.25.007	AACTGGGGACGCCTCTGTTCCAAAT	726
Hu.DMD.Exon52.25.007.2	GCCTCTGTTCCAAATCCTGCATTGT	727
Hu.DMD.Exon52.25.008	CAGCGGTAATGAGTTCTTCCAACTG	728
Hu.DMD.Exon52.25.008.2	CTTCCAACTGGGGACGCCTCTGTTC	729
Hu.DMD.Exon52.25.009	CTTGTTTTTCAAATTTTGGGCAGCG	730
Hu.DMD.Exon52.25.010	CTAGCCTCTTGATTGCTGGTCTTGT	731
Hu.DMD.Exon52.25.010.2	TTTTCAAATTTTGGGCAGCGGTAAT	732
Hu.DMD.Exon52.25.011	TTCGATCCGTAATGATTGTTCTAGC	733
Hu.DMD.Exon52.25.011.2	GATTGCTGGTCTTGTTTTTCAAATT	734
Hu.DMD.Exon52.25.012	CTTACTTCGATCCGTAATGATTGTT	735
Hu.DMD.Exon52.25.012.2	TTGTTCTAGCCTCTTGATTGCTGGT	736
Hu.DMD.Exon52.25.013	AAAAACTTACTTCGATCCGTAATGA	737
Hu.DMD.Exon52.25.014	TGTTAAAAAACTTACTTCGATCCGT	738
Hu.DMD.Exon52.25.015	ATGCTTGTTAAAAAACTTACTTCGA	739
Hu.DMD.Exon52.25.016	GTCCCATGCTTGTTAAAAAACTTAC	740
Hu.DMD.Exon52.20.001	AGAACAAATATCCCTTAGTA	741
Hu.DMD.Exon52.20.002	GTAAGAACAAATATCCCTTA	742
Hu.DMD.Exon52.20.003	TGCCTGTAAGAACAAATATC	743

Hu.DMD.Exon52.20.004	ATTGTTGCCTGTAAGAACAA	744
Hu.DMD.Exon52.20.005	CCTGCATTGTTGCCTGTAAG	745
Hu.DMD.Exon52.20.006	CAAATCCTGCATTGTTGCCT	746
Hu.DMD.Exon52.20.007	GCCTCTGTTCCAAATCCTGC	747
Hu.DMD.Exon52.20.008	CTTCCAACTGGGGACGCCTC	748
Hu.DMD.Exon52.20.009	CAGCGGTAATGAGTTCTTCC	749
Hu.DMD.Exon52.20.010	TTTTCAAATTTTGGGCAGCG	750
Hu.DMD.Exon52.20.011	GATTGCTGGTCTTGTTTTTC	751
Hu.DMD.Exon52.20.012	TTGTTCTAGCCTCTTGATTG	752
Hu.DMD.Exon52.20.013	TTCGATCCGTAATGATTGTT	753
Hu.DMD.Exon52.20.014	CTTACTTCGATCCGTAATGA	754
Hu.DMD.Exon52.20.015	AAAAACTTACTTCGATCCGT	755
Hu.DMD.Exon52.20.016	TGTTAAAAAACTTACTTCGA	756
Hu.DMD.Exon52.20.017	ATGCTTGTTAAAAAACTTAC	757
Hu.DMD.Exon52.20.018	GTCCCATGCTTGTTAAAAAA	758
Hu.DMD.Exon53.25.001	CTAGAATAAAAGGAAAAATAAATAT	759
Hu.DMD.Exon53.25.002	AACTAGAATAAAAGGAAAAATAAAT	760
Hu.DMD.Exon53.25.002.2	TTCAACTAGAATAAAAGGAAAAATA	761
Hu.DMD.Exon53.25.003	CTTTCAACTAGAATAAAAGGAAAAA	762
Hu.DMD.Exon53.25.003.2	ATTCTTTCAACTAGAATAAAAGGAA	763
Hu.DMD.Exon53.25.004	GAATTCTTTCAACTAGAATAAAAGG	764
Hu.DMD.Exon53.25.004.2	TCTGAATTCTTTCAACTAGAATAAA	765
Hu.DMD.Exon53.25.005	ATTCTGAATTCTTTCAACTAGAATA	766
Hu.DMD.Exon53.25.005.2	CTGATTCTGAATTCTTTCAACTAGA	767
Hu.DMD.Exon53.25.006	CACTGATTCTGAATTCTTTCAACTA	768
Hu.DMD.Exon53.25.006.2	TCCCACTGATTCTGAATTCTTTCAA	769
Hu.DMD.Exon53.25.007	CATCCCACTGATTCTGAATTCTTTC	770
Hu.DMD.Exon53.25.008	TACTTCATCCCACTGATTCTGAATT	771
Hu.DMD.Exon53.25.008.2	CTGAAGGTGTTCTTGTACTTCATCC	772
Hu.DMD.Exon53.25.009	CGGTTCTGAAGGTGTTCTTGTACT	773
Hu.DMD.Exon53.25.009.2	CTGTTGCCTCCGGTTCTGAAGGTGT	774
Hu.DMD.Exon53.25.010	TTTCATTCAACTGTTGCCTCCGGTT	775
Hu.DMD.Exon53.25.010.2	TAACATTTCATTCAACTGTTGCCTC	776
Hu.DMD.Exon53.25.011	TTGTGTTGAATCCTTTAACATTTCA	777
Hu.DMD.Exon53.25.012	TCTTCCTTAGCTTCCAGCCATTGTG	778
Hu.DMD.Exon53.25.012.2	CTTAGCTTCCAGCCATTGTGTTGAA	779
Hu.DMD.Exon53.25.013	GTCCTAAGACCTGCTCAGCTTCTTC	780
Hu.DMD.Exon53.25.013.2	CTGCTCAGCTTCTTCCTTAGCTTCC	781
Hu.DMD.Exon53.25.014	CTCAAGCTTGGCTCTGGCCTGTCCT	782
Hu.DMD.Exon53.25.014.2	GGCCTGTCCTAAGACCTGCTCAGCT	783
Hu.DMD.Exon53.25.015	TAGGGACCCTCCTTCCATGACTCAA	784
Hu.DMD.Exon53.25.016	TTTGGATTGCATCTACTGTATAGGG	785
Hu.DMD.Exon53.25.016.2	ACCCTCCTTCCATGACTCAAGCTTG	786
Hu.DMD.Exon53.25.017	CTTGGTTTCTGTGATTTTCTTTTGG	787
Hu.DMD.Exon53.25.017.2	ATCTACTGTATAGGGACCCTCCTTC	788
Hu.DMD.Exon53.25.018	CTAACCTTGGTTTCTGTGATTTTCT	789

Hu.DMD.Exon53.25.018.2	TTTCTTTTGGATTGCATCTACTGTA	790
Hu.DMD.Exon53.25.019	TGATACTAACCTTGGTTTCTGTGAT	791
Hu.DMD.Exon53.25.020	ATCTTTGATACTAACCTTGGTTTCT	792
Hu.DMD.Exon53.25.021	AAGGTATCTTTGATACTAACCTTGG	793
Hu.DMD.Exon53.25.022	TTAAAAAGGTATCTTTGATACTAAC	794
Hu.DMD.Exon53.20.001	ATAAAAGGAAAAATAAATAT	795
Hu.DMD.Exon53.20.002	GAATAAAAGGAAAAATAAAT	796
Hu.DMD.Exon53.20.003	AACTAGAATAAAAGGAAAAA	797
Hu.DMD.Exon53.20.004	CTTTCAACTAGAATAAAAGG	798
Hu.DMD.Exon53.20.005	GAATTCTTTCAACTAGAATA	799
Hu.DMD.Exon53.20.006	ATTCTGAATTCTTTCAACTA	800
Hu.DMD.Exon53.20.007	TACTTCATCCCACTGATTCT	801
Hu.DMD.Exon53.20.008	CTGAAGGTGTTCTTGTACT	802
Hu.DMD.Exon53.20.009	CTGTTGCCTCCGGTTCTGAA	803
Hu.DMD.Exon53.20.010	TAACATTTCATTCAACTGTT	804
Hu.DMD.Exon53.20.011	TTGTGTTGAATCCTTTAACA	805
Hu.DMD.Exon53.20.012	CTTAGCTTCCAGCCATTGTG	806
Hu.DMD.Exon53.20.013	CTGCTCAGCTTCTTCCTTAG	807
Hu.DMD.Exon53.20.014	GGCCTGTCCTAAGACCTGCT	808
Hu.DMD.Exon53.20.015	CTCAAGCTTGGCTCTGGCCT	809
Hu.DMD.Exon53.20.016	ACCCTCCTTCCATGACTCAA	810
Hu.DMD.Exon53.20.017	ATCTACTGTATAGGGACCCT	811
Hu.DMD.Exon53.20.018	TTTCTTTTGGATTGCATCTA	812
Hu.DMD.Exon53.20.019	CTTGGTTTCTGTGATTTTCT	813
Hu.DMD.Exon53.20.020	CTAACCTTGGTTTCTGTGAT	814
Hu.DMD.Exon53.20.021	TGATACTAACCTTGGTTTCT	815
Hu.DMD.Exon53.20.022	ATCTTTGATACTAACCTTGG	816
Hu.DMD.Exon53.20.023	AAGGTATCTTTGATACTAAC	817
Hu.DMD.Exon53.20.024	TTAAAAAGGTATCTTTGATA	818
Hu.DMD.Exon54.25.001	CTATAGATTTTTATGAGAAAGAGA	819
Hu.DMD.Exon54.25.002	AACTGCTATAGATTTTTATGAGAAA	820
Hu.DMD.Exon54.25.003	TGGCCAACTGCTATAGATTTTTATG	821
Hu.DMD.Exon54.25.004	GTCTTTGGCCAACTGCTATAGATTT	822
Hu.DMD.Exon54.25.005	CGGAGGTCTTTGGCCAACTGCTATA	823
Hu.DMD.Exon54.25.006	ACTGGCGGAGGTCTTTGGCCAACTG	824
Hu.DMD.Exon54.25.007	TTTGTCTGCCACTGGCGGAGGTCTT	825
Hu.DMD.Exon54.25.008	AGTCATTTGCCACATCTACATTTGT	826
Hu.DMD.Exon54.25.008.2	TTTGCCACATCTACATTTGTCTGCC	827
Hu.DMD.Exon54.25.009	CCGGAGAAGTTTCAGGGCCAAGTCA	828
Hu.DMD.Exon54.25.010	GTATCATCTGCAGAATAATCCCGGA	829
Hu.DMD.Exon54.25.010.2	TAATCCCGGAGAAGTTTCAGGGCCA	830
Hu.DMD.Exon54.25.011	TTATCATGTGGACTTTTCTGGTATC	831
Hu.DMD.Exon54.25.012	AGAGGCATTGATATTCTCTGTTATC	832
Hu.DMD.Exon54.25.012.2	ATGTGGACTTTTCTGGTATCATCTG	833
Hu.DMD.Exon54.25.013	CTTTTATGAATGCTTCTCCAAGAGG	834
Hu.DMD.Exon54.25.013.2	ATATTCTCTGTTATCATGTGGACTT	835

Hu.DMD.Exon54.25.014	CATACCTTTTATGAATGCTTCTCCA	836
Hu.DMD.Exon54.25.014.2	CTCCAAGAGGCATTGATATTCTCTG	837
Hu.DMD.Exon54.25.015	TAATTCATACCTTTTATGAATGCTT	838
Hu.DMD.Exon54.25.015.2	CTTTTATGAATGCTTCTCCAAGAGG	839
Hu.DMD.Exon54.25.016	TAATGTAATTCATACCTTTTATGAA	840
Hu.DMD.Exon54.25.017	AGAAATAATGTAATTCATACCTTTT	841
Hu.DMD.Exon54.25.018	GTTTTAGAAATAATGTAATTCATAC	842
Hu.DMD.Exon54.20.001	GATTTTTATGAGAAAGAGA	843
Hu.DMD.Exon54.20.002	CTATAGATTTTTATGAGAAA	844
Hu.DMD.Exon54.20.003	AACTGCTATAGATTTTTATG	845
Hu.DMD.Exon54.20.004	TGGCCAACTGCTATAGATTT	846
Hu.DMD.Exon54.20.005	GTCTTTGGCCAACTGCTATA	847
Hu.DMD.Exon54.20.006	CGGAGGTCTTTGGCCAACTG	848
Hu.DMD.Exon54.20.007	TTTGTCTGCCACTGGCGGAG	849
Hu.DMD.Exon54.20.008	TTTGCCACATCTACATTTGT	850
Hu.DMD.Exon54.20.009	TTCAGGGCCAAGTCATTTGC	851
Hu.DMD.Exon54.20.010	TAATCCCGGAGAAGTTTCAG	852
Hu.DMD.Exon54.20.011	GTATCATCTGCAGAATAATC	853
Hu.DMD.Exon54.20.012	ATGTGGACTTTTCTGGTATC	854
Hu.DMD.Exon54.20.013	ATATTCTCTGTTATCATGTG	855
Hu.DMD.Exon54.20.014	CTCCAAGAGGCATTGATATT	856
Hu.DMD.Exon54.20.015	CTTTTATGAATGCTTCTCCA	857
Hu.DMD.Exon54.20.016	CATACCTTTTATGAATGCTT	858
Hu.DMD.Exon54.20.017	TAATTCATACCTTTTATGAA	859
Hu.DMD.Exon54.20.018	TAATGTAATTCATACCTTTT	860
Hu.DMD.Exon54.20.019	AGAAATAATGTAATTCATAC	861
Hu.DMD.Exon54.20.020	GTTTTAGAAATAATGTAATT	862
Hu.DMD.Exon55.25.001	CTGCAAAGGACCAAATGTTCAGATG	863
Hu.DMD.Exon55.25.002	TCACCCTGCAAAGGACCAAATGTTC	864
Hu.DMD.Exon55.25.003	CTCACTCACCCTGCAAAGGACCAAA	865
Hu.DMD.Exon55.25.004	TCTCGCTCACTCACCCTGCAAAGGA	866
Hu.DMD.Exon55.25.005	CAGCCTCTCGCTCACTCACCCTGCA	867
Hu.DMD.Exon55.25.006	CAAAGCAGCCTCTCGCTCACTCACC	868
Hu.DMD.Exon55.25.007	TCTTCCAAAGCAGCCTCTCGCTCAC	869
Hu.DMD.Exon55.25.007.2	TCTATGAGTTTCTTCCAAAGCAGCC	870
Hu.DMD.Exon55.25.008	GTTGCAGTAATCTATGAGTTTCTTC	871
Hu.DMD.Exon55.25.008.2	GAACTGTTGCAGTAATCTATGAGTT	872
Hu.DMD.Exon55.25.009	TTCCAGGTCCAGGGGGAACTGTTGC	873
Hu.DMD.Exon55.25.010	GTAAGCCAGGCAAGAAACTTTTCCA	874
Hu.DMD.Exon55.25.010.2	CCAGGCAAGAAACTTTTCCAGGTCC	875
Hu.DMD.Exon55.25.011	TGGCAGTTGTTTCAGCTTCTGTAAG	876
Hu.DMD.Exon55.25.011.2	TTCAGCTTCTGTAAGCCAGGCAAGA	877
Hu.DMD.Exon55.25.012	GGTAGCATCCTGTAGGACATTGGCA	878
Hu.DMD.Exon55.25.012.2	GACATTGGCAGTTGTTTCAGCTTCT	879
Hu.DMD.Exon55.25.013	TCTAGGAGCCTTTCCTTACGGGTAG	880
Hu.DMD.Exon55.25.014	CTTTTACTCCCTTGGAGTCTTCTAG	881

Hu.DMD.Exon55.25.014.2	GAGCCTTTCCTTACGGGTAGCATCC	882
Hu.DMD.Exon55.25.015	TTGCCATTGTTTCATCAGCTCTTTT	883
Hu.DMD.Exon55.25.015.2	CTTGGAGTCTTCTAGGAGCCTTTCC	884
Hu.DMD.Exon55.25.016	CTTACTTGCCATTGTTTCATCAGCT	885
Hu.DMD.Exon55.25.016.2	CAGCTCTTTTACTCCCTTGGAGTCT	886
Hu.DMD.Exon55.25.017	CCTGACTTACTTGCCATTGTTTCAT	887
Hu.DMD.Exon55.25.018	AAATGCCTGACTTACTTGCCATTGT	888
Hu.DMD.Exon55.25.019	AGCGGAAATGCCTGACTTACTTGCC	889
Hu.DMD.Exon55.25.020	GCTAAAGCGGAAATGCCTGACTTAC	890
Hu.DMD.Exon55.20.001	AAGGACCAAATGTTCAGATG	891
Hu.DMD.Exon55.20.002	CTGCAAAGGACCAAATGTTC	892
Hu.DMD.Exon55.20.003	TCACCCTGCAAAGGACCAAA	893
Hu.DMD.Exon55.20.004	CTCACTCACCCTGCAAAGGA	894
Hu.DMD.Exon55.20.005	TCTCGCTCACTCACCCTGCA	895
Hu.DMD.Exon55.20.006	CAGCCTCTCGCTCACTCACC	896
Hu.DMD.Exon55.20.007	CAAAGCAGCCTCTCGCTCAC	897
Hu.DMD.Exon55.20.008	TCTATGAGTTTCTTCCAAAG	898
Hu.DMD.Exon55.20.009	GAACTGTTGCAGTAATCTAT	899
Hu.DMD.Exon55.20.010	TTCCAGGTCCAGGGGGAACT	900
Hu.DMD.Exon55.20.011	CCAGGCAAGAAACTTTTCCA	901
Hu.DMD.Exon55.20.012	TTCAGCTTCTGTAAGCCAGG	902
Hu.DMD.Exon55.20.013	GACATTGGCAGTTGTTTCAG	903
Hu.DMD.Exon55.20.014	GGTAGCATCCTGTAGGACAT	904
Hu.DMD.Exon55.20.015	GAGCCTTTCCTTACGGGTAG	905
Hu.DMD.Exon55.20.016	CTTGGAGTCTTCTAGGAGCC	906
Hu.DMD.Exon55.20.017	CAGCTCTTTTACTCCCTTGG	907
Hu.DMD.Exon55.20.018	TTGCCATTGTTTCATCAGCT	908
Hu.DMD.Exon55.20.019	CTTACTTGCCATTGTTTCAT	909
Hu.DMD.Exon55.20.020	CCTGACTTACTTGCCATTGT	910
Hu.DMD.Exon55.20.021	AAATGCCTGACTTACTTGCC	911
Hu.DMD.Exon55.20.022	AGCGGAAATGCCTGACTTAC	912
Hu.DMD.Exon55.20.023	GCTAAAGCGGAAATGCCTGA	913
H50A(+02+30)-AVI-5656	CCACTCAGAGCTCAGATCTTCTAACTTCC	914
H50D(+07−18)-AVI-5915	GGGATCCAGTATACTTACAGGCTCC	915
H50A(+07+33)	CTTCCACTCAGAGCTCAGATCTTCTAA	916
H51A(+61+90)-AVI-4657	ACATCAAGGAAGATGGCATTTCTAGTTTGG	917
H51A(+66+95)-AVI-4658	CTCCAACATCAAGGAAGATGGCATTTCTAG	918
H51A(+111+134)	TTCTGTCCAAGCCCGGTTGAAATC	919
H51A(+175+195)	CACCCACCATCACCCTCYGTG	920
H51A(+199+220)	ATCATCTCGTTGATATCCTCAA	921
H51A(+66+90)	ACATCAAGGAAGATGGCATTTCTAG	922
H51A(−01+25)	ACCAGAGTAACAGTCTGAGTAGGAGC	923
h51AON1	TCAAGGAAGATGGCATTTCT	924
h51AON2	CCTCTGTGATTTTATAACTTGAT	925
H51D(+08−17)	ATCATTTTTTCTCATACCTTCTGCT	926
H51D(+16−07)	CTCATACCTTCTGCTTGATGATC	927

hAON#23	TGGCATTTCTAGTTTGG	928
hAON#24	CCAGAGCAGGTACCTCCAACATC	929
H44A(+61+84)	TGTTCAGCTTCTGTTAGCCACTGA	930
H44A(+85+104)	TTTGTGTCTTTCTGAGAAAC	931
h44AON1	CGCCGCCATTTCTCAACAG	932
H44A(−06+14)	ATCTGTCAAATCGCCTGCAG	933
H45A(+71+90)	TGTTTTTGAGGATTGCTGAA	934
h45AON1	GCTGAATTATTTCTTCCCC	935
h45AON5	GCCCAATGCCATCCTGG	936
H45A(−06+20)	CCAATGCCATCCTGGAGTTCCTGTAA	937
H53A(+39+69)	CATTCAACTGTTGCCTCCGGTTCTGAAGGTG	938
H53A(+23+47)	CTGAAGGTGTTCTTGTACTTCATCC	939
h53AON1	CTGTTGCCTCCGGTTCTG	940
H53A(−12+10)	ATTCTTTCAACTAGAATAAAAG	941
huEx45.30.66	GCCATCCTGGAGTTCCTGTAAGATACCAAA	942
huEx45.30.71	CCAATGCCATCCTGGAGTTCCTGTAAGATA	943
huEx45.30.79	GCCGCTGCCCAATGCCATCCTGGAGTTCCT	944
huEx45.30.83	GTTTGCCGCTGCCCAATGCCATCCTGGAGT	945
huEx45.30.88	CAACAGTTTGCCGCTGCCCAATGCCATCCT	946
huEx45.30.92	CTGACAACAGTTTGCCGCTGCCCAATGCCA	947
huEx45.30.96	TGTTCTGACAACAGTTTGCCGCTGCCCAAT	948
huEx45.30.99	CAATGTTCTGACAACAGTTTGCCGCTGCCC	949
huEx45.30.103	CATTCAATGTTCTGACAACAGTTTGCCGCT	950
huEx45.30.120	TATTTCTTCCCCAGTTGCATTCAATGTTCT	951
huEx45.30.127	GCTGAATTATTTCTTCCCCAGTTGCATTCA	952
huEx45.30.132	GGATTGCTGAATTATTTCTTCCCCAGTTGC	953
huEx45.30.137	TTTGAGGATTGCTGAATTATTTCTTCCCCA	954
huEx53.30.84	GTACTTCATCCCACTGATTCTGAATTCTTT	955
huEx53.30.88	TCTTGTACTTCATCCCACTGATTCTGAATT	956
huEx53.30.91	TGTTCTTGTACTTCATCCCACTGATTCTGA	957
huEx53.30.103	CGGTTCTGAAGGTGTTCTTGTACTTCATCC	958
huEx53.30.106	CTCCGGTTCTGAAGGTGTTCTTGTACTTCA	959
huEx53.30.109	TGCCTCCGGTTCTGAAGGTGTTCTTGTACT	960
huEx53.30.112	TGTTGCCTCCGGTTCTGAAGGTGTTCTTGT	961
huEx53.30.115	AACTGTTGCCTCCGGTTCTGAAGGTGTTCT	962
huEx53.30.118	TTCAACTGTTGCCTCCGGTTCTGAAGGTGT	963

[0450] **Step 1: Antibody conjugation with maleimide-PEG-NHS followed by siRNA-*DMD* conjugates**

[0451] Anti-dystrophin antibody is exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SMCC linker or maleimide-PEGxkDa-NHS (x = 1, 5, 10, 20) is added and rotated for 4 hours at room temperature. Unreacted maleimide-PEG is removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS pH 7.4. The antibody-PEG-Mal conjugate is collected and transferred into a reaction vessel. Various siRNA conjugates are synthesized using sequences listed in **Tables 13-17**. siRNA-*DMD* conjugates (2 equivalents) is added at

RT to the antibody-PEG-maleimide in PBS and rotated overnight. The reaction mixture is analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody and siRNA is seen.

[0452] **Step 2: Purification**

[0453] The crude reaction mixture is purified by AKTA explorer FPLC using anion exchange chromatography. Fractions containing the antibody-PEG-DMD conjugate are pooled, concentrated and buffer exchanged with PBS, pH 7.4. Antibody siRNA conjugates with SMCC linker, PEG1kDa, PEG5kDa and PEG10kDa are separated based on the siRNA loading.

[0454] **Step-3: Analysis of the purified conjugate**

[0455] The isolated conjugate is characterized by either mass spec or SDS-PAGE. The purity of the conjugate is assessed by analytical HPLC using anion exchange chromatography.

Example 8. Additional Sequences

[0456] Table 18 illustrates additional polynucleic acid molecule sequences described herein.

