NUCLEIC ACID COMPOUNDS FOR BINDING GROWTH DIFFERENTIATION FACTOR 11

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority of US Provisional Application No. 62/083,592, filed November 24, 2014, and US Provisional Application No. 62/113,864, filed February 9, 2015, each of which is incorporated by reference herein in its entirety for any purpose.

SEQUENCE LISTING

[002] The present application is filed with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled "2015-11-23_01137-0011-00PCT_ST25.txt" created on November 23, 2015, which is 122,816 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD

[003] The present disclosure relates generally to the field of nucleic acids, and more specifically, to aptamers capable of binding to growth differentiation factor 11 (GDF11) protein; compositions comprising a GDF11 binding aptamer with a GDF11 protein; and methods of making and using the same.

BACKGROUND

[004] Growth differentiation factor 11 (GDF11) or bone morphogenetic protein 11 (BMP-11) belongs to the transforming growth factor beta superfamily that controls anterior-posterior patterning during development by regulating the expression of Hox genes. GDF11 has been shown to play a role in the regulation of cardiacmyocite proliferation, kidney organogenesis, pancreatic development, act as a negative regulator of neurogenesis and chondrogenesis.

[005] GDF11 is closely related to myostatin (GDF8), which is a negative regulator of muscle growth. Like GDF11, myostatin is involved in the regulation of cardiacmyocyte proliferation. The similarities between GDF11 and myostatin imply a likelihood that the same regulatory mechanisms are used to control tissue size during both muscular and neural development. Mechanistically, the actions of GDF11 are likely regulated by WFIKKN2, a large extracellular multidomain protein consisting of follistatin, immunoglobulin, protease inhibitor, and NTR domains. WFIKKN2 has a high affinity for GDF11, and previously has been found to inhibit the biological activities of myostatin.

[006] Both GDF11 and GDF8 (myostatin) play important roles in the course of development, and in the regulation of cell growth and differentiation in adult tissues. The ability to localize and/or measure these two proteins is important to further understand and distinguish their contributions in development and adult tissues (e.g., cardiac and skeletal muscle). However, due to their homology it is difficult to distinguish the presence and/or levels of these proteins with current protein binding reagents (e.g., antibodies). Thus, there is a need for protein binding reagents that are capable of distinguishing the proteins GDF11 and GDF8. The present disclosure meets such needs by providing aptamers having binding specificity to a GDF11 protein.

SUMMARY

[007] The present disclosure describes aptamers capable of binding to growth differentiation factor 11 (GDF11) protein. In some embodiment, aptamers that bind GDF11 with an equilibrium binding constant (K_d) of less than 100 nM are provided. In another embodiment, the K_d is from about 0.1 nM to about 100 nM (or from about 0.1 nM to about 50 nM, or from about 0.1 nM to about 10 nM, or from abou 0.5 nM to about 10 nM, or from about 0.5 nM to about 5 nM).

[007a] Disclosed is an aptamer that binds GDF11 with an affinity of less than 10 nM, wherein, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8.

[008] In some embodiments, an aptamer that binds GDF11 with an affinity of less than 10 nM is provided. In some embodiments, the aptamer binds GDF11 with an affinity of less than 10 nM, and, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11. In some embodiments, the aptamer does not bind GDF8. In some embodiments, the aptamer binds GDF8 with an affinity that is at least 20-fold weaker, or at least 30-fold weaker, or at least 50-fold weaker than the affinity for GDF11. In some embodiments, the aptamer binds GDF8 with an affinity greater than 50 nM, or greater than 100 nM, or greater than 150 nM, or greater than 200 nM, or greater than 250 nM, or greater than 300 nM. In some embodiments, the aptamer binds GDF11 with an affinity of less than 8 nM, or less than 7 nM, or less than 6 nM, or less than 5 nM, or less than 4 nM, or less than