Exon	AO name (h,H:Human; M:mouse)	Location from acceptor site	Sequence	SEQ ID NO:
2	hEx2_Ac12	12	CCA UUU UGU GAA UGU UUU CUU UUG AAC AUC	964
2	hEx2_Ac19	19	CCC AUU UUG UGA AUG UUU UCU UUU	965
2	hEx2_Ac32	32	UUG UGC AUU UAC CCA UUU UGU G	966
2	hEx2_Ac35	35	GAA AAU UGU GCA UUU ACC CAU UUU	967
3	hEx3_Ac20	20	GUA GGU CAC UGA AGA GGU UCU	968
4	hEx4_Ac11	11	UGU UCA GGG CAU GAA CUC UUG UGG AUC CUU	969
5	hEx5_Ac25	25	UCA GUU UAU GAU UUC CAU CUA CGA UGU CAG U	970
6	hEx6_Ac69	69	UAC GAG UUG AUU GUC GGA CCC AG	971
7	hEx_Ac45	45	UGC AUG UUC CAG UCG UUG UGU GG	972
8	hEx8_Ac-6	-6	GAU AGG UGG UAU CAA CAU CUG UAA	973
8	hEx8_Ac26	26	CUU CCU GGA UGG CUU CAA U	974
8	hEx8_Ac84	84	GUA CAU UAA GAU GGA CUU C	975
9	hEx9_Ac-6	-6	CCC UGU GCU AGA CUG ACC GUG AUC UGC AG	976
10	hEx10_Ac-5	-5	CAG GAG CUU CCA AAU GCU GCA	977
10	hEx10_Ac98	98	UCC UCA GCA GAA AGA AGC CAC G	978
11	hEx11_Ac75	75	CAU CUU CUG AUA AUU UUC CUG UU	979
12	hEx12_Ac52	52	UCU UCU GUU UUU GUU AGC CAG UCA	980
13	hEx13_Ac77	77	CAG CAG UUG CGU GAU CUC CAC UAG	981
14	hEx14_Ac32	32	GUA AAA GAA CCC AGC GGU CUU CUG UCC AUC	982
15	hEx15_Ac48	48	UCU UUA AAG CCA GUU GUG UGA AUC	983
16	hEx16_Ac12	12	CUA GAU CCG CUU UUA AAA CCU GUU AAA ACA A	984
16	hEx16_Ac11	11	GAU UGC UUU UUC UUU UCU AGA UCC G	985
17	hEx17_Ac-7	-7	UGA CAG CCU GUG AAA UCU GUG AG	986

17	hEx17_Ac36	36	CCA UUA CAG UUG UCU GUG UU	987
17	hEx17_Ac132	132	UAA UCU GCC UCU UCU UUU GG	988
18	hEx18_Ac24	24	CAG CUU CUG AGC GAG UAA UCC AGC UGU GAA	989
19	hEx19_Ac35	35	GCC UGA GCU GAU CUG CUG GCA UCU UGC AGU U	990
19	hEx19_Ac39	39	UCU GCU GGC AUC UUG C	991
20	hEx20_Ac23	23	GUU CAG UUG UUC UGA GGC UUG UUU G	992
20	mEx20_Ac23	23	GUU CAG UUG UUC UGA AGC UUG UCU G	993
20	hEx20_Ac44	44	CUG GCA GAA UUC GAU CCA CCG GCU GUU C	994
20	mEx20_Ac44	44	UUG GCA GAA UUC UGU CCA CCG GCU GUU C	995
20	hEx20_Ac140	140	AGU AGU UGU CAU CUG CUC CAA UUG U	996
20	mEx20_Ac140	140	AGU AGU UGU CAU CUG UUC CAA UUG U	997
20	hEx20_Ac147	147	CAG CAG UAG UUG UCA UCU GCU C	998
20	mEx20_Ac147	147	CGG CAG UAG UUG UCA UCU GUU C	999
21	hEx21_Ac85	85	CUG CAU CCA GGA ACA UGG GUC C	1000
21	mEx21_Ac85	85	CUG CAU CCA GAA ACA UUG GCC C	1001
21	hEx21_Ac86	86	GUC UGC AUC CAG GAA CAU GGG UC	1002
22	mEx22_Ac8	8	AUG UCC ACA GAC CUG UAA UU	1003
22	hEx22_Ac8	8	AUA UUC ACA GAC CUG CAA UU	1004
22	hEx22_Ac125	125	CUG CAA UUC CCC GAG UCU CUG C	1005
22	mEx22_Ac125	125	CUG UAA UUU CCC GAG UCU CUC C	1006
23	mEx23_Ac7	7	GGC CAA ACC UCG GCU UAC CUG AAA U	1007
23	hEx23_Ac7	7	AGU AAA AUC UUG AAU UAC CUG AAU U	1008
23	hEx23_Ac69	69	CGG CUA AUU UCA GAG GGC GCU UUC UUC GAC	1009
23	mEx23_Ac69	69	UGG CAU AUU UCU GAA GGU GCU UUC UUG GCC	1010
24	mEx24_Ac16	16	CAA CUU CAG CCA UCC AUU UCU GUA A	1011
24	hEx24_Ac16	16	CAA CUU CAG CCA UCC AUU UCU UCA G	1012
24	hEx24_Ac51	51	CAA GGG CAG GCC AUU CCU CCU UC	1013
24	mEx24_Ac51	51	CCA GGG CAG GCC AUU CCU CUU UC	1014
24	mEx24_Ac78	78	GAG CUG UUU UUU CAG GAU UUC AGC A	1015
24	hEx24_Ac78	78	CAG CUG CUU UUU UAG AAU UUC UGA A	1016
25	hEx25_Ac95	95	UUG AGU UCU GUC UCA AGU CUC GAA G	1017
25	mEx25_Ac95	95	CUA AGU UCU GUC UCC AGU CUG GAU G	1018
26	hEx26_Ac-7	-7	CCU CCU UUC UGG CAU AGA CCU UCC AC	1019
27	hEx27_Ac82	82	UUA AGG CCU CUU GUG CUA CAG GUG	1020

			G	
28	hEx28_Ac99	99	CAG AGA UUU CCU CAG CUC CGC CAG GA	1021
29	hEx29_Ac15	15	UAU CCU CUG AAU GUC GCA UC	1022
29	hEx29_Ac18	18	GGU UAU CCU CUG AAU GUC GC	1023
29	hEx29_Ac45	45	UCU GUG CCA AUA UGC GAA UC	1024
29	hEx29_Ac57	57	UCC GCC AUC UGU UAG GGU CUG UGC C	1025
29	hEx29_Ac59	59	CCA UCU GUU AGG GUC UGU G	1026
29	hEx29_Ac105	105	UUA AAU GUC UCA AGU UCC	1027
29	hEx29_Ac127	127	GUA GUU CCC UCC AAC G	1028
29	hEx29_Ac131	131	CAU GUA GUU CCC UCC	1029
30	hEx30_Ac25	25	UCC UGG GCA GAC UGG AUG CUC UGU UC	1030
31	hEx31_Ac3	3	UAG UUU CUG AAA UAA CAU AUA CCU G	1031
32	hEx32_Ac44	44	CUU GUA GAC GCU GCU CAA AAU UGG CUG GUU	1032
33	hEx33_Ac64	64	CCG UCU GCU UUU UCU GUA CAA UCU G	1033
34	hEx34_Ac46	46	CAU UCA UUU CCU UUC GCA UCU UAC G	1034
34	hEx34_Ac95	95	AUC UCU UUG UCA AUU CCA UAU CUG UA	1035
35	hEx35_Ac24	24	UCU GUG AUA CUC UUC AGG UGC ACC UUC UGU	1036
36	hEx36_Ac22	22	UGU GAU GUG GUC CAC AUU CUG GUC AAA AGU	1037
37	hEx37_Ac134	134	UUC UGU GUG AAA UGG CUG CAA AUC	1038
38	hEx38_Ac88	88	UGA AGU CUU CCU CUU UCA GAU UCA C	1039
39	hEx39_Ac62	62	UUU CCU CUC GCU UUC UCU CAU CUG UGA UUC	1040
40	hEx40_Ac-5	-5	CUU UGA GAC CUC AAA UCC UGU U	1041
40	hEx40_Ac13	13	GAG CCU UUU UUC UUC UUU G	1042
40	hEx40_Ac127	127	UCC UUU CAU CUC UGG GCU C	1043
41	hEx41_Ac44	44	CAA GCC CUC AGC UUG CCU ACG CAC UG	1044
41	hEx41_Ac18	18	CUC CUC UUU CUU CUU CUG C	1045
41	hEx41_Ac145	145	CUU CGA AAC UGA GCA AAU UU	1046
42	hEx42_Ac4	4	AUC GUU UCU UCA CGG ACA GUG UGC UGG	1047
42	hEx42_Ac90	90	CUU GUG AGA CAU GAG UG	1048
42	hEx42_Ac175	175	CAG AGA CUC CUC UUG CUU	1049
43	hEx43_Ac52	52	UGC UGC UGU CUU CUU GCU	1050
43	hEx43_Ac90	90	CUG UAG CUU CAC CCU UUC C	1051
43	hEx43_Ac101	101	GGA GAG AGC UUC CUG UAG CU	1052
43	hEx43_Ac132	132	UGU UAA CUU UUU CCC AUU GG	1053
43	hEx43_Ac134	134	UUG UUA ACU UUU UCC AUU	1054
43	hEx43_Ac137	137	CAU UUU GUU AAC UUU UUC CC	1055
44	hEx44_Ac0	0	CGC CAT TTC TCA ACA GAT CTG TCA	1056

			AAT CGC	
44	hEx44_Ac1	1	CCG CCA TTT CTC AAC AGA TCTGTC AAA TCG	1057
44	hEx44_Ac2	2	GCC GCC ATT TCT CAA CAG ATC TGT CAA ATC	1058
44	hEx44_Ac3	3	AGC CGC CAT TTC TCA ACA GAT CTG TCA AAT	1059
44	hEx44_Ac4	4	AAG CCG CCA TTT CTC AAC AGA TCT GTC AAA	1060
44	hEx44_Ac5	5	AAA GCC GCC ATT TCT CAA CAG ATC TGT CAA	1061
44	hEx44_Ac6	6	AAA AGC CGC CAT TTC TCA ACA GAT CTG TCA	1062
44	hEx44_Ac7	7	AAA ACG CCG CCA TTT CTC AAC AGA TCT GTC	1063
44	hEx44_Ac8	8	GAA AAC GCC GCC ATT TCT CAA CAG ATC TGT	1064
44	hEx44_Ac9	9	TGA AAA CGC CGC CAT TTC TCA ACA GAT CTG	1065
44	hEx44_Ac10	10	ATG AAA ACG CCG CCA TTT CTC AAC AGA TCT	1066
44	hEx44_Ac14	14	CAT AAT GAA AAC GCC GCC ATT TCT CAA CAG	1067
44	hEx44_Ac15	15	CGC CGC CAU UUC UCA ACA G	1068
44	hEx44_Ac18	18	ATA TCA TAA TGA AAA CGC CGC CAT TTC TCA	1069
44	hEx44_Ac19	19	TAT ATC ATA ATG AAA ACG CCG CCA TTT CTC	1070
44	hEx44_54	54	TGT TCA GCT TCT GTT AGC CAC TGA TTA AAT	1071
44	hEx44_Ac56	56	ACT GTT CAG CTT CTG TTA GCC ACT GAT TAA	1072
44	hEx44_Ac59	59	GAA ACT GTT CAG CTT CTG TTA GCC ACT GAT	1073
44	hEx44_Ac61	61	UGU UCA GCU UCU GUU AGC CAC UGA	1074
44	hEx44_Ac69	69	GTC TTT CTG AGA AAC TGT TCA GCT TCT GTT	1075
44	hEx44_Ac87	87	UUU GUA UUU AGC AUG UUC CC	1076
45	hEx45_Ac-6	-6	CCA AUG CCA UCC UGG AGU UCC UGU AA	1077
45	hEx45_Ac0	0	TTG CCG CTG CCC AAT GCC ATC CTG GAG TTC	1078
45	hEx45_Ac1	1	TTT GCC GCT GCC CAA TGC CAT CCT GGA GTT	1079
45	hEx45_Ac2	2	GTT TGC CGC TGC CCA ATG CCA TCC TGG AGT	1080
45	hEx45_Ac3	3	AGT TTG CCG CTG CCC AAT GCC ATC CTG GAG	1081
45	hEx45_Ac4	4	CAG TTT GCC GCT GCC CAA TGC CAT CCT GGA	1082
45	hEx45_Ac6	6	GCC CAA UGC CAU CCU GG	1083
45	hEx45_Ac7	7	CAA CAG TTT GCC GCT GCC CAA TGC CAT CCT	1084
45	hEx45_Ac8	8	ACA ACA GTT TGC CGC TGC CCA ATG CCA TCC	1085

45	hEx45_Ac9	9	GAC AAC AGT TTG CCG CTG CCC AAT GCC ATC	1086
45	hEx45_Ac10	10	TGA CAA CAG TTT GCC GCT GCC CAA TGC CAT	1087
45	hEx45_Ac11	11	CTG ACA ACA GTT TGC CGC TGC CCA ATG CCA	1088
45	hEx45_Ac12	12	TCT GAC AAC AGT TTG CCG CTG CCC AAT GCC	1089
45	hEx45_Ac58	58	GCU GAA UUA UUU CUU CCC C	1090
45	hEx45_Ac75	75	UCU GUU UUU GAG GAU UGC	1091
45	hEx45_Ac122	122	CCA CCG CAG AUU CAG GC	1092
45	hEx45_Ac137	137	UUU GCA GAC CUC CUG CC	1093
45	hEx45_Ac154	154	UUU UUC UGU CUG ACA GCU G	1094
46	hEx46_Ac14	14	CUG ACA AGA UAU UCU U	1095
46	hEx46_Ac15	15	GAA AUU CUG ACA AGA UAU UCU	1096
46	hEx46_Ac45	45	CTT CCT CCA ACC ATA AAA CAA ATT CAT TTA	1097
46	hEx46_Ac46	46	GCT TCC TCC AAC CAT AAA ACA AAT TCA TTT	1098
46	hEx46_Ac47	47	TGC TTC CTC CAA CCA TAA AAC AAA TTC ATT	1099
46	hEx46_Ac47	47	UAA AAC AAA UUC AUU	1100
46	hEx46_Ac48	48	CTG CTT CCT CCA ACC ATA AAA CAA ATT CAT	1101
46	hEx46_Ac49	49	TCT GCT TCC TCC AAC CAT AAA ACA AAT TCA	1102
46	hEx46_Ac50	50	ATC TGC TTC CTC CAA CCA TAA AAC AAA TTC	1103
46	hEx46_Ac51	51	TAT CTG CTT CCT CCA ACC ATA AAA CAA ATT	1104
46	hEx46_Ac52	52	TTA TCT GCT TCC TCC AAC CAT AAA ACA AAT	1105
46	hEx46_Ac53	53	GTT ATC TGC TTC CTC CAA CCA TAA AAC AAA	1106
46	hEx46_Ac54	54	TGT TAT CTG CTT CCT CCA ACC ATA AAA CAA	1107
46	hEx46_Ac55	55	ATG TTA TCT GCT TCC TCC AAC CAT AAA ACA	1108
46	hEx46_Ac56	56	AAT GTT ATC TGC TTC CTC CAA CCA TAA AAC	1109
46	hEx46_Ac57	57	CAA TGT TAT CTG CTT CCT CCA ACC ATA AAA	1110
46	hEx46_Ac58	58	GCA ATG TTA TCT GCT TCC TCC AAC CAT AAA	1111
46	hEx46_Ac59	59	AGC AAT GTT ATC TGC TTC CTC CAA CCA TAA	1112
46	hEx46_Ac60	60	TAG CAA TGT TAT CTG CTT CCT CCA ACC ATA	1113
46	hEx46_Ac61	61	CTA GCA ATG TTA TCT GCT TCC TCC AAC CAT	1114
46	hEx46_Ac62	62	ACT AGC AAT GTT ATC TGC TTC CTC CAA CCA	1115
46	hEx46_Ac63	63	GUU AUC UGC UUC CUC CAA CC	1116
46	hEx46_Ac88	88	AGG UUC AAG UGG GAU ACU A	1117

46	hEx46_Ac90	90	UCC AGG UUC AAG UGG GAU AC	1118
46	hEx46_Ac96	96	UUC CAG GUU CAA GUG	1119
46	hEx46_Ac107	107	CAA GCU UUU CUU UUA GUU GCU GCU CUU UUC C	1120
46	hEx46_Ac111	111	UUA GUU GCU GCU CUU	1121
46	hEx46_Ac115	115	GCU UUU CUU UUA GUU GCU GC	1122
46	hEx46_Ac122	122	UCA AGC UUU UCU UUU AG	1123
47	hEx47_Ac-6	-6	CAG GGG CAA CUC UUC CAC CAG UAA CUG AAA	1124
47	hEx47_Ac39	39	UCC AGU UUC AUU UAA UUG UUU G	1125
47	hEx47_Ac63	63	AGC ACU UAC AAG CAC GGG U	1126
47	hEx47_Ac87	87	UCU UGC UCU UCU GGG CUU	1127
47	hEx47_Ac94	94	UUC AAG UUU AUC UUG CUC UUC	1128
47	hEx47_Ac101	101	CUU GAG CUU AUU UUC AAG UUU	1129
47	hEx47_Ac103	103	CUG CUU GAG CUU AUU UUC AAG UU	1130
48	hEx48_Ac-7	-7	UUC UCA GGU AAA GCU CUG GAA ACC UGA AAG	1131
48	hEx48_Ac2	2	CUU CAA GCU UUU UUU CAA GCU	1132
48	hEx48_Ac19	19	UUU CUC CUU GUU UCU C	1133
48	hEx48_Ac23	23	GCU UCA AUU UCU CCU UGU U	1134
48	hEx48_Ac32	32	UUU AUU UGA GCU UCA AUU U	1135
48	hEx48_Ac37	37	GGU CUU UUA UUU GAG CUU C	1136
48	hEx48_Ac48	48	GCU GCC CAA GGU CUU UU	1137
48	hEx48_Ac71	71	CUU CAA GGU CUU CAA GCU UUU	1138
48	hEx48_Ac79	79	UAA CUG CUC UUC AAG GUC UUC	1139
48	hEx48_Ac133	133	UUA UAA AUU UCC AAC UGA UUC	1140
49	hEx49_Ac-11	-11	CUG CUA UUU CAG UUU CCU GGG GAA AAG	1141
49	hEx49_Ac25	25	CUU CCA CAU CCG GUU GUU U	1142
49	hEx49_Ac60	60	GUG GCU GGU UUU UCC UUG U	1143
50	hEx50_Ac2	2	CCA CUC AGA GCU CAG AUC UUC UAA CUU CC	1144
50	hEx50_Ac11	11	CUC AGA GCU CAG AUC UU	1145
50	hEx50_Ac36	36	GGC UGC UUU GCC CUC	1146
51	hEx51_Ac0	0	GTG TCA CCA GAG TAA CAG TCT GAG TAG GAG	1147
51	hEx51_Ac5	5	AGG TTG TGT CAC CAG AGT AAC AGT CTG AGT	1148
51	hEx51_Ac9	9	CCA CAG GTT GTG TCA CCA GAG TAA CAG TCT	1149
51	hEx51_Ac26	26	GGC AGT TTC CTT AGT AAC CAC AGG TTG TGT	1150
51	hEx51_Ac30	30	AGA TGG CAG TTT CCT TAG TAA CCA CAG GTT	1151
51	hEx51_Ac48	48	ATG GCA TTT CTA GTT TGG AGA TGG CAG TTT	1152
51	hEx51_Ac65	65	CTC CAA CAT CAA GGA AGA TGG CAT TTC TAG	1153
51	hEx51_Ac66	66	ACA UCA AGG AAG AUG GCA UUU CUA G	1154
51	hEx51_Ac67	67	TCA AGG AAG ATG GCA TTT CT	1155

51	hEx51_Ac68	68	UCA AGG AAG AUG GCA UUU CU	1156
51	hEx51_Ac132	132	GAA AGC CAG UCG GUA AGU UC	1157
51	hEx51_Ac141	141	TTA TAA CTT GAT CAA GCA GAG AAA GCC AGT	1158
51	hEx51_Ac160	160	CCU CUG UGA UUU UAU AAC UUG AU	1159
51	hEx51_Ac181	181	CAC CCA CCA UCA CCC	1160
51	hEx51_Ac191	191	UGA UAU CCU CAA GGU CAC CC	1161
51	hEx51_Ac207	207	ATA CCT TCT GCT TGA TGA TCA TCT CGT TGA	1162
52	hEx52_Ac12	12	UCC AAC UGG GGA CGC CUC UGU UCC AAA UCC	1163
52	mEx52_Ac12	12	UCC AAU UGG GGG CGU CUC UGU UCC AAA UCU	1164
52	mEx52_Ac17	17	UCC AAU UGG GGG CGU CUC UGU UCC A	1165
52	hEx52_Ac17	17	UCC AAC UGG GGA CGC CUC UGU UCC A	1166
52	hEx52_Ac18	18	UUC CAA CUG GGG ACG CCU CUG UUC C	1167
52	hEx52_Ac24	24	GGT AAT GAG TTC TTC CAA CTG GGG ACG CCT	1168
52	mEx52_Ac42	42	UUC AAA UUC UGG GCA GCA GUA AUG AGU UCU	1169
52	hEx52_Ac42	42	UUC AAA UUU UGG GCA GCG GUA AUG AGU UCU	1170
52	hEx52_Ac69	69	UUG CUG GUC UUG UUU UUC	1171
52	hEx52_Ac97	97	CCG UAA UGA UUG UUC U	1172
53	hEx53_Ac1	1	ACT TCA TCC CAC TGA TTC TGA ATT CTT TCA	1173
53	hEx53_Ac2	2	TAC TTC ATC CCA CTG ATT CTG AAT TCT TTC	1174
53	hEx53_Ac3	3	GTA CTT CAT CCC ACT GAT TCT GAA TTC TTT	1175
53	hEx53_Ac4	4	TGT ACT TCA TCC CAC TGA TTC TGA ATT CTT	1176
53	mEx53_Ac5	5	UUU UAA AGA UAU GCU UGA CAC UAA CCU UGG	1177
53	hEx53_Ac5	5	UUA AAA AGG UAU CUU UGA UAC UAA CCU UGG	1178
53	hEx53_Ac5	5	TTG TAC TTC ATC CCA CTG ATT CTG AAT TCT	1179
53	hEx53_Ac6	6	CTT GTA CTT CAT CCC ACT GAT TCT GAA TTC	1180
53	hEx53_Ac7	7	TCT TGT ACT TCA TCC CAC TGA TTC TGA ATT	1181
53	hEx53_Ac8	8	TTC TTG TAC TTC ATC CCA CTG ATT CTG AAT	1182
53	hEx53_Ac9	9	GTT CTT GTA CTT CAT CCC ACT GAT TCT GAA	1183
53	hEx53_Ac10	10	TGT TCT TGT ACT TCA TCC CAC TGA TTC TGA	1184
53	hEx53_Ac11	11	GTG TTC TTG TAC TTC ATC CCA CTG ATT CTG	1185
53	hEx53_Ac12	12	GGT GTT CTT GTA CTT CAT CCC ACT	1186

			GAT TCT	
53	hEx53_Ac13	13	AGG TGT TCT TGT ACT TCA TCC CAC TGA TTC	1187
53	hEx53_Ac14	14	AAG GTG TTC TTG TAC TTC ATC CCA CTG ATT	1188
53	hEx53_Ac15	15	GAA GGT GTT CTT GTA CTT CAT CCC ACT GAT	1189
53	hEx53_Ac16	16	TGA AGG TGT TCT TGT ACT TCA TCC CAC TGA	1190
53	hEx53_Ac17	17	CTG AAG GTG TTC TTG TAC TTC ATC CCA CTG	1191
53	hEx53_Ac18	18	TCT GAA GGT GTT CTT GTA CTT CAT CCC ACT	1192
53	hEx53_Ac19	19	TTC TGA AGG TGT TCT TGT ACT TCA TCC CAC	1193
53	hEx53_Ac20	20	GTT CTG AAG GTG TTC TTG TAC TTC ATC CCA	1194
53	hEx53_Ac21	21	GGT TCT GAA GGT GTT CTT GTA CTT CAT CCC	1195
53	hEx53_Ac22	22	CGG TTC TGA AGG TGT TCT TGT ACT TCA TCC	1196
53	hEx53_Ac23	23	CCG GTT CTG AAG GTG TTC TTG TAC TTC ATC	1197
53	hEx53_Ac24	24	TCC GGT TCT GAA GGT GTT CTT GTA CTT CAT	1198
53	hEx53_Ac25	25	CTC CGG TTC TGA AGG TGT TCT TGT ACT TCA	1199
53	hEx53_Ac26	26	CCT CCG GTT CTG AAG GTG TTC TTG TAC TTC	1200
53	hEx53_Ac27	27	GCC TCC GGT TCT GAA GGT GTT CTT GTA CTT	1201
53	hEx53_Ac28	28	TGC CTC CGG TTC TGA AGG TGT TCT TGT ACT	1202
53	hEx53_Ac20	29	TTG CCT CCG GTT CTG AAG GTG TTC TTG TAC	1203
53	hEx53_Ac30	30	GTT GCC TCC GGT TCT GAA GGT GTT CTT GTA	1204
53	hEx53_Ac39	39	CAU UCA ACU GUU GCC UCC GGU UCU GAA GGU G	1205
53	mEx53_Ac39	39	CAU UCA ACU GUU GUC UCC UGU UCU GCA GCU G	1206
53	hEx53_Ac45	45	CUG UUG CCU CCG GUU CUG	1207
53	hEx53_Ac69	69	CAG CCA UUG UGU UGA AUC CUU UAA CAU UUC	1208
53	hEx53_Ac128	128	UUG GCU CUG GCC UGU CCU	1209
53	mEx53_Ac151	151	CUA CUG UGU GAG GAC CUU CUU UCC AUG AGU	1210
53	mEx53_Ac176	176	UCU GUG AUC UUC UUU UGG AUU GCA UCU ACU	1211
54	hEx54_Ac21	21	UAC AUU UGU CUG CCA CUG G	1212
54	hEx54_Ac42	42	GAG AAG TTT CAG GGC CAA GTC ATT TGC CAC	1213
54	hEx54_Ac58	58	CCC GGA GAA GUU UCA GGG	1214
54	hEx54_Ac67	67	UCU GCA GAA UAA UCC CGG AGA AG	1215

55	hEx55_Ac0	0	TCT TCC AAA GCA GCC TCT CGC TCA CTC ACC	1216
55	hEx55_Ac29	29	UGC AGU AAU CUA UGA GUU UC	1217
55	hEx55_Ac33	33	CUG UUG CAG UAA UCU AUG AG	1218
55	hEx55_Ac104	104	UCC UGU AGG ACA UUG GCA GU	1219
55	hEx55_Ac139	139	GAG UCU UCU AGG AGC CUU	1220
55	hEx55_Ac141	141	CUU GGA GUC UUC UAG GAG CC	1221
55	hEx55_Ac167	167	UGC CAU UGU UUC AUC AGC UCU UU	1222
56	hEx56_Ac48	48	UUU UUU GGC UGU UUU CAU CC	1223
56	hEx56_Ac69	69	CCU UCC AGG GAU CUC AGG	1224
56	hEx56_Ac102	102	GUU AUC CAA ACG UCU UUG UAA CAG G	1225
56	hEx56_Ac129	129	GUU CAC UCC ACU UGA AGU UC	1226
57	hEx57_Ac-12	-12	CUG GCU UCC AAA UGG GAC CUG AAA AAG AAC	1227
57	hEx57_Ac64	64	UUC AGC UGU AGC CAC ACC	1228
57	hEx57_Ac97	97	UAG GUG CCU GCC GGC UU	1229
57	hEx57_Ac118	118	CUG AAC UGC UGG AAA GUC GCC	1230
58	hEx58_Ac9	9	UUC UUU AGU UUU CAA UUC CCU C	1231
58	hEx58_Ac21	21	ACU CAU GAU UAC ACG UUC UUU AGU U	1232
58	hEx58_Ac86	86	GAG UUU CUC UAG UCC UUC C	1233
59	hEx59_Ac6	6	UCC UCA GGA GGC AGC UCU AAA U	1234
59	hEx59_Ac66	66	GAG UUU CUC UAG UCC UUC C	1235
59	hEx59_Ac134	134	UUG AAG UUC CUG GAG UCU U	1236
60	hEx60_Ac19	19	GUU CUC UUU CAG AGG CGC	1237
60	hEx60_Ac37	37	CUG GCG AGC AAG GUC CUU GAC GUG GCU CAC	1238
60	hEx60_Ac92	92	GUG CUG AGG UUA UAC GGU G	1239
61	hEx61_Ac10	10	GGG CUU CAU GCA GCU GCC UGA CUC GGU CCU C	1240
61	hEx61_Ac31	31	GUC CCU GUG GGC UUC AUG	1241
61	hEx61_Ac51	51	GUG CUG AGA UGC UGG ACC	1242
62	hEx62_Ac8	8	GAG AUG GCU CUC UCC CAG GGA CCC UGG	1243
62	hEx62_Ac15	15	UGG CUC UCU CCC AGG G	1244
62	hEx62_Ac37	37	GGG CAC UUU GUU UGG CG	1245
63	hEx63_Ac11	11	UGG GAU GGU CCC AGC AAG UUG UUU G	1246
63	hEx63_Ac11	11	GGU CCC AGC AAG UUG UUU G	1247
63	hEx63_Ac33	33	GUA GAG CUC UGU CAU UUU GGG	1248
64	hEx64_Ac47	47	GCA AAG GGC CUU CUG CAG UCU UCG GAG	1249
65	hEx65_Ac-11	-11	GCU CAA GAG AUC CAC UGC AAA AAA C	1250
65	mEx65_Ac-11	–11	GCU CAA GAG AUC CAC UGC AAA AAA G	1251
65	hEx65_Ac15	15	GCC AUA CGU ACG UAU CAU AAA CAU UC	1252
65	hEx65_Ac26	26	GUU GUG CUG GUC CAA GGC AUC ACA U	1253

65	mEx65_Ac26	26	GUU GUG CUG GUC CAG GGC AUC ACA U	1254
65	hEx65_Ac63	63	UCU GCA GGA UAU CCA UGG GCU GGU C	1255
65	hEx65_Ac63	63	UCU GCA GGA UAU CCA UGG GCU GGU C	1256
66	hEx66_Ac-8	-8	GAU CCU CCC UGU UCG UCC CCU AUU AUG	1257
67	hEx67_Ac22	22	GCG CUG GUC ACA AAA UCC UGU UGA AC	1258
68	hEx68_Ac22	22	CAU CCA GUC UAG GAA GAG GGC CGC UUC	1259
69	hEx69_Ac-6	-6	UGC UUU AGA CUC CUG UAC CUG AUA	1260
70	hEx70_Ac98	98	CCU CUA AGA CAG UCU GCA CUG GCA	1261
71	hEx71_Ac-3	-3	AAG UUG AUC AGA GUA ACG GGA CUG	1262
71	hEx71_Ac8	8	GCC AGA AGU UGA UCA GAG U	1263
71	hEx71_Ac16	16	UCU ACU GGC CAG AAG UUG	1264
72	hEx72_Ac2	2	GUG UGA AAG CUG AGG GGA CGA GGC AGG	1265
72	hEx72_Ac20	20	UGA GUA UCA UCG UGU GAA AG	1266
72	hEx72_Ac42	42	GCA UAA UGU UCA AUG CGU G	1267
73	hEx73_Ac6	6	GAU CCA UUG CUG UUU UCC AUU UCU G	1268
73	hEx73_Ac13	13	GAU CCA UUG CUG UUU UCC	1269
73	hEx73_Ac31	31	GAG AUG CUA UCA UUU AGA UAA	1270
74	hEx74_Ac48	48	CGA GGC UGG CUC AGG GGG GAG UCC U	1271
74	hEx74_Ac51	51	CUG GCU CAG GGG GGA GU	1272
74	hEx74_Ac72	72	UCC CCU CUU UCC UCA CUC U	1273
75	hEx75_Ac34	34	GGA CAG GCC UUU AUG UUC GUG CUG C	1274
75	hEx75_Ac33	33	CCU UUA UGU UCG UGC UGC U	1275
75	hEx75_Ac144	144	GGC GGC CUU UGU GUU GAC	1276
76	hEx76_Ac53	53	GCU GAC UGC UGU CGG ACC UCU GUA GAG	1277
76	hEx76_Ac37	37	GAG AGG UAG AAG GAG AGG A	1278
76	hEx76_Ac65	65	AUA GGC UGA CUG CUG UCG G	1279
77	hEx77_Ac16	16	CUG UGC UUG UGU CCU GGG GAG GAC UGA	1280
77	hEx77_Ac20	20	UUG UGU CCU GGG GAG GA	1281
77	hEx77_A47	47	UGC UCC AUC ACC UCC UCU	1282
78	hEx78_Ac4	4	UCU CAU UGG CUU UCC AGG GGU AUU UC	1283
78	hEx78_Ac4	4	GCU UUC CAG GGG UAU UUC	1284
78	hEx78_Ac10	10	CAU UGG CUU UCC AGG GG	1285

Example 9. Screening of DMD exon 44 and 45 skipping PMOs in transfected primary human skeletal muscle cells

[0457] Primary, pre-differentiated human skeletal muscle cells (Gibco, #A11440) were plated on collagen Type 1 coated 24-well plates (Gibco, #1970788) in DMEM supplemented with 2% horse serum)

and 1x ITS (Gibco, #1933286) according to the manufacturer's instructions. Cells were grown in 37°C + 5% CO_2 for 2 days to establish myotubes. These cells were then treated with defined concentrations of PMOs in water and 2 uM Endo-Porter (Gene Tools, #EP6P1-1) to facilitate PMO uptake into cells. Cell were harvested 48 hours after treatment by aspirating the culture medium and addition of 300 ul TRIZOL per well. Cells were frozen at -80°C before RNA was prepared using Direct-zol™-96 RNA kit (Zymo Research, #R2056). Total RNA concentration was quantified spectroscopically. Between 100-200 ng total RNA was reverse transcribed using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, #4368813). RT PCR reactions were incubated at 25°C for 10 min, 37°C for 120 min, 85°C for 5 min, and then held at 4°C. Reactions were diluted 1:1 with water. For quantification of exon skipping by gel electrophoresis DNA fragments representing total (non-skipped +skipped) and skipped mRNAs were amplified by qPCR using Taqman Fast Advanced Master mix (Applied Biosystems, #4444558) and specific primer pairs (see Table 19). qPCR reactions were incubated at 95°C for 20 sec, followed by 32 cycles of 95°C for 1 sec and 60°C for 20 sec using a QuantStudio 7 Flex (Applied Biosystems). PCR products were diluted 4:1 with TAE loading buffer and loaded onto 24-well 4% TAE gels (Embi Tec, #GG3807) containing GelGreen. PCR products were separated by electrophoresis (50 V for 2 hrs). The intensity of bands corresponding to total DMD and skipped DMD products were quantified by densiometry using ChemiDoc™ XRS+ (Bio-Rad).

[0458] Taqman qPCR primers and probes are illustrated in Table 19.

hDMD Ex44 skipped	Forward:	5'-CTGTGGAAAGGGTGAAGCTA-3'
	Reverse:	5'-GACAAGGGAACTCCAGGATG-3
	Probe:	5'-AGCTCTCTCCCAGCTTGATTTCCA-3'
hDMD Ex45 skipped	Forward:	5'-CAGTGGCTAACAGAAGCTGA-3'
	Reverse:	5'-CAAATGGTATCTTAAGGCTAGAAGAAC-3'
	Probe:	5'-ACACAAATTCCTGAGAATTGGGAACATGC-3'

[0459] hDMD total Hs01049401_m1, human DMD VIC-MGB, 360 rxns (Thermo Fisher Scientific)

[0460] Table 20A illustrates exon skipping activity of PMOs (30mer) targeting DMD exon 45 in transfected primary human skeletal muscle cells.

	PMO conc	**% Skipping (skipped/total)**	
	uM	AVG	STDEV
hEx45_Ac1	10.0	43.5	6.4
	3.0	38.5	9.2
	1.0	29.5	3.5
hEx45_Ac2	10.0	67.0	14.1
	3.0	71.5	14.8
	1.0	38.0	7.8
	0.1	10.0	
hEx45_Ac3	10.0	69.5	2.1
	3.0	56.5	10.6
	1.0	34.0	8.5

hEx45_Ac4	10.0	51.7	10.4
	3.0	49.0	1.4
	1.0	34.0	5.3
	0.1	18.0	
hEx45_Ac7	10.0	72.0	11.4
	3.0	62.5	2.1
	1.0	43.3	4.9
	0.1	18.0	
hEx45_Ac8	10.0	76.0	8.5
	3.0	69.5	12.0
	1.0	43.5	19.1
hEx45_Ac9	10.0	73.7	6.0
	3.0	62.5	9.2
	1.0	47.3	8.3
	0.1	20.0	
hEx45_Ac10	10.0	53.0	0.0
	3.0	56.5	10.6
	1.0	35.5	0.7
hEx45_Ac11	10.0	54.5	2.1
	3.0	53.0	1.4
	1.0	34.0	4.2
hEx45_Ac12	10.0	52.0	21.2
	3.0	40.0	14.1
	1.0	26.5	10.6
No PMO	0	10.5	6.4

[0461] Table 20B illustrates exon skipping activity of PMOs (30mer) targeting DMD exon 44 in transfected primary human skeletal muscle cells.

	PMO conc	**% Skipping (skipped/total)**	
	uM	AVG	STDEV
hEx44_Ac0	10	83.8	11.3
	3	79.7	3.5
	1	67.5	7.8
	0.1	31.5	0.7
hEx44_Ac1	10	77.7	8.3
	3	79.5	0.7
	1	68.3	8.5
	0.1	32.0	
hEx44_Ac2	10	88.7	4.5
	3	96.0	7.1
	1	70.0	13.2
	0.1	31.0	
hEx44_Ac3	10	75.0	14.1
	3	89.0	
	1	62.0	8.5
	0.1	26.0	
hEx44_Ac4	10	84.0	17.0
	3	88.0	

	1	67.0	15.6
	0.1	23.0	
hEx44_Ac5	10	63.0	0.0
	3	68.0	
	1	54.0	8.5
	0.1	18.0	
hEx44_Ac6	10	74.0	12.7
	3	81.0	
	1	58.5	17.7
	0.1	20.0	
hEx44_Ac7	10	84.3	19.5
	3	85.0	4.2
	1	59.3	13.0
	0.1	23.0	
hEx44_Ac8	10	76.0	0.0
	3	70.0	
	1	53.5	2.1
	0.1	27.0	
hEx44_Ac9	10	76.5	2.1
	3	73.0	
	1	59.0	15.6
	0.1	32.0	
hEx44_Ac10	10	85.0	18.4
	3	79.0	
	1	45.5	6.4
	0.1	23.0	
hEx44_Ac14	10	86.5	19.1
	3	80.0	11.8
	1	62.0	9.0
	0.1	31.5	0.7
No PMO		8.3	3.8

[0462] Fig. 15 illustrates exon skipping activity of different lengths of hEx45_Ac9 PMOs in transfected primary human skeletal muscle cells.

Example 10. Synthesis and purification of human TfR1 PMO conjugates

[0463] An anti-human transferrin receptor antibody was produced. PMOs (28-mers) were synthesized by GeneTools. Antibody (10 mg/ml) in borate buffer (25 mM sodium tetraborate, 25 mM NaCl, 1 mM Diethylene triamine pentaacetic acid, pH 8.0) was reduced by adding 4 equivalents of tris(2-carboxyethyl)phosphine (TCEP) in water and incubating at 37°C for 4 hours. 4(N-Maleimidomethyl)cyclohexanecarboxylic acid N-hydroxysuccinimide ester (SMCC) was coupled to the primary amine on the 3' end of the PMO by incubating the PMO (50 mg/ml) in DMSO with 10 equivalents of SMCC (10 mg/ml) in DMSO for one hour. Unconjugated SMCC was removed by ultrafiltration using Amicon Ultra-15 centrifugal filter units with a MWCO of 3 kDa. The PMO-SMCC was washed three times with acetate buffer (10 mM sodium acetate, pH 6.0) and used immediately. The reduced antibody was mixed with 2.25 equivalents of PMO-SMCC and incubated overnight at 4°C. The pH of the reaction mixture was then reduced to 7.5 and 8 equivalents of N-Ethylmaleimide was added to

the mixture at room temperature for 30 minutes to quench unreacted cysteines. Analysis of the reaction mixture by hydrophobic interaction chromatography (HIC) method-2 showed antibody-PMO conjugates along with unreacted antibody and PMO.

[0464] The reaction mixture was purified with an AKTA Explorer FPLC using HIC method-1. Dependent on the conjugate, fractions containing either conjugates with a drug to antibody ratio of one (DAR 1), two (DAR 2), and three (DAR 3), or fractions containing conjugates with a drug to antibody ratio of 3+ (DAR 3+), or 4+ (DAR 4+) were combined and concentrated with Amicon Ultra-15 centrifugal filter units with a MWCO of 50 kDa. Concentrated conjugates were buffer exchanged with PBS (pH 7.4) using Amicon Ultra-15 centrifugal filter units prior to analysis.

[0465] Hydrophobic interaction chromatography (HIC) method-1.

1. Column: GE, HiScreen Butyl HP, 4.7ml
2. Solvent A: 50 mM phosphate buffer, 0.7M Ammonium Sulfate, pH 7.0; Solvent B: 80% 50 mM phosphate buffer, 20% IPA, pH 7.0; Flow Rate: 1.0 ml/min
3. Gradient:

	%A	%B	Column Volume
a.			
b.	100	0	1
c.	70	30	25
d.	0	100	1
e.	0	100	2

[0466] Binding of hTfR1.mAb-PMO conjugates to human Transferrin Receptor

[0467] Antibody conjugate (AOC) binding was measured by ELISA. Recombinant human Transferrin Receptor (Sino Biological 11020-H07H) was coated onto high bind plates (Costar 3690) at 1 ng/uL in PBS overnight. Plates were washed and AOC or mAb samples were added at concentrations up to 10 nM. Color was developed through HRP conjugated secondary antibody (Jackson Immunoresearch 109-035-006) and TMB substrate (ThermoFisher 34028) stopped with 2N sulfuric acid. Kd was determined using GraphPad Prism.

[0468] Fig. 16 illustrates binding of hTfR1.mAb-PMO conjugates to human Transferrin Receptor in vitro.

[0469] Activity of TfR1 mAb-PMO conjugates in primary human skeletal muscle cells

[0470] Primary, pre-differentiated human skeletal muscle cells (Gibco, #A11440) were plated on collagen Type 1 coated 24-well plates (Gibco, #1970788) in DMEM supplemented with 2% horse serum and 1x ITS (Gibco, #1933286) according to the manufacturer's instructions. Cells were grown in 37°C + 5% CO_2 for 2 days to establish myotubes. Immortalized human skeletal muscle cells from healthy donors (Myology Institute Paris) were plated on collagen Type 1 coated 24-well plates (Gibco, #1970788) in Skeletal Muscle Cell Growth medium (Promocell, C-23160) supplemented with 5 % FBS. After myoblasts reached confluency, myotube formation was induced in differentiation medium containing DMEM supplemented with gentamycin (50 ug/ml) (Invitrogen, 15750-045) and insulin (10 ug/ml) (sigma, 91077). Myotubes were then treated with defined concentrations of AOCs in the respective medium. Cell were harvested 72 hours after treatment by aspirating the culture medium, followed by

addition of 300 ul TRIZOL per well. RNA isolation and quantification of DMD exon skipping was performed as detailed in example 9.

[0471] Fig. 17 illustrates exon skipping activity of hTfR1.mAb-PMO (28-mer) conjugates in primary human skeletal muscle cells.

[0472] Fig. 18 illustrates exon skipping activity of hTfR1.mAb-PMO conjugates in myotubes of primary and immortalized human skeletal muscle cells.

[0473] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A polynucleic acid conjugate comprising an anti-human transferrin receptor antibody or its binding fragment thereof and at least one polynucleic acid molecule, wherein the polynucleic acid molecule hybridizes to a target region in exon 44 or exon 45 or at or around an exon-intron junction of exon 44 or exon 45, wherein the polynucleic acid molecule comprises at least one phosphorodiamidate morpholino oligonucleotide (PMO) modified non-natural nucleotide and induces splicing out of the exon 44 or the exon 45 from a pre-mRNA transcript to generate an mRNA transcript that encodes a functional dystrophin protein in a human muscle cell.
2. The polynucleic acid conjugate of claim 1, wherein the functional dystrophin protein is a truncated form of the dystrophin protein.
3. The polynucleic acid conjugate of claim 1 or 2, wherein the polynucleic acid molecule is a single stranded oligonucleotide.
4. The polynucleic acid conjugate of any one of claims 1-3, wherein the target region is at the exon-intron junction, wherein the exon is the exon that is to be spliced out, or the target region is an intronic region upstream or downstream of the exon-intron junction.
5. The polynucleic acid conjugate of any one of claims 1-3, wherein the exon-intron junction is located at the 5' of the exon that is to be spliced out.
6. The polynucleic acid conjugate of any one of claims 1-3, wherein the exon-intron junction is located at the 3' of the exon that is to be spliced out.
7. The polynucleic acid conjugate of any one of the claims 1-6, wherein the polynucleic acid molecule is from about 10 to about 50 nucleotides in length.
8. The polynucleic acid conjugate of any one of claims 1-7, wherein the polynucleic acid molecule comprises at least 26 contiguous nucleotides from a nucleic acid sequence selected from SEQ ID NOs: 1056-1067, 1069-1073, 1075, 1077-1082, and 1084-1089.
9. The polynucleic acid conjugate of claim 8, wherein the polynucleic acid molecule comprises at least 26 contiguous nucleotides from a nucleic acid sequence selected from SEQ ID NOs: 1056-1067, 1079-1082, and 1084-1089.

10. The polynucleic acid conjugate of claim 9, wherein the polynucleic acid molecule comprises at least 26 contiguous nucleotides from a nucleic acid sequence selected from SEQ ID NOs: 1063 and 1086.

11. The polynucleic acid conjugate of any one of claims 1-10, wherein the anti-human transferrin receptor antibody is conjugated with two or more, three or more, four or more, five or more, six or more, or eight or more polynucleic acid molecules.

12. The polynucleic acid conjugate of any one of claims 1-11, wherein the polynucleic acid molecule comprises at least one non-natural nucleotide with a 2'-modification, at least one modified internucleotide linkage, at least one inverted abasic moiety, at least one 5'-vinylphosphonate modified non-natural nucleotide, or a combination thereof.

13. The polynucleic acid conjugate of claim 12, wherein the 2'-modification is selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide.

14. The polynucleic acid conjugate of any one of claims 1-13, wherein anti-human transferrin receptor antibody or its binding fragment thereof comprises a humanized antibody or antigen binding fragment thereof, chimeric antibody or antigen binding fragment thereof, monoclonal antibody or antigen binding fragment thereof, monovalent Fab', divalent Fab2, single chain variable fragment (scFv), diabody, minibody, nanobody, single domain antibody (sdAb), or camelid antibody or antigen binding fragment thereof.

15. The polynucleic acid conjugate of any one of the claims 1-14, wherein the polynucleic acid conjugate comprises

A-(X^1-B)n

Formula (V)

wherein,

A comprises the anti-human transferrin receptor antibody or its binding fragment thereof;

B consists of the polynucleic acid molecule;

X^1 consists of a bond or first non-polymeric linker; and

n is an averaged value selected from 1-12.

16. A method of treating a disease or condition characterized with a defective *DMD* mRNA in a subject in need thereof, wherein the method comprises administering to the subject the polynucleic acid conjugate of any one of claims 1-15, wherein the polynucleic acid conjugate induces skipping of exon 44 or exon 45 that leads to the defective *DMD* mRNA to generate a processed *DMD* mRNA encoding the functional dystrophin protein, thereby treating the disease or condition in the subject.

17. The method of claim 16, wherein the disease or condition is Duchenne muscular dystrophy or Becker muscular dystrophy.

18. A method of inducing exon skipping of exon 44 or 45 in a targeted pre-mRNA transcript of a DMD gene in a human muscle cell, comprising:
a) contacting the human muscle cell with a polynucleic acid conjugate of any one of claims 1-15;
b) hybridizing the polynucleic acid conjugate to exon 44 or exon 45 of the targeted pre-mRNA transcript; and
c) translating an mRNA transcript produced from the targeted pre-mRNA transcript processed in step b) in the muscle cell to generate a functional dystrophin protein.

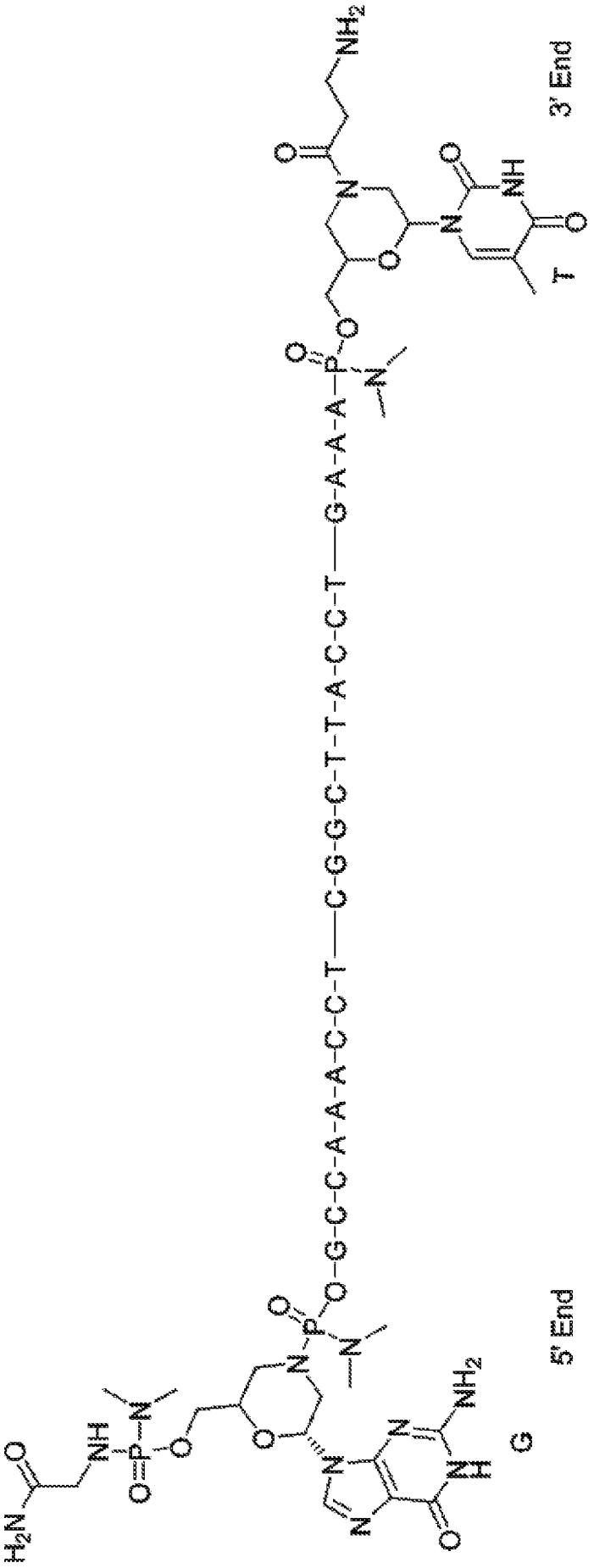


FIG. 1

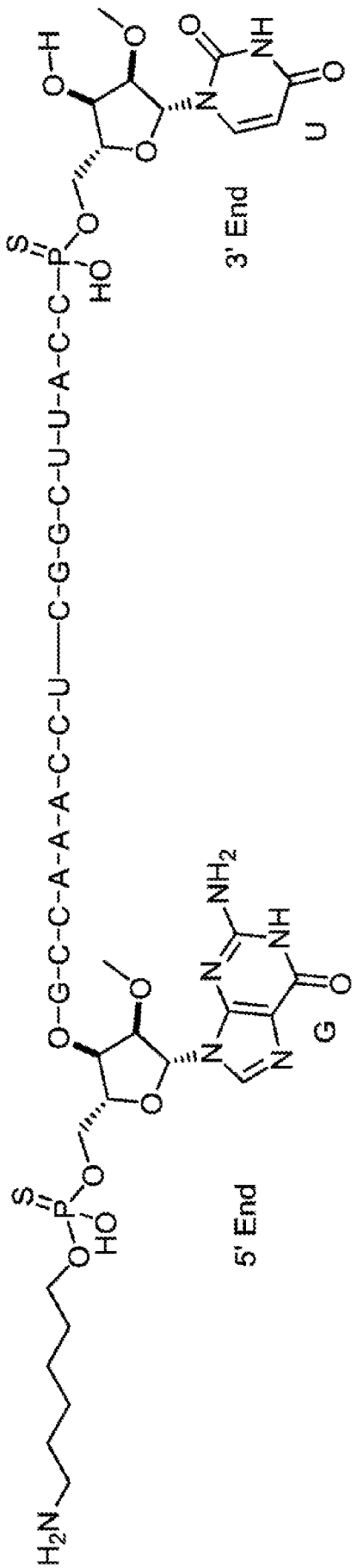


FIG. 2A

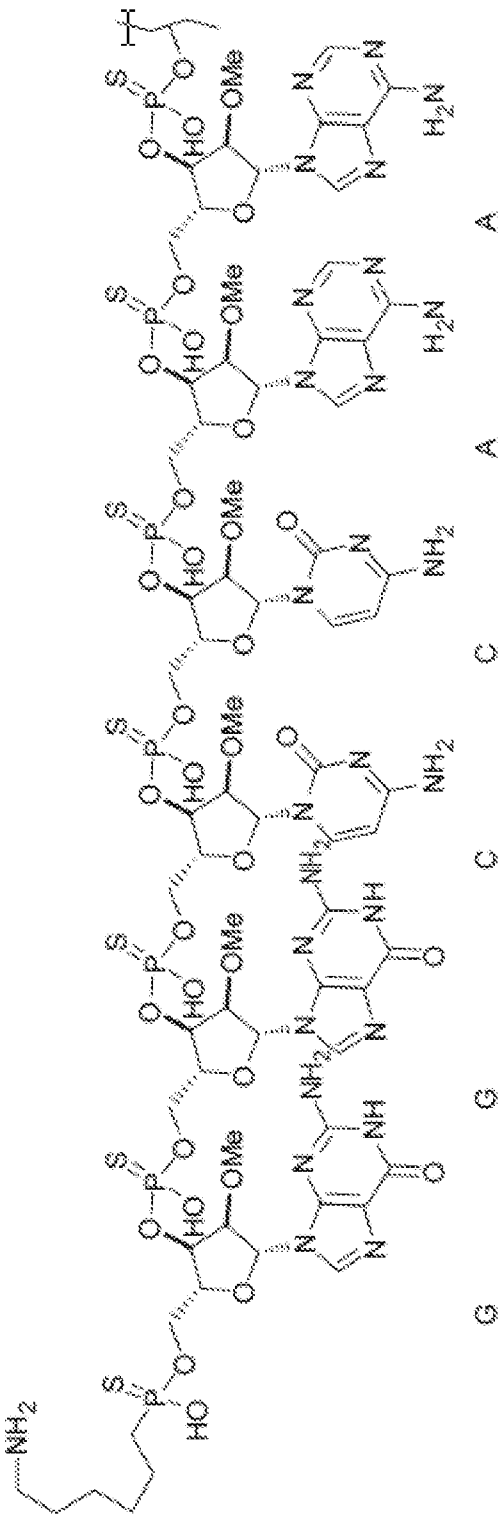


FIG. 2B

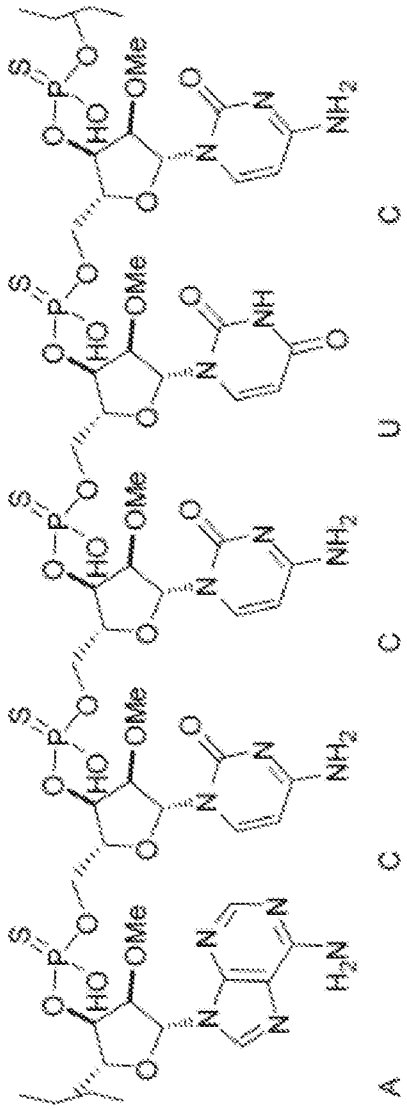


FIG. 2B (Cont.1)

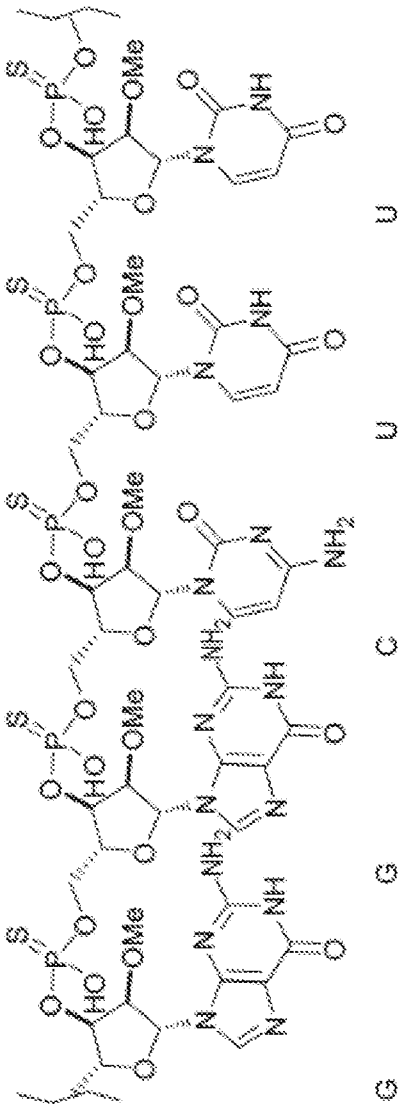


FIG. 2B (Cont.2)

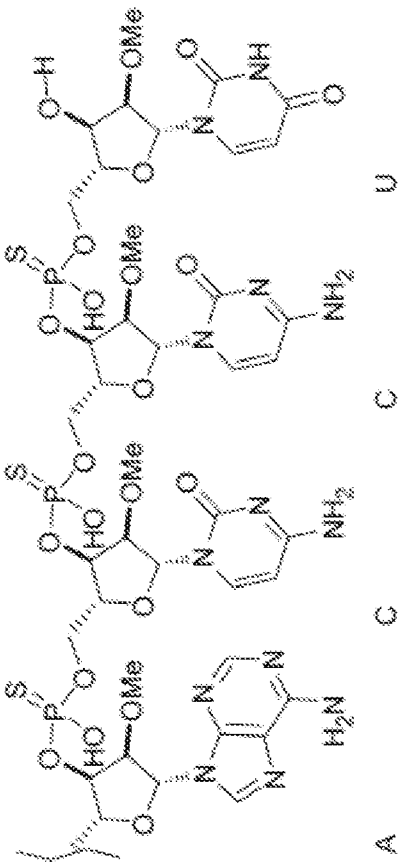


FIG. 2B (Cont.3)

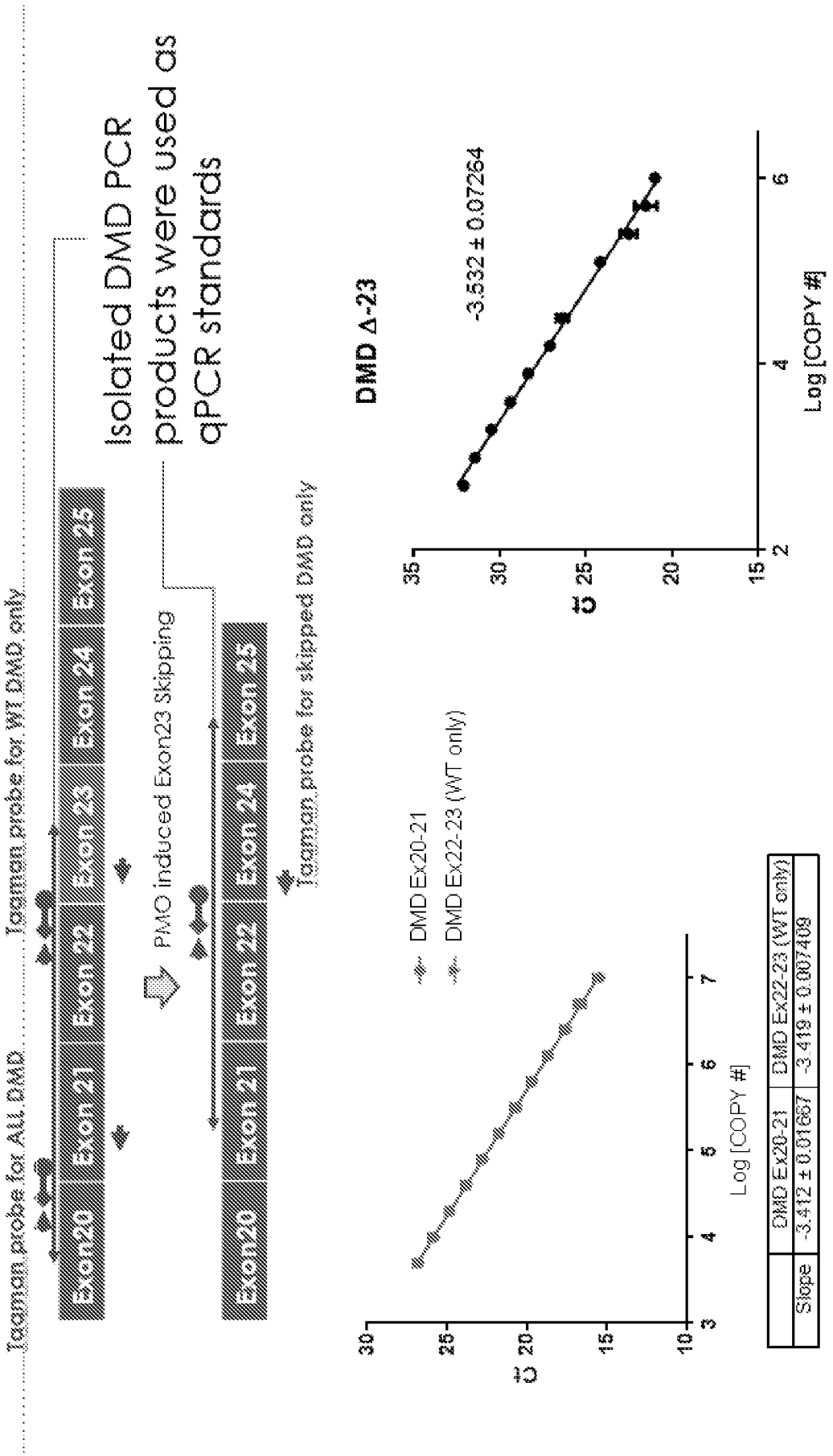

Taqman probe for ALL DMD
Taqman probe for WT DMD only
Exon20
Exon 21
Exon 22
Exon 23
Exon 24
Exon 25
PMO induced Exon23 Skipping
Taqman probe for skipped DMD only
Isolated DMD PCR products were used as qPCR standards
DMD Ex20-21
DMD Ex22-23 (WT only)
Log [COPY #]
Ct
DMD Δ-23
-3.532 ± 0.07264
Log [COPY #]
Ct
Slope
DMD Ex20-21
DMD Ex22-23 (WT only)
-3.412 ± 0.01667
-3.419 ± 0.007409

FIG. 3

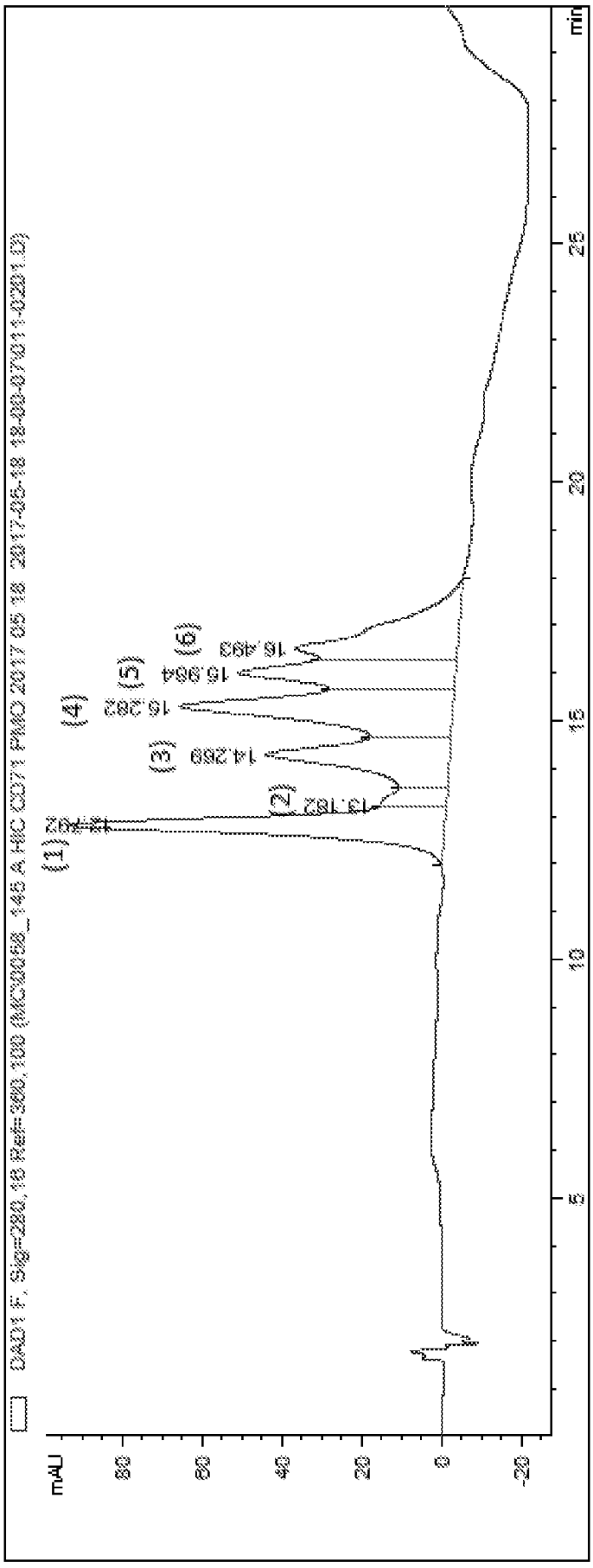

DAD1 F, Sig=220,16 Ref=360,100 (MC\0058_146 A HIC CD71 PMO 2017 05 18 2017-05-18 18-00-07\011-0201.D)
mAU
min
(1)
(2) 13.182
(3) 14.269
(4) 16.282
(5) 16.964
(6) 16.493

FIG. 4

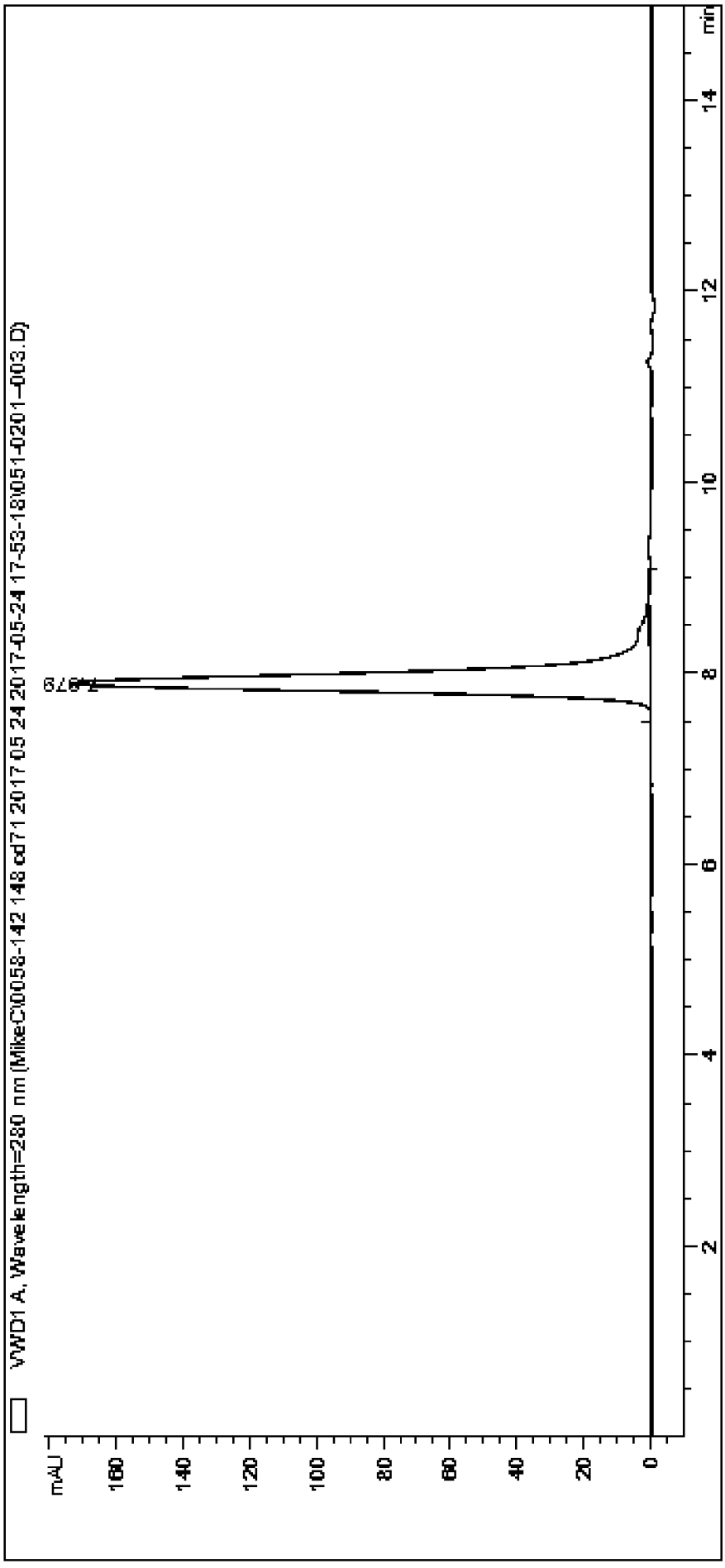

VWD1 A, Wavelength=280 nm (MikeCW0058-142 148 cd71 2017 05 24 2017-05-24 17-53-18\051-0201-003.D)
mAU
min
7.878

FIG. 5A

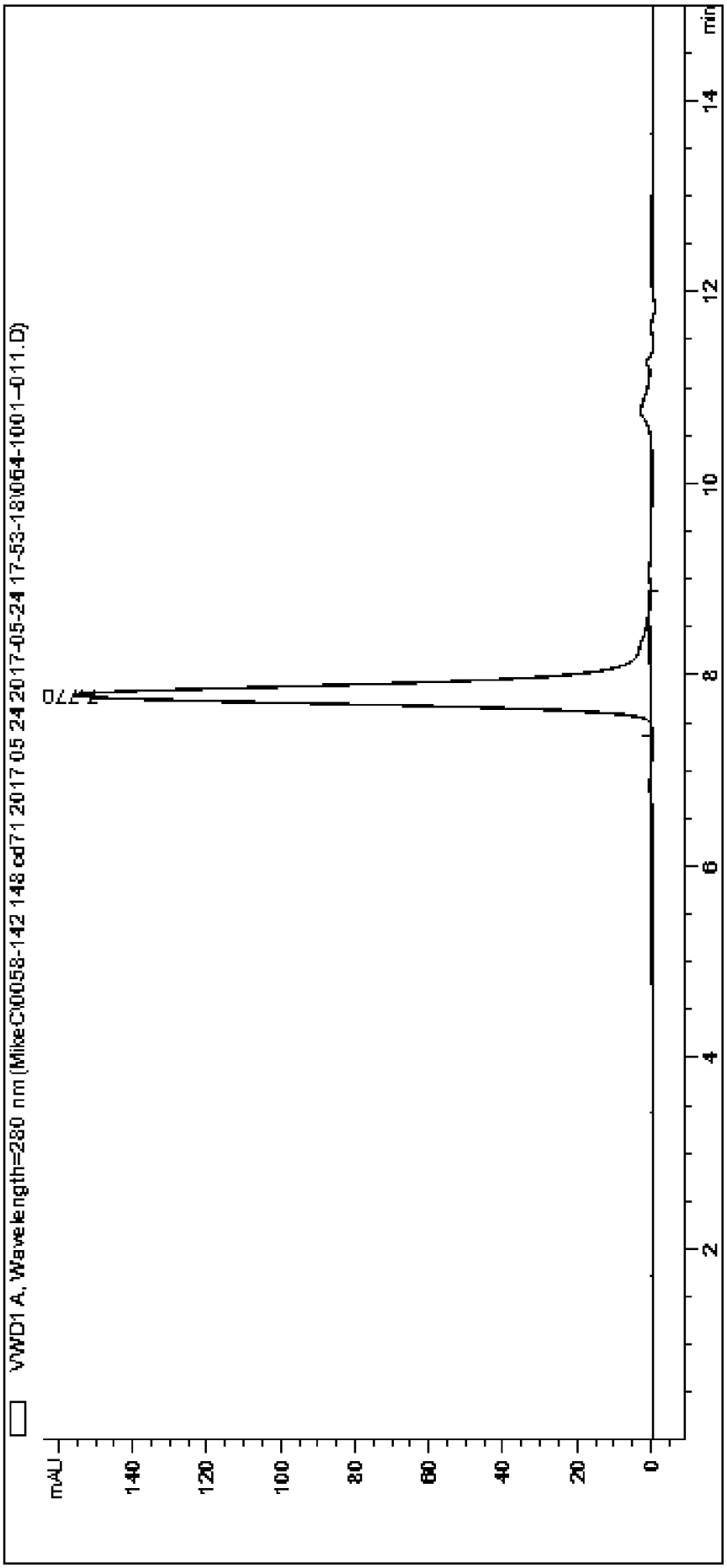

VWD1 A, Wavelength=280 nm (MikeC\0058-142 148 cd71 2017 05 24 2017-05-24 17-53-18\064-1001-011.D)
mAU
140
120
100
80
60
40
20
0
2
4
6
8
10
12
14
min

FIG. 5B

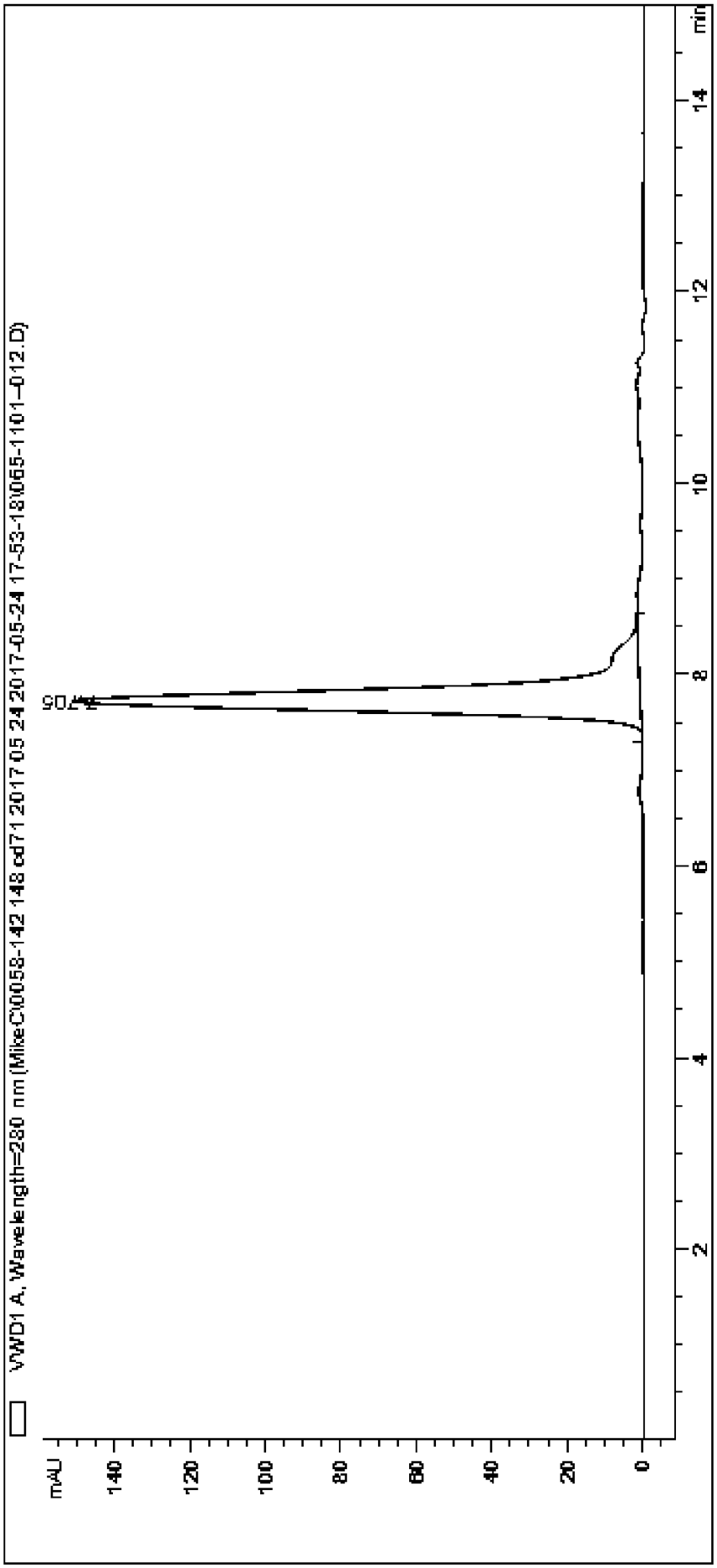

VWD1 A, Wavelength=280 nm (MikeC\0058-142 148 cd71 2017 05 24 2017-05-24 17-53-18\065-1101-012.D)
mAU
140
120
100
80
60
40
20
0
2
4
6
8
10
12
14
min

FIG. 5C

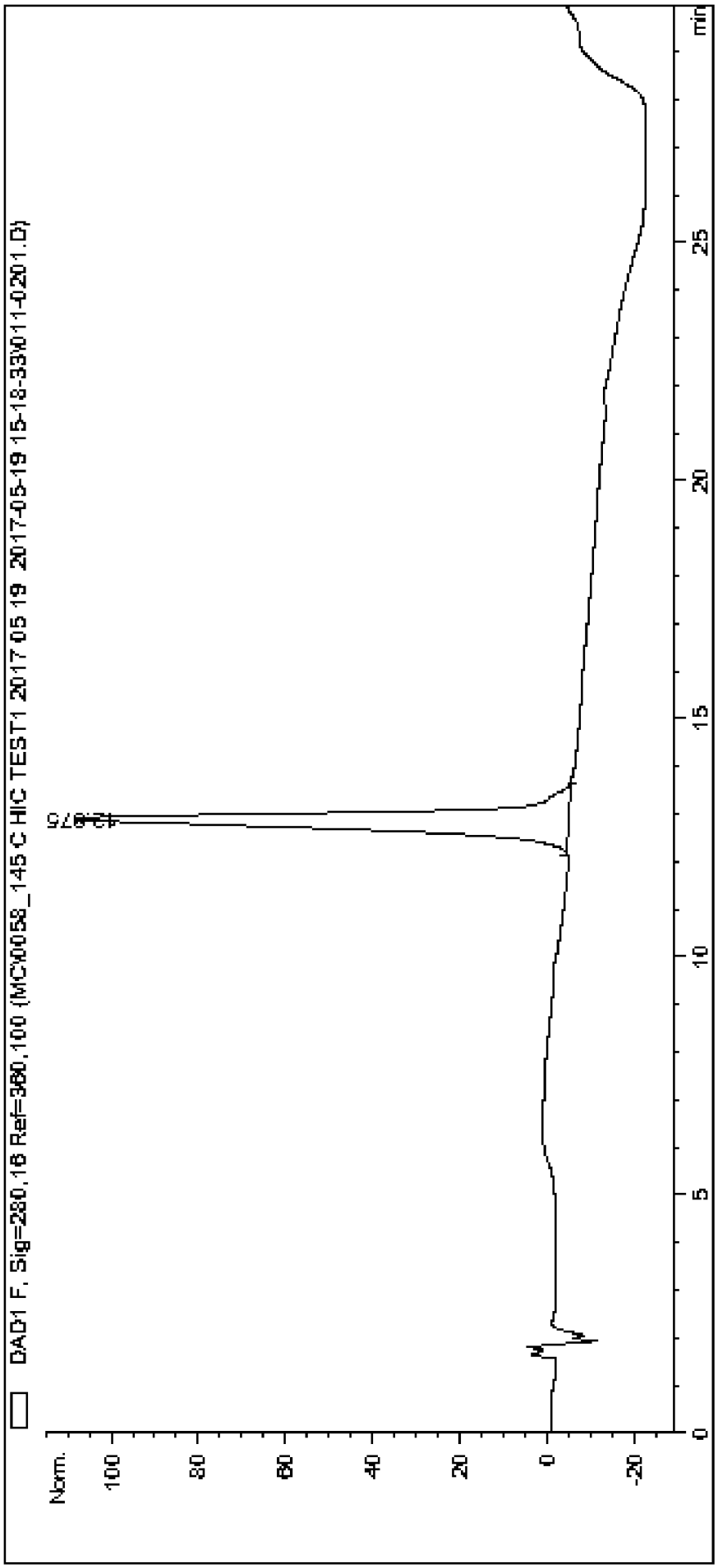

DAD1 F, Sig=280,16 Ref=360,100 (MC\00058_145 C HIC TEST1 2017 05 19 2017-05-19 15-18-33\011-0201.D)
Norm.
100
80
60
40
20
0
-20
0
5
10
15
20
25
min

FIG. 6A

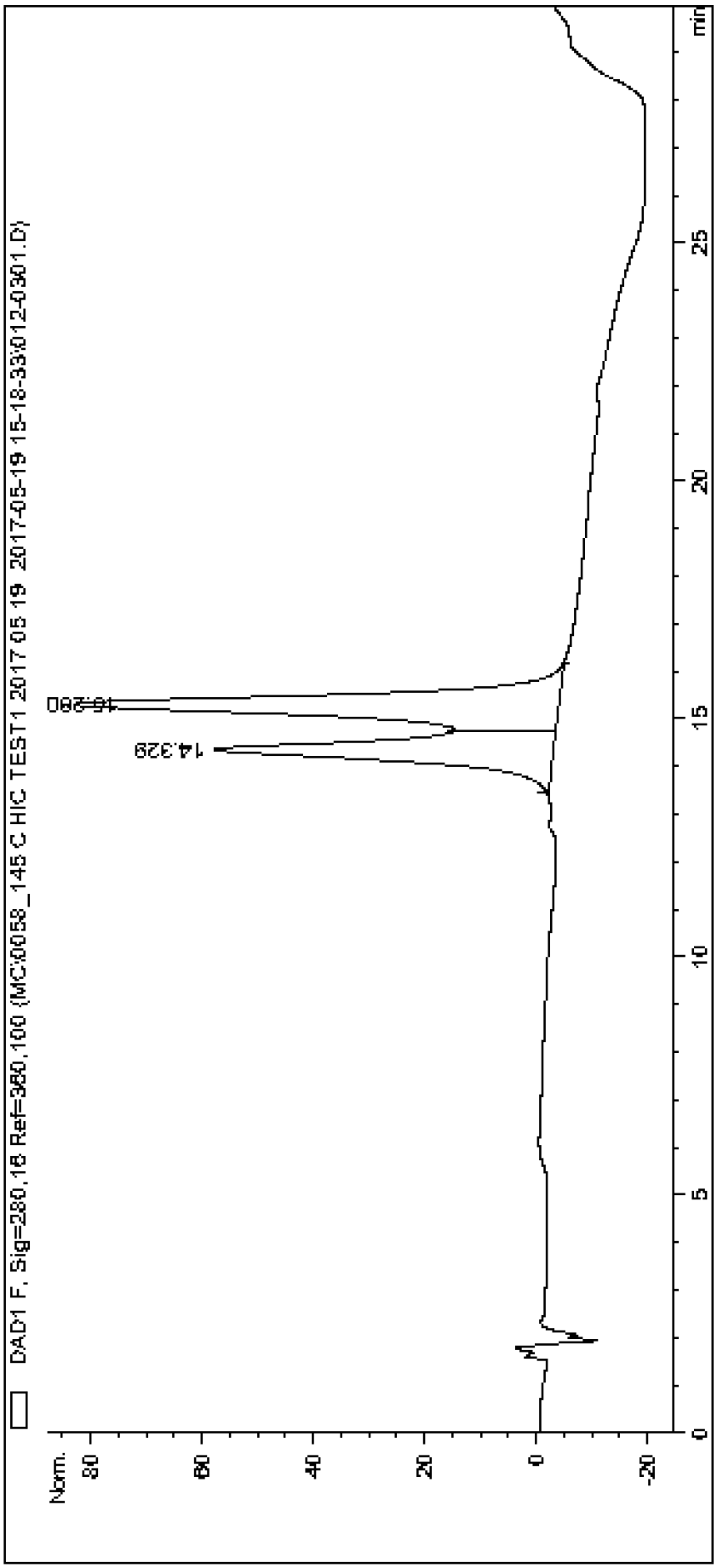

DAD1 F, Sig=280,16 Ref=360,100 (MC\0058_145 C HIC TEST1 2017 05 19 2017-05-19 15-18-33\012-0301.D)
Norm.
80
60
40
20
0
-20
0
5
10
15
20
25
min
14.329

FIG. 6B

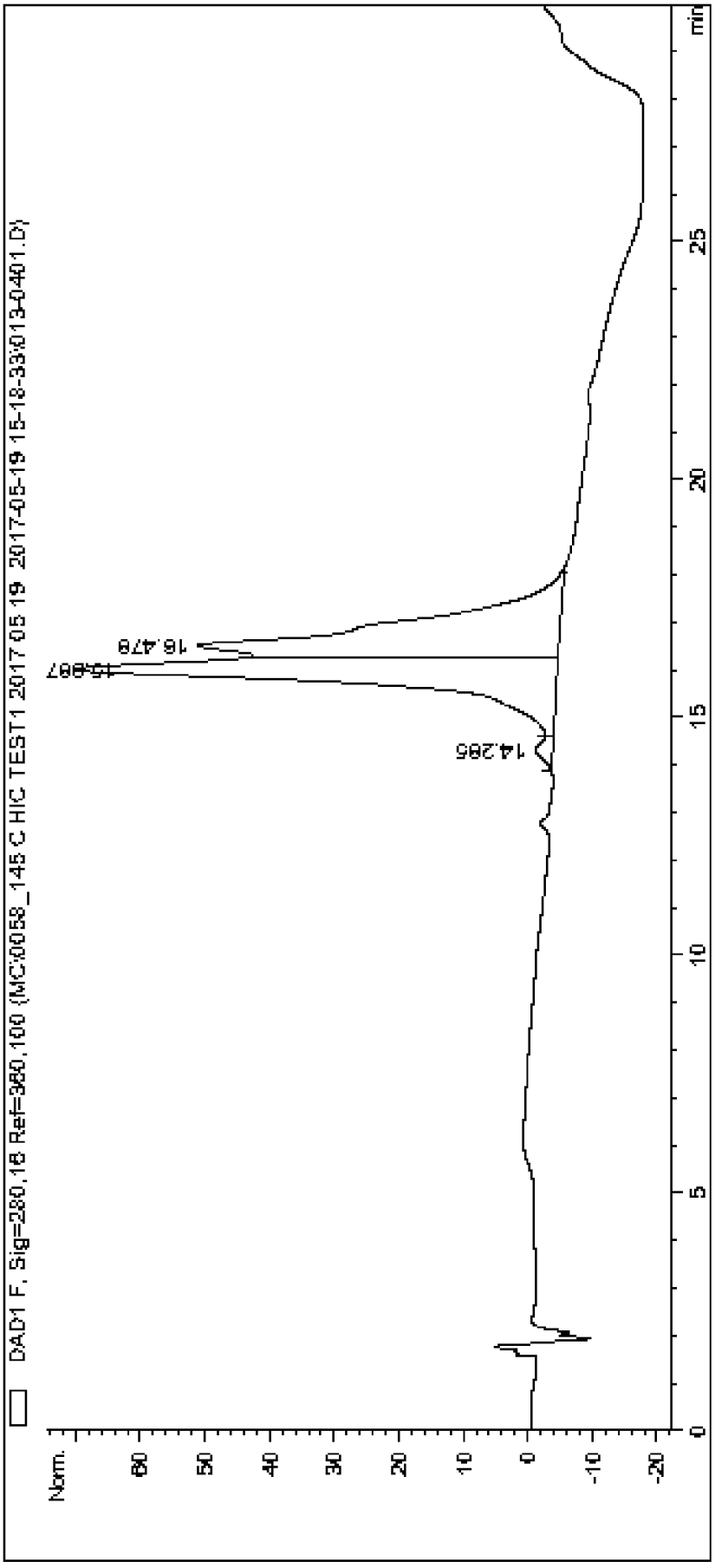

DAD1 F, Sig=280,16 Ref=360,100 (MC:0058_145 C HIC TEST1 2017 05 19 2017-05-19 15-18-33\013-0401.D)
Norm.
80
60
40
30
20
10
0
-10
-20
0
5
10
15
20
25
min
14.285
15.887
16.478

FIG. 6C

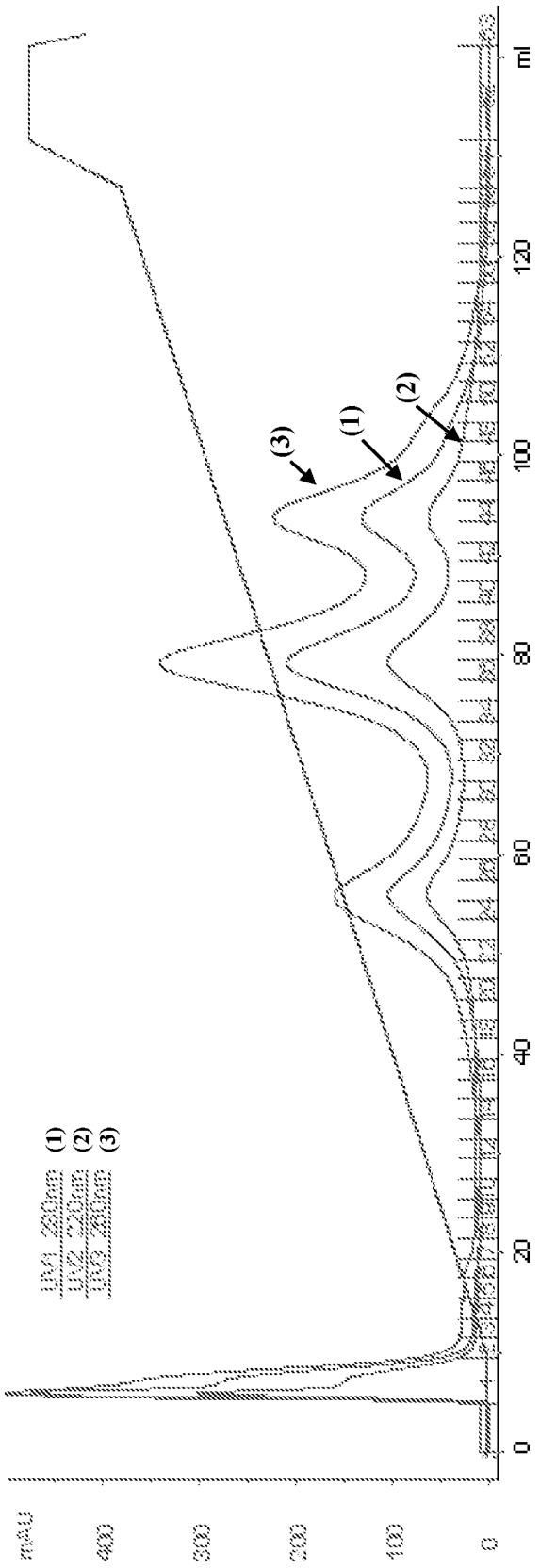

mAU
400
300
200
100
0
0
20
40
60
80
100
120
ml
(1)
(2)
(3)
UV1 280nm
UV2 220nm
UV3 260nm

FIG. 7A

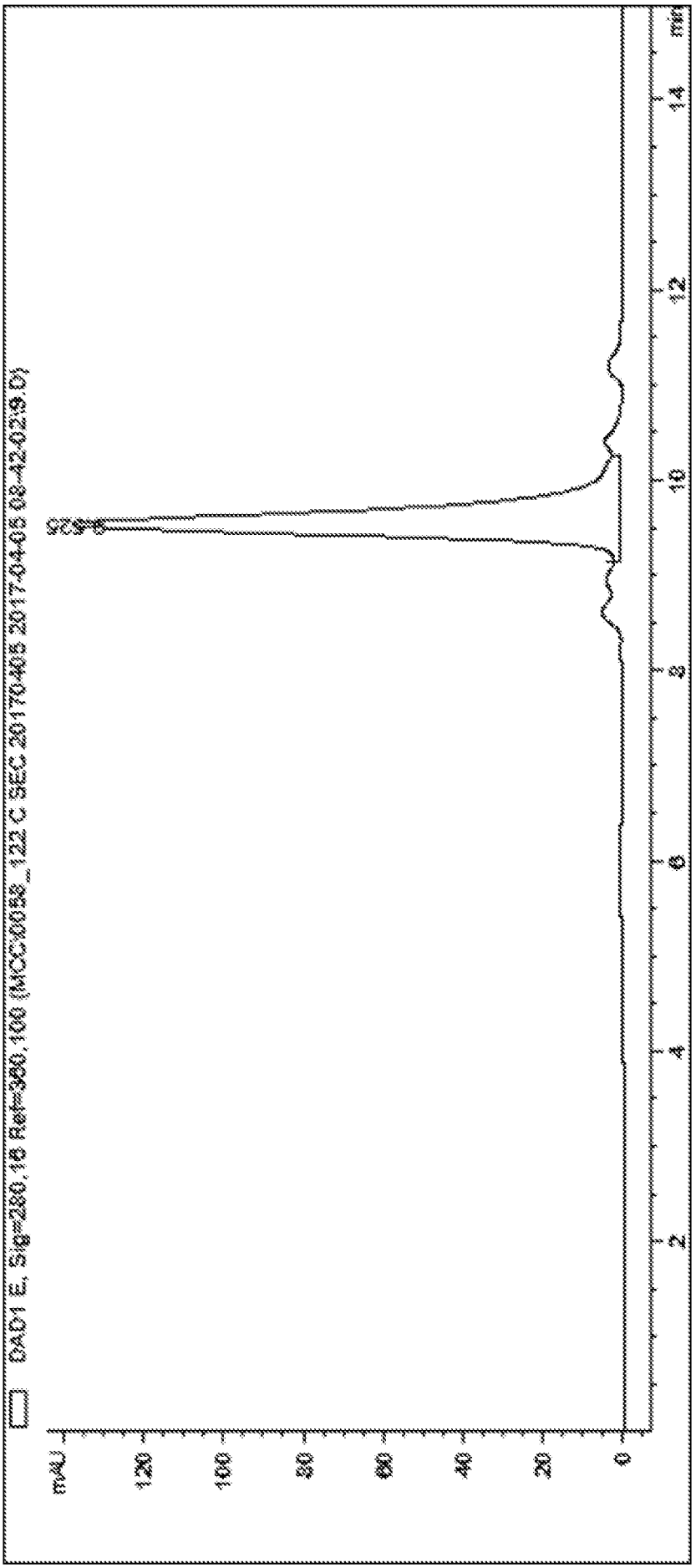

DAD1 E, Sig=280,16 Ref=360,100 (MCC\00058_122 C SEC 20170405 2017-04-05 08-42-0219.D)
mAU
120
100
80
60
40
20
0
2
4
6
8
10
12
14
min

FIG. 7B

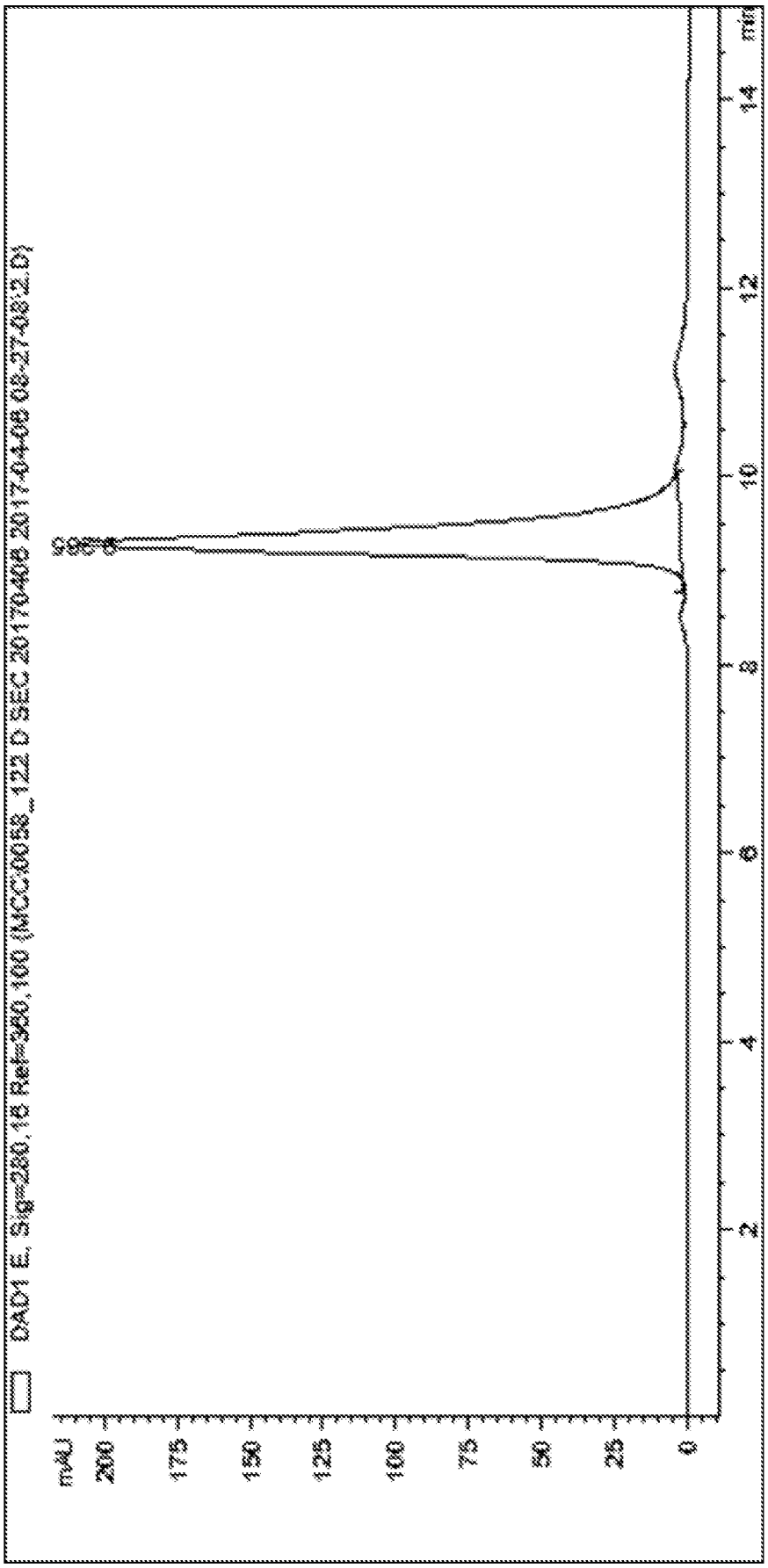

DAD1 E, Sig=280,16 Ref=360,100 (MCC\0058_122 D SEC 20170408 2017-04-08 08-27-48\2.D)
mAU
200
175
150
125
100
75
50
25
0
2
4
6
8
10
12
14
min

FIG. 7C

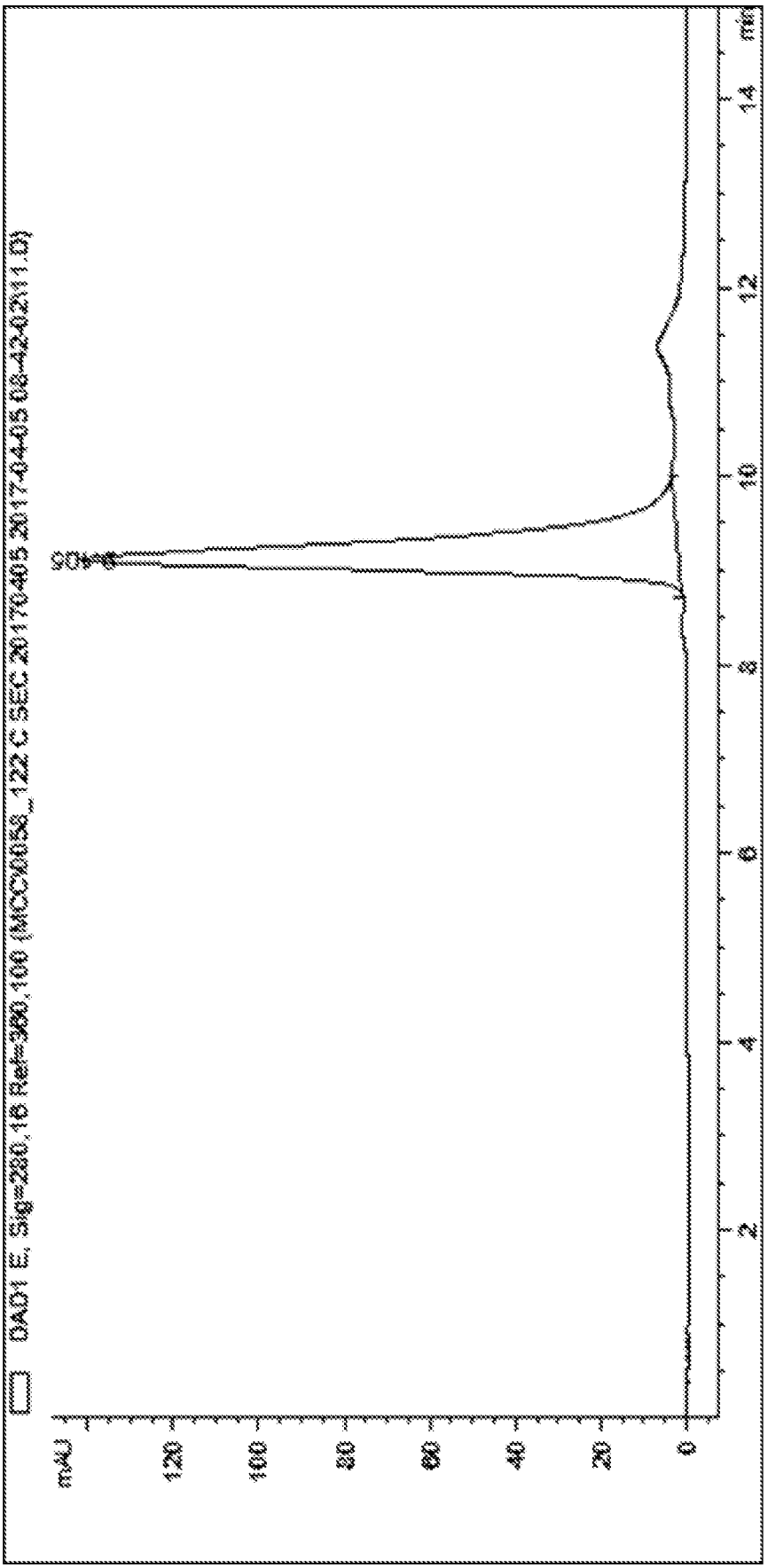

DAD1 E, Sig=280,16 Ref=360,100 (MCCM0058_122 C SEC 20170405 2017-04-05 08-42-02\1.D)
mAU
120
100
80
60
40
20
0
2
4
6
8
10
12
14
min

FIG. 7D

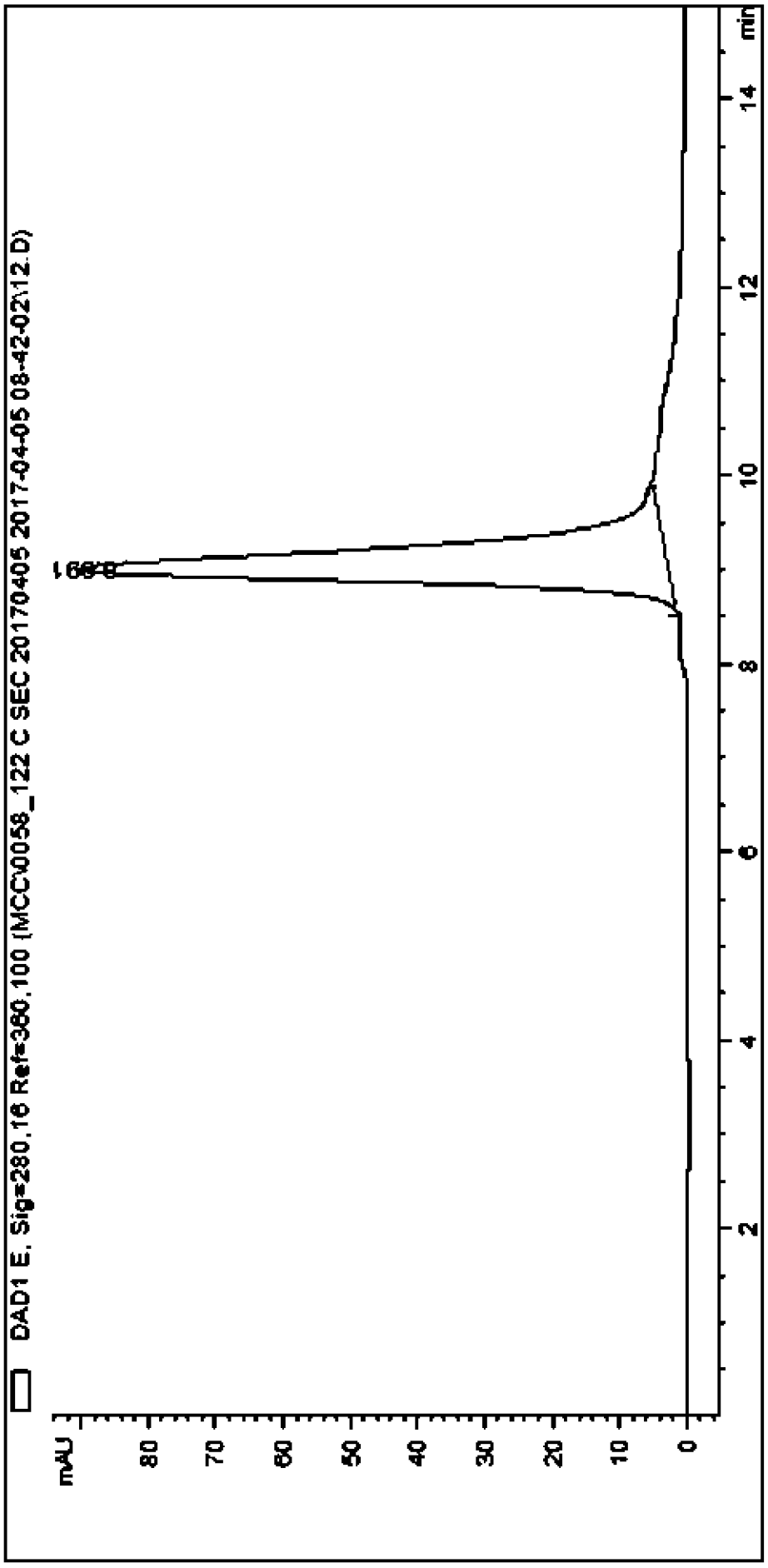

DAD1 E, Sig=280,16 Ref=360,100 (MCC\0058_122 C SEC 20170405 2017-04-05 08-42-02\12.D)
mAU
min
0
10
20
30
40
50
60
70
80
2
4
6
8
10
12
14

FIG. 7E

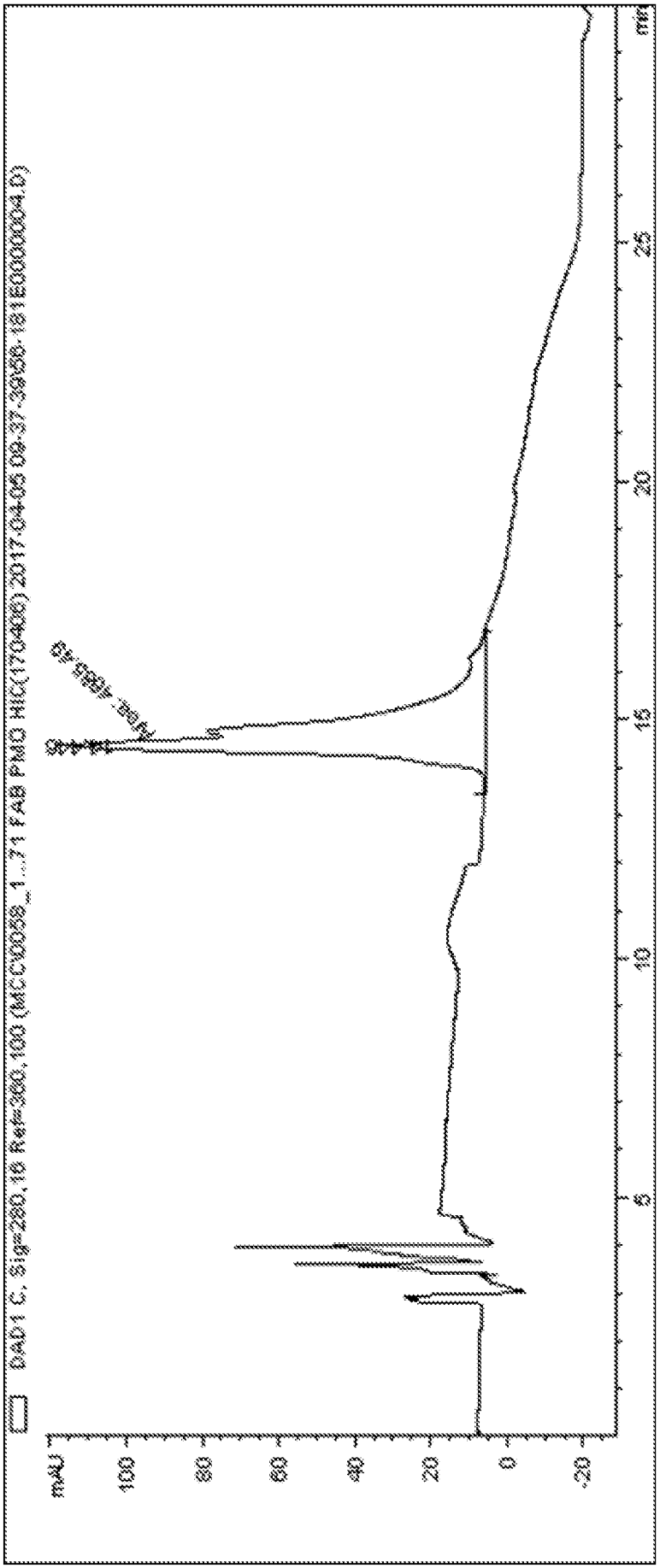


FIG. 7F

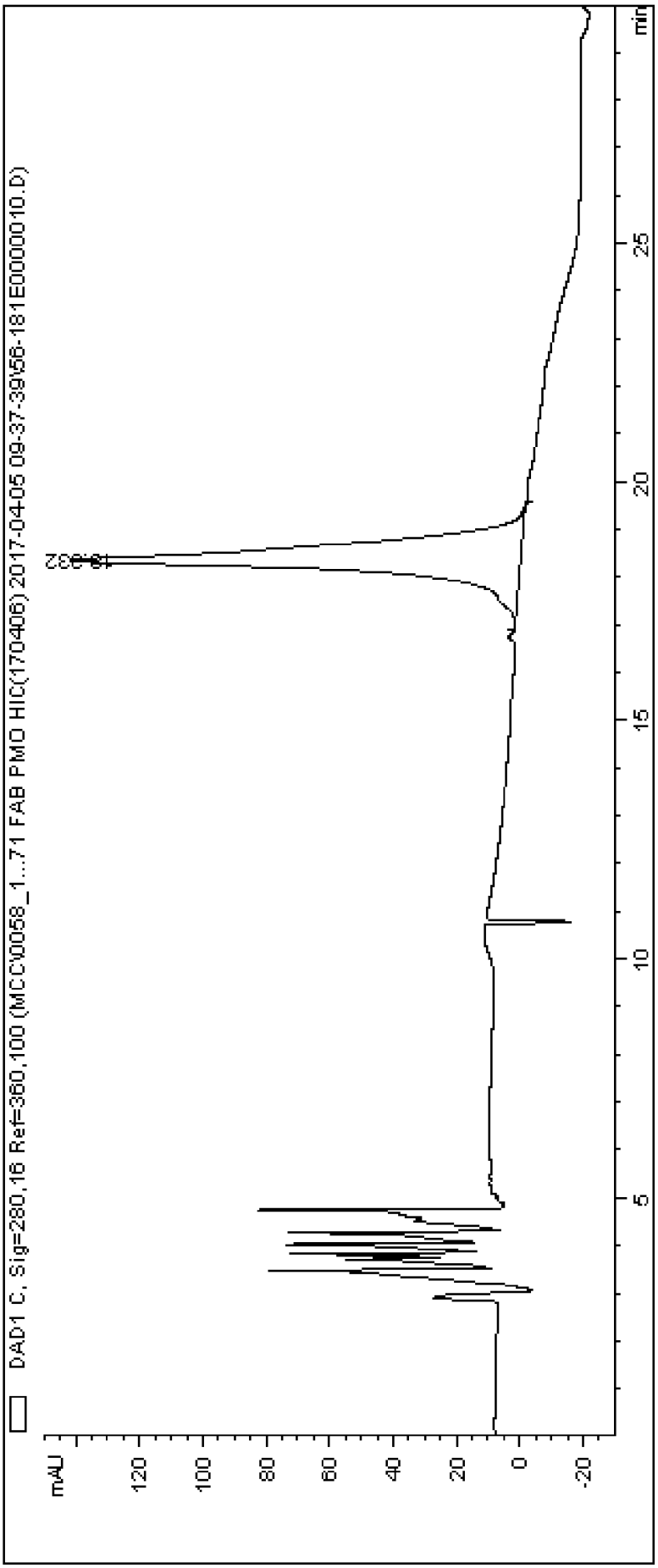

DAD1 C, Sig=280,16 Ref=360,100 (MCC\0058_1...71 FAB PMO HIC(170406) 2017-04-05 09-37-39\56-181E0000010.D)
mAU
120
100
80
60
40
20
0
-20
min
5
10
15
20
25

FIG. 7G

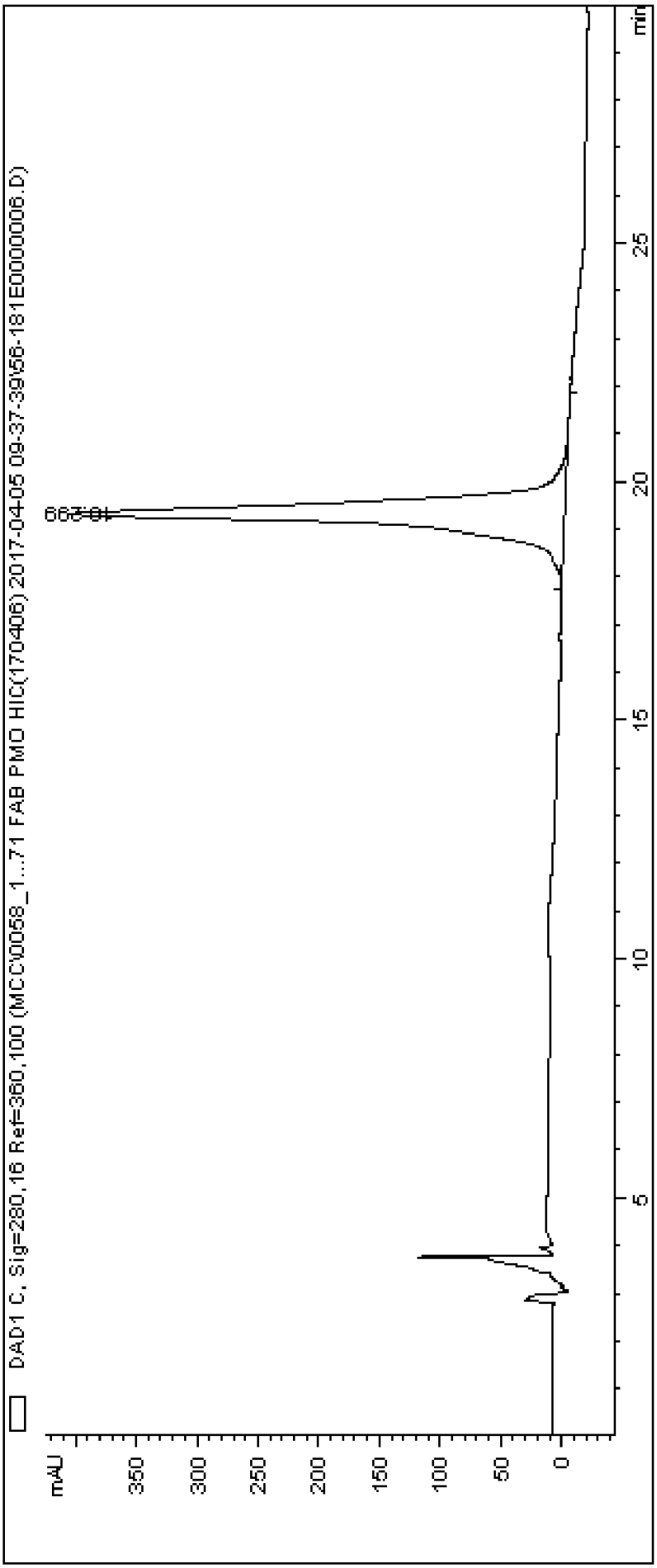

DAD1 C, Sig=280,16 Ref=360,100 (MCC\0058_1...71 FAB PMO HIC(170406) 2017-04-05 09-37-39\56-181E0000006.D)
mAU
350
300
250
200
150
100
50
0
5
10
15
20
25
min

FIG. 7H

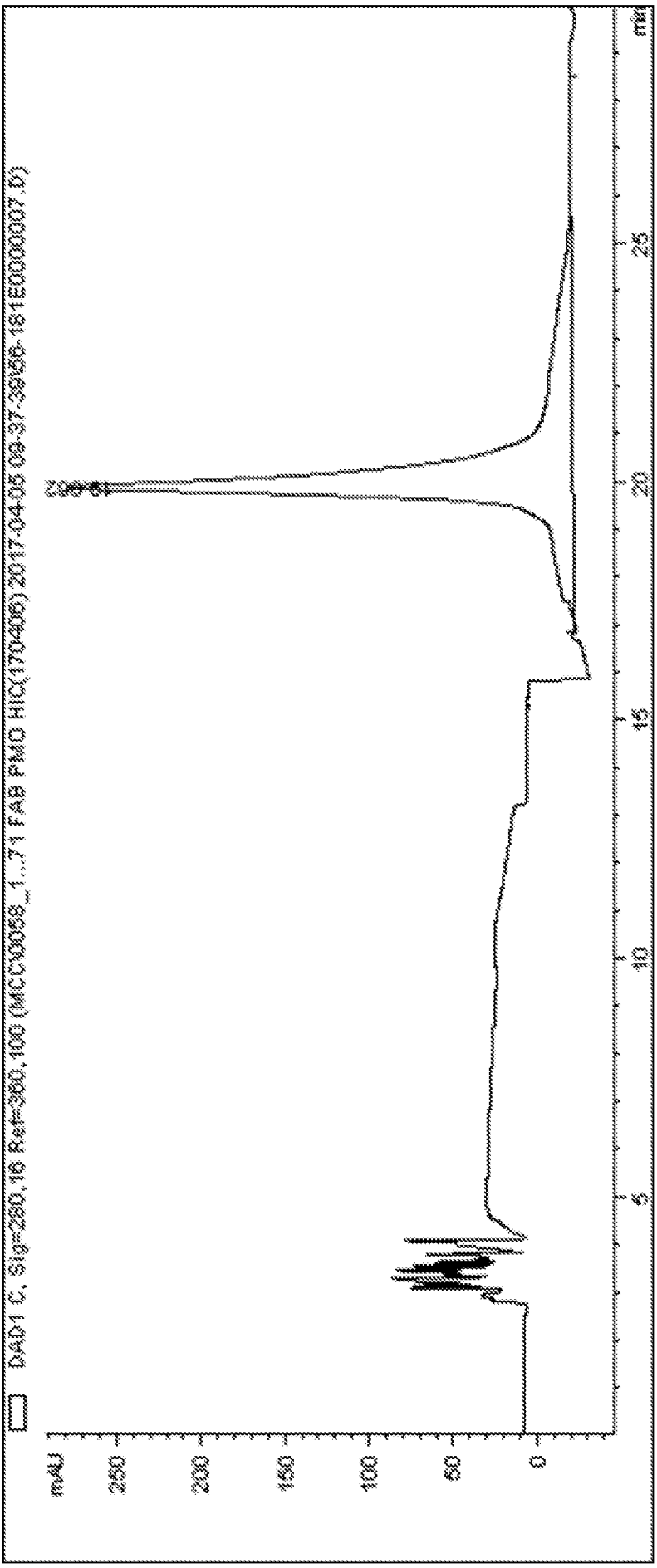


FIG. 7I

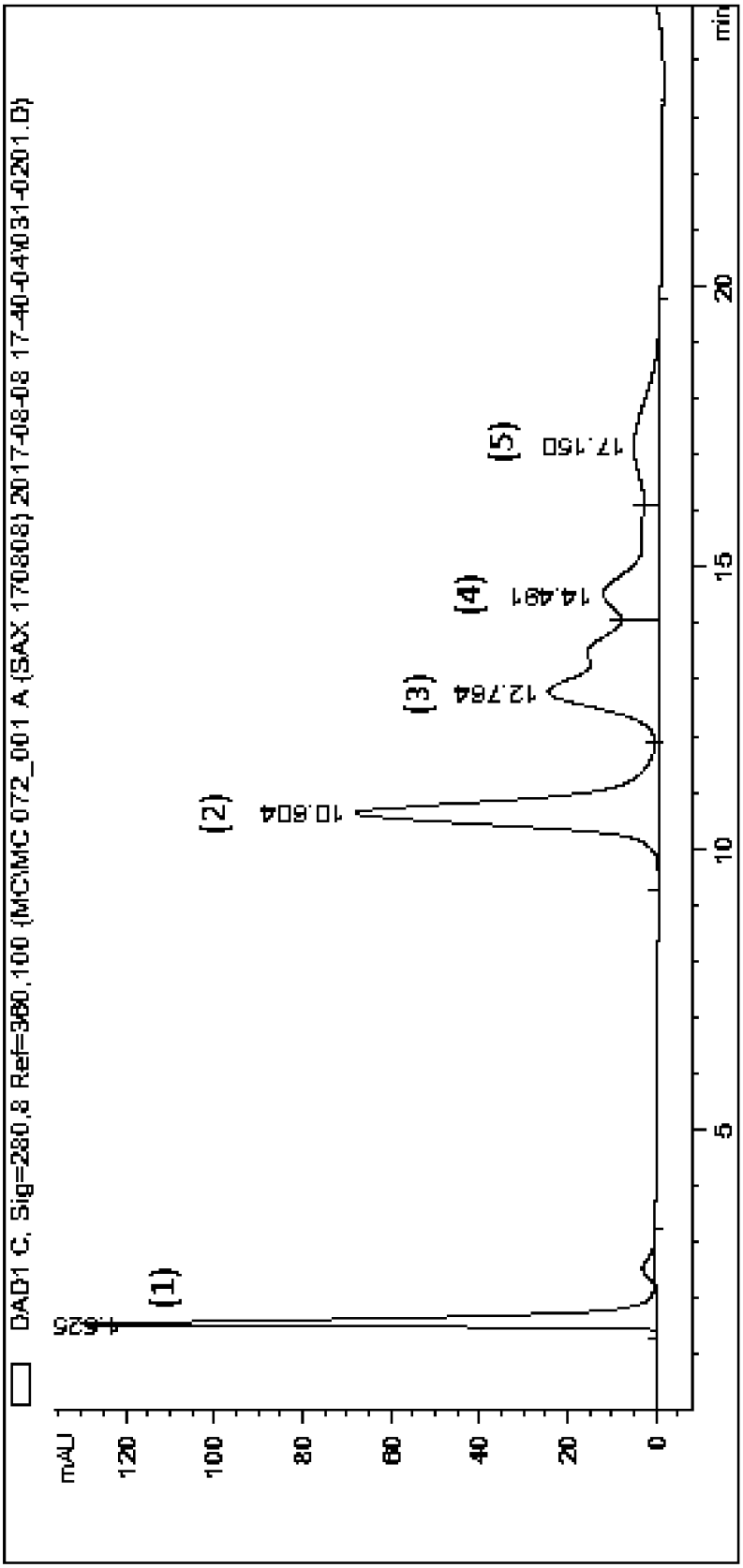

DAD1 C, Sig=280,8 Ref=360,100 (MC\MC 072_001 A (SAX 1708008) 2017-08-08 17-40-04\031-0201.D)
mAU
min
(1)
(2)
(3)
(4)
(5)
10.804
12.784
14.491
17.150

FIG. 8A

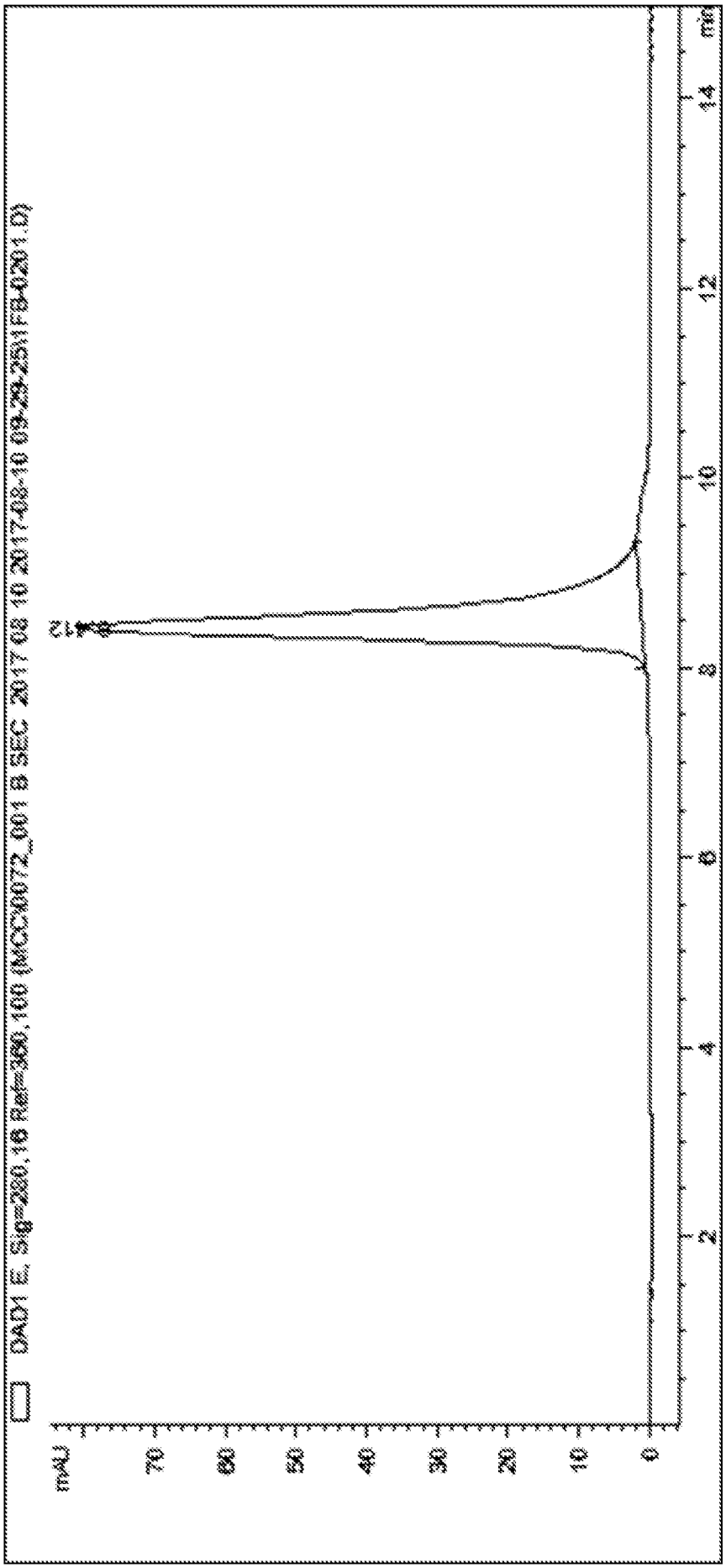

DAD1 E, Sig=280,16 Ref=360,100 (MCC\0072_001 B SEC 2017 08 10 2017-08-10 09-29-25\FB-0201.D)
mAU
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
min

FIG. 8B

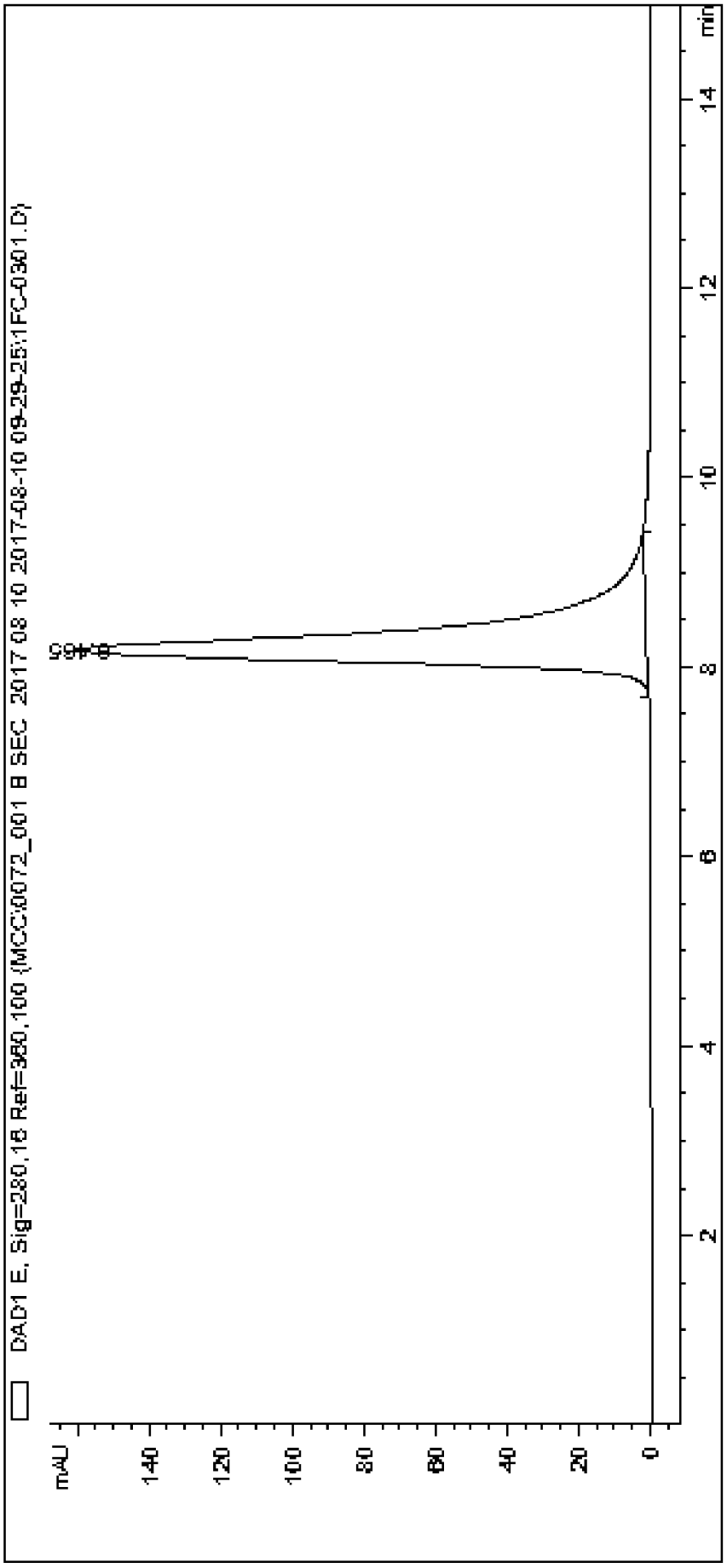

DAD1 E, Sig=280,16 Ref=360,100 (MCC\0072_001 B SEC 2017 08 10 2017-08-10 09-29-25\1FC-0301.D)
mAU
140
120
100
80
60
40
20
0
2
4
6
8
10
12
14
min

FIG. 8C

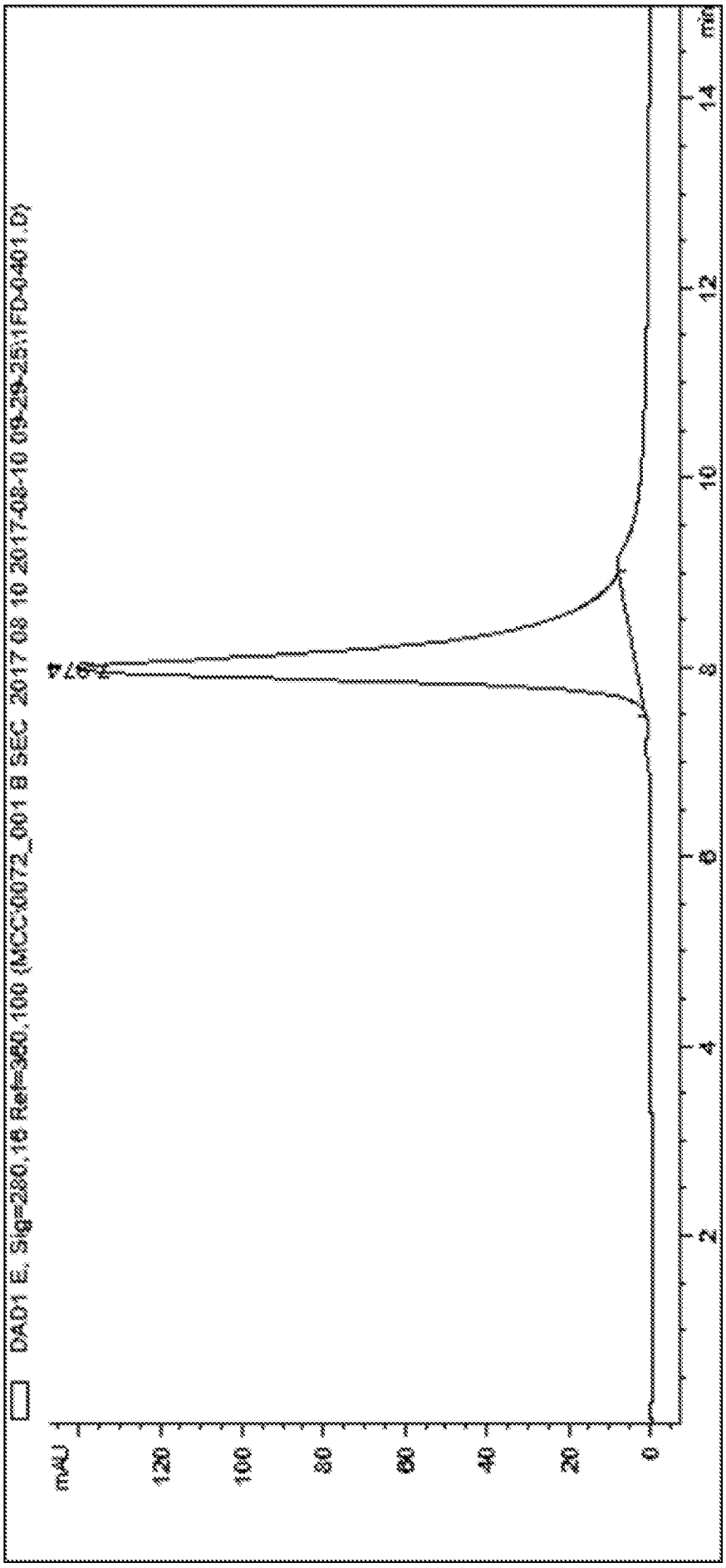

DAD1 E, Sig=280,16 Ref=360,100 (MCC-0072_001 B SEC 2017 08 10 2017-08-10 09-29-25\FD-0401.D)
mAU
120
100
80
60
40
20
0
2
4
6
8
10
12
14
min

FIG. 8D

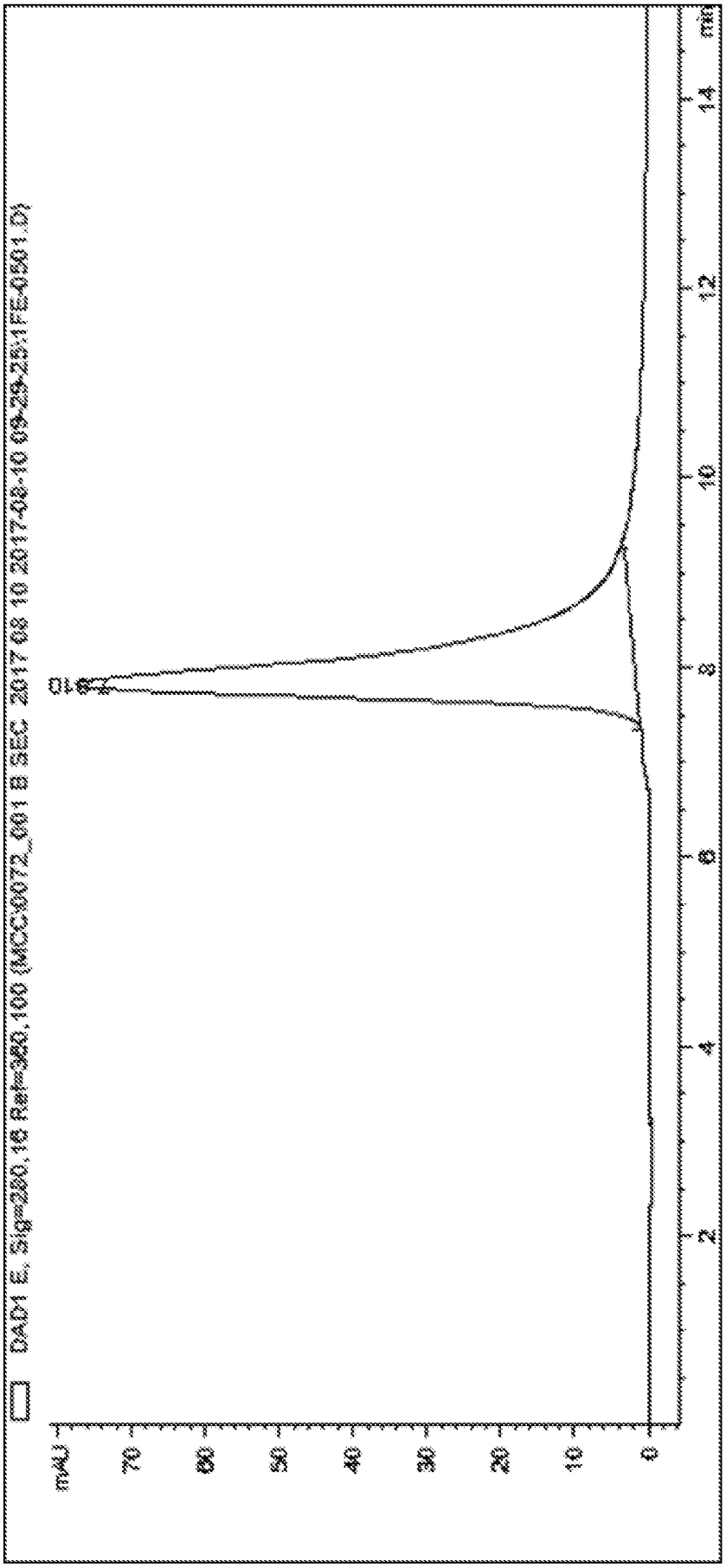

DAD1 E, Sig=280,16 Ref=360,100 (MCC\0072_001 8 SEC 2017 08 10 2017-08-10 09-29-25\1FE-0501.D)
mAU
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
min

FIG. 8E

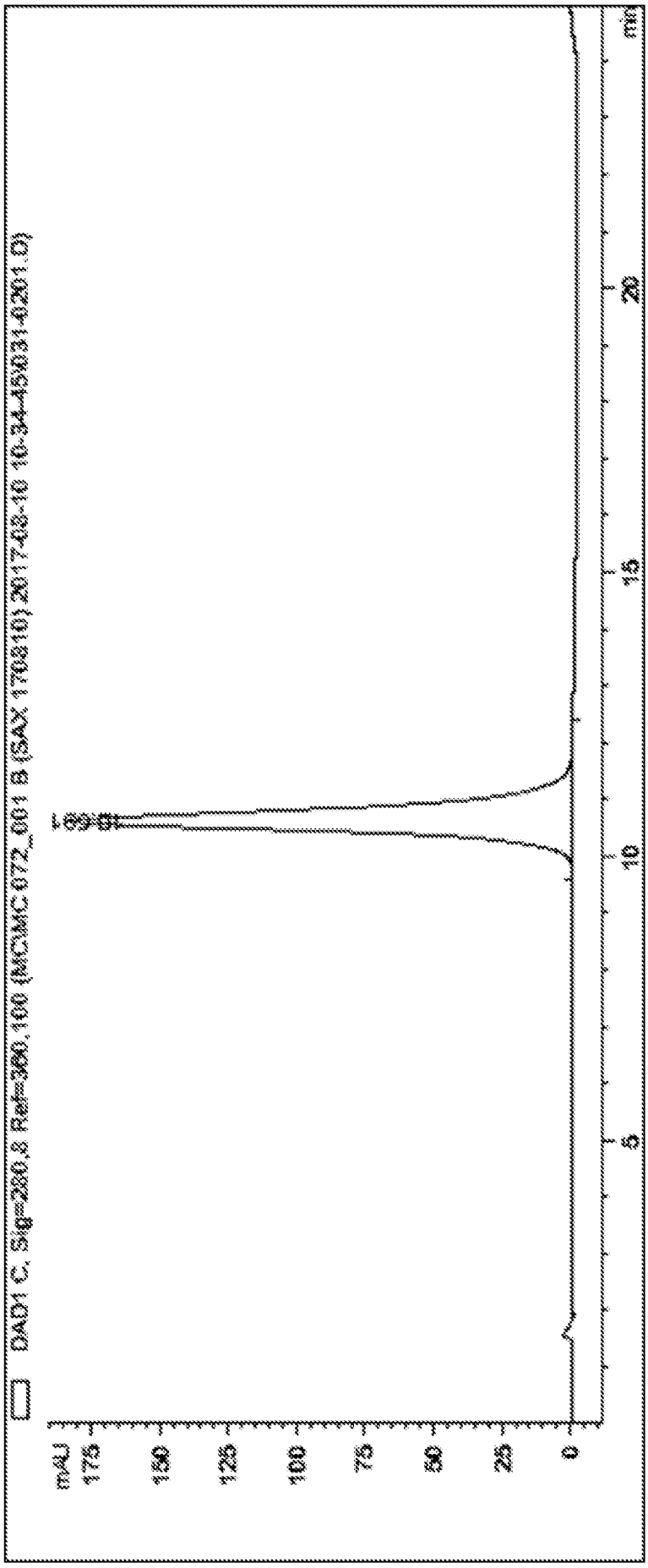

DAD1 C, Sig=280,8 Ref=360,100 (MCMC 072_001 B (SAX 170810) 2017-08-10 10-34-45\031-0201.D)
mAU
175
150
125
100
75
50
25
0
5
10
15
20
min

FIG. 8F

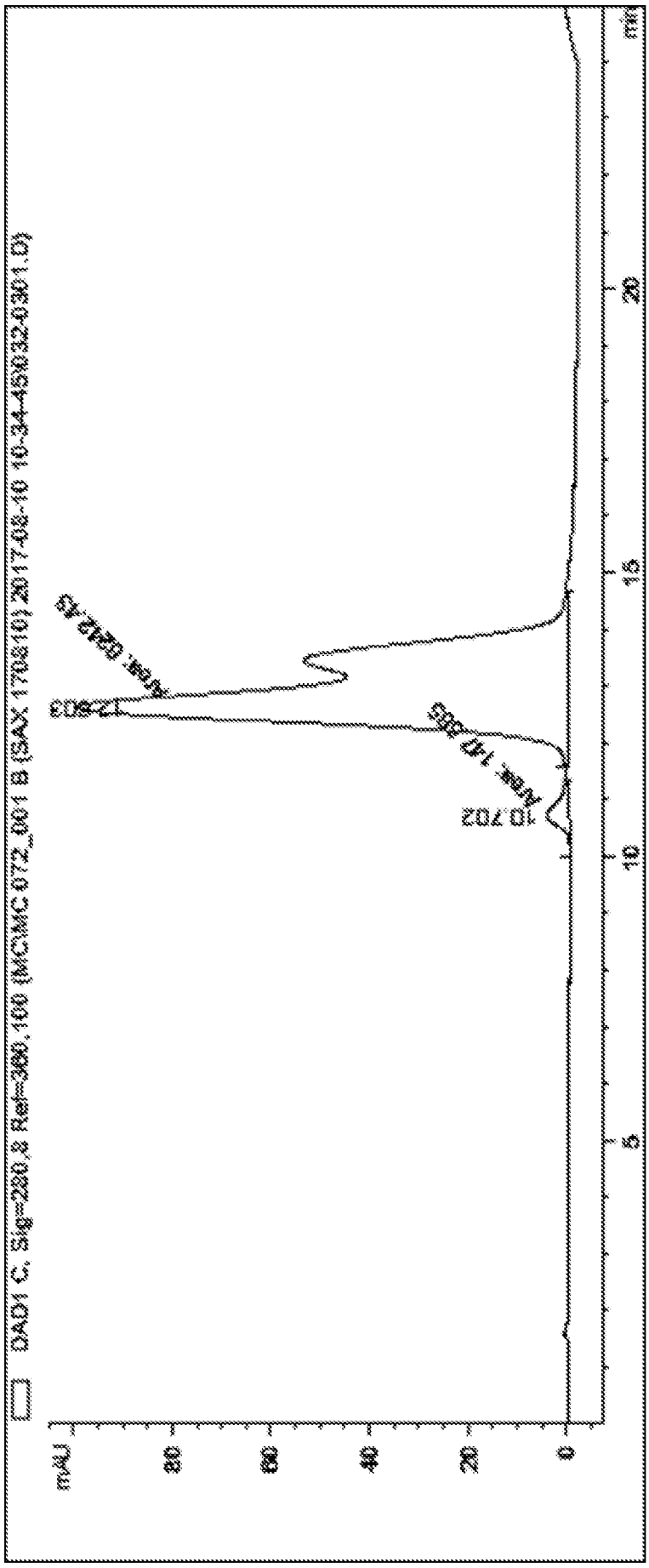

DAD1 C, Sig=280,8 Ref=360,100 (MC\MC 072_001 B (SAX 170210) 2017-09-10 10-34-45\032-0301.D)
mAU
80
60
40
20
0
5
10
15
20
min
12.803
Type: BB
10.702
Type: BB

FIG. 8G

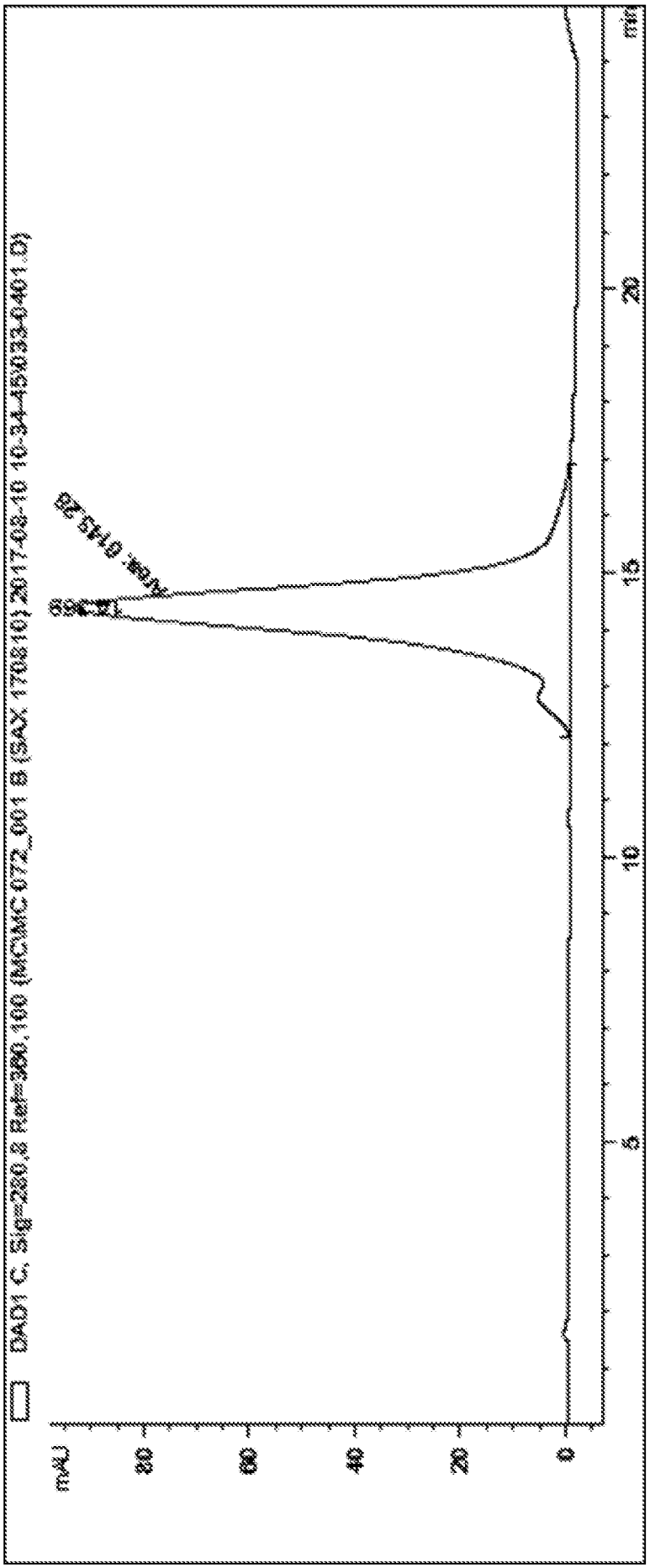


FIG. 8H

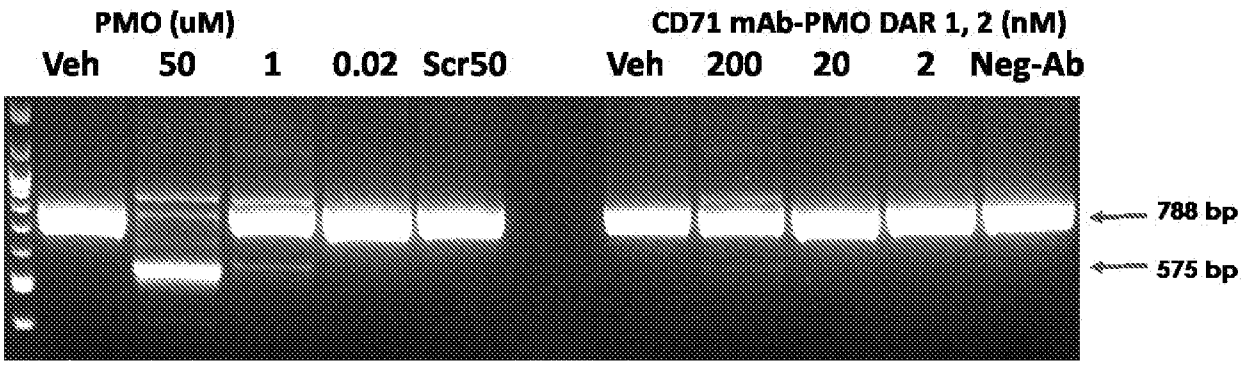


FIG. 9

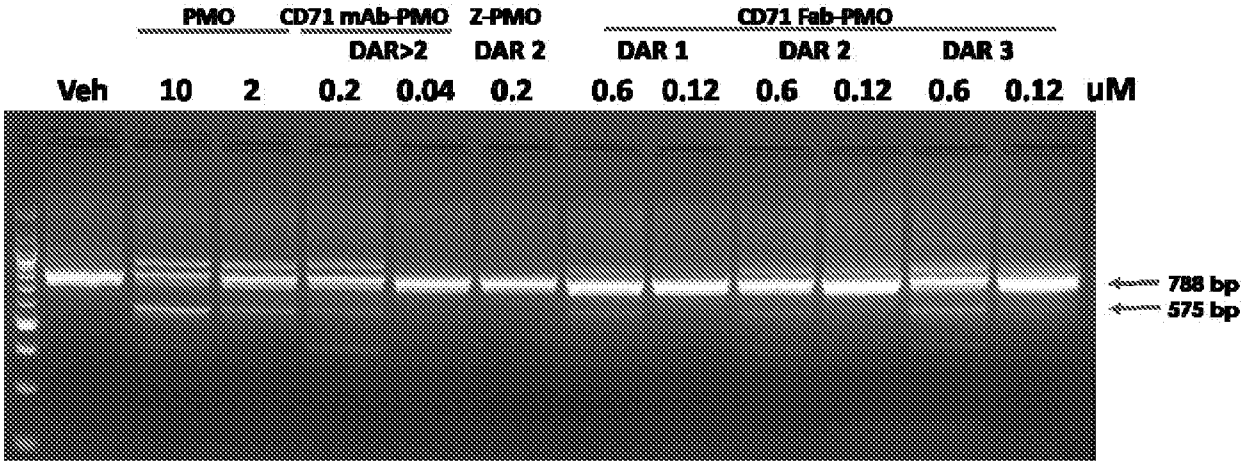


FIG. 10

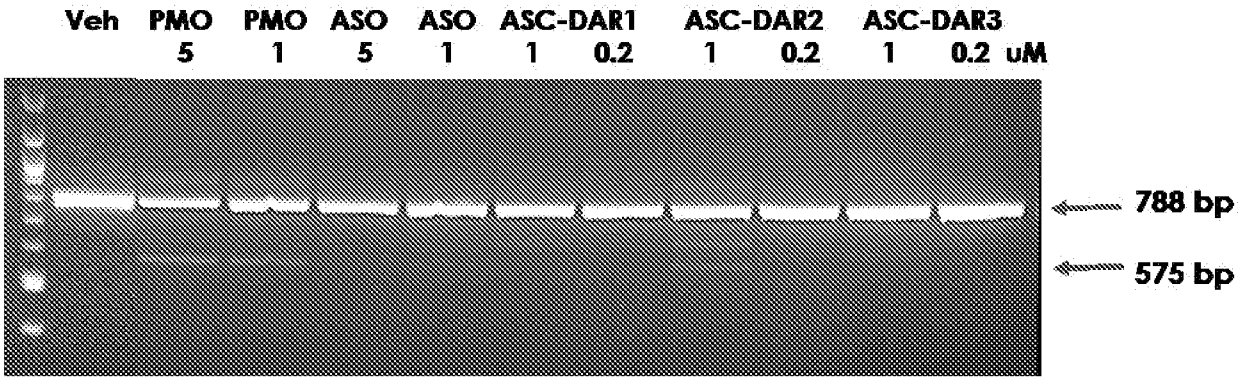


FIG. 11

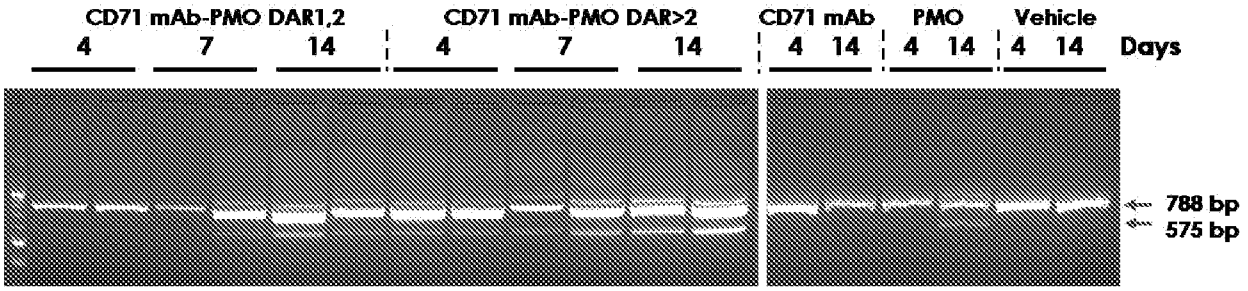


FIG. 12A

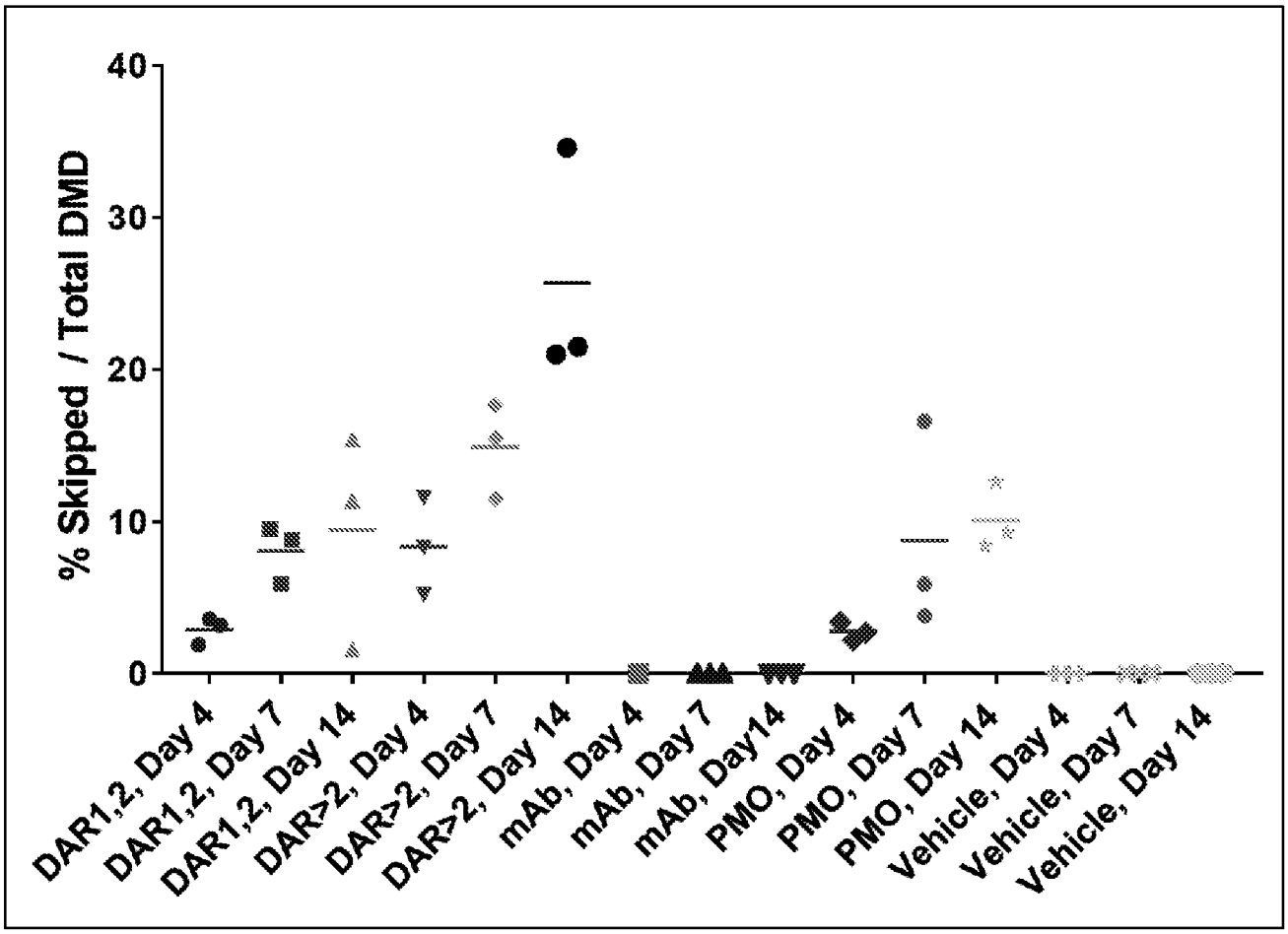


FIG. 12B

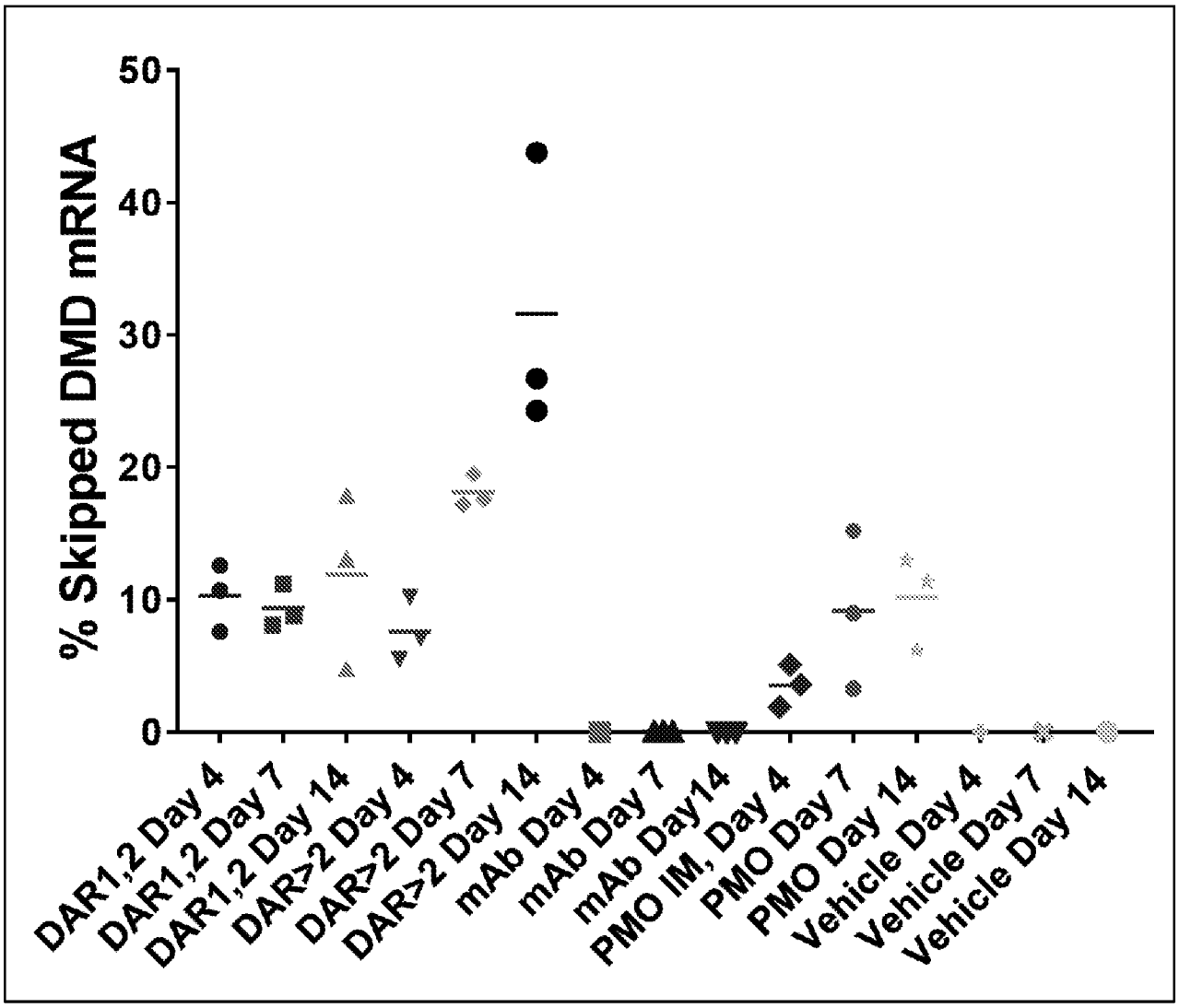


FIG. 12C

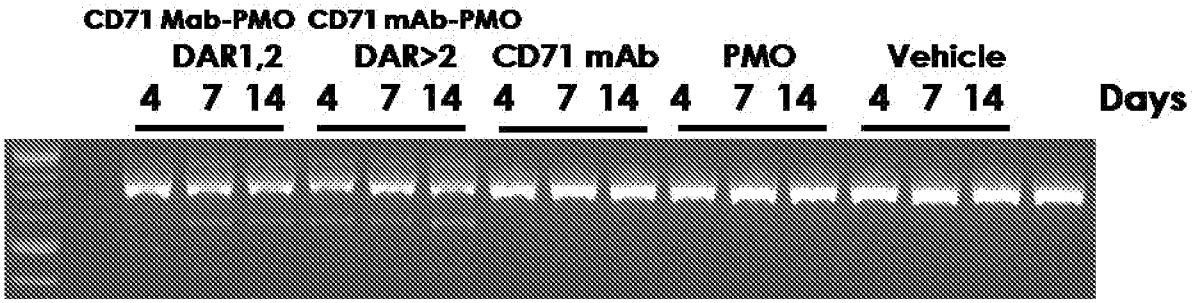


FIG. 13A

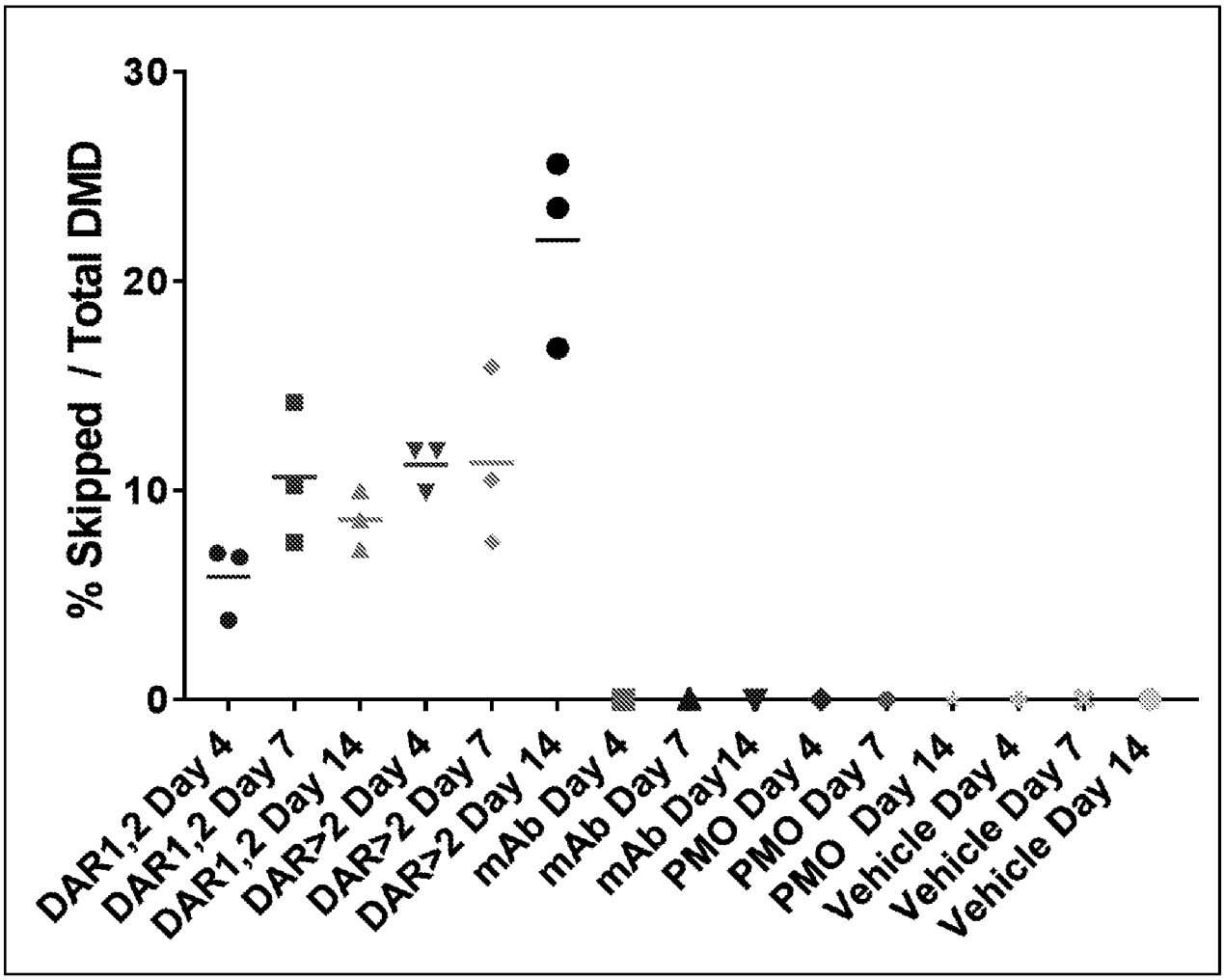


FIG. 13B

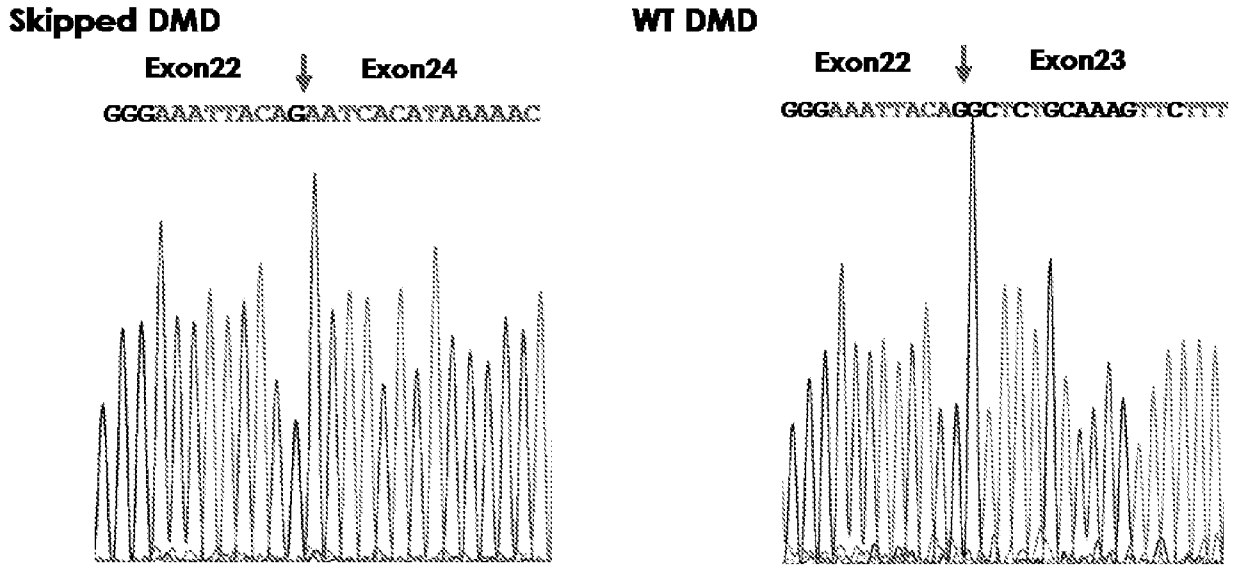


FIG. 14

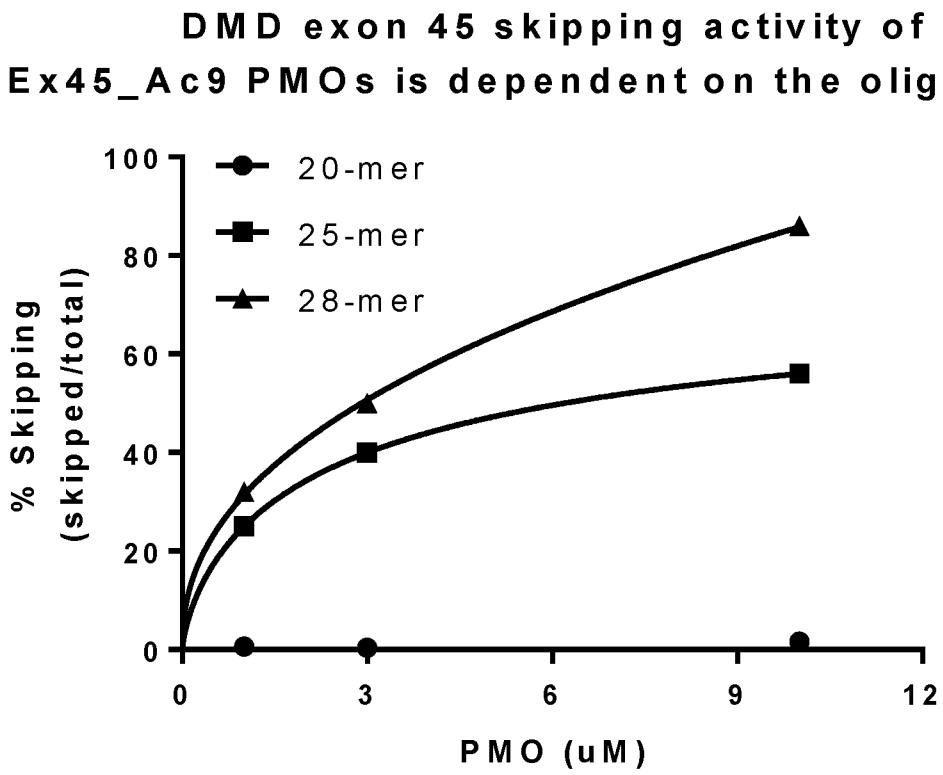

DMD exon 45 skipping activity of
hEx45_Ac9 PMOs is dependent on the oligo length
20-mer
25-mer
28-mer
% Skipping
(skipped/total)
100
80
60
40
20
0
0
3
6
9
12
PMO (uM)

FIG. 15

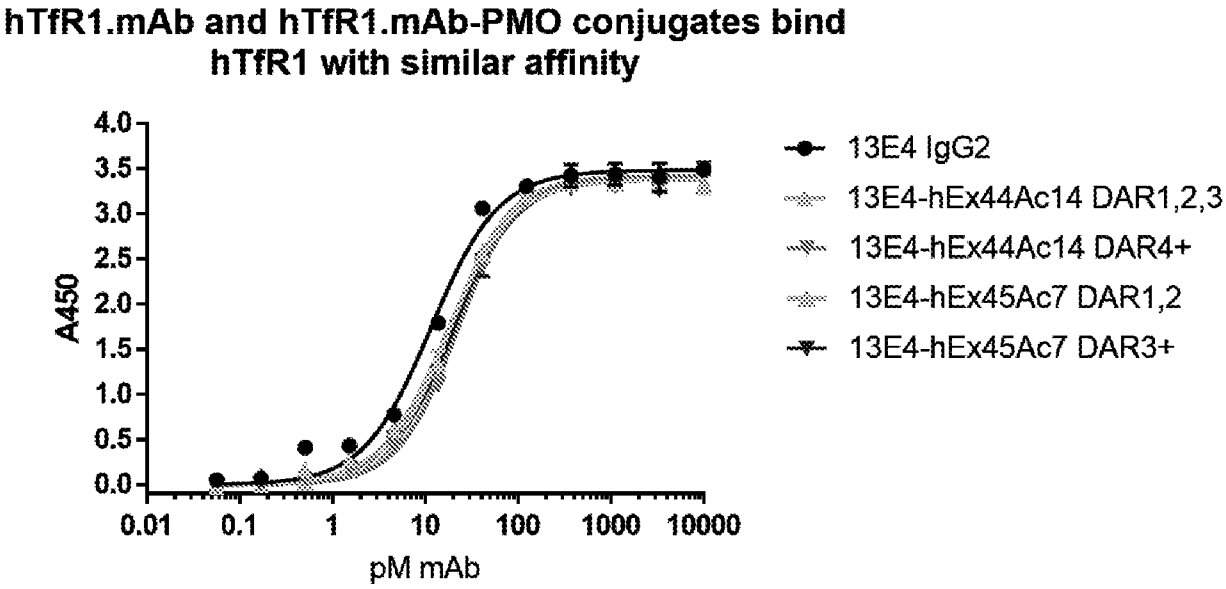

hTfR1.mAb and hTfR1.mAb-PMO conjugates bind
hTfR1 with similar affinity
13E4 IgG2
13E4-hEx44Ac14 DAR1,2,3
13E4-hEx44Ac14 DAR4+
13E4-hEx45Ac7 DAR1,2
13E4-hEx45Ac7 DAR3+
A450
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
1000
10000
pM mAb

FIG. 16

hTfR1.mAb-PMO conjugates mediate DMD exon 44 skipping in primary human skeletal muscle cells

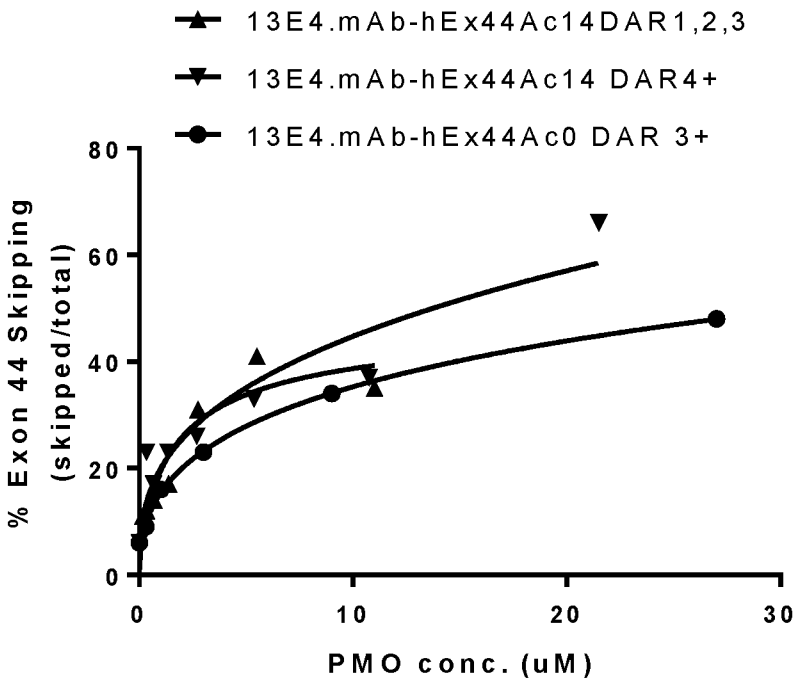


FIG. 17

hTfR1.mAb-PMO conjugates show similar DMD exon skipping activity in myotubes of primary and immortalized human skeletal muscle cells

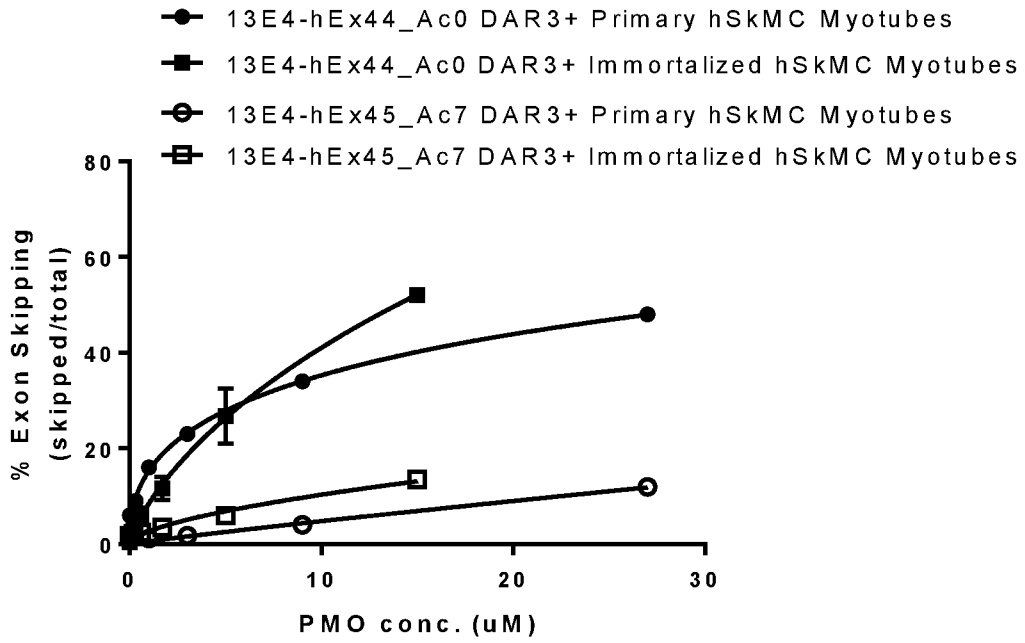


FIG. 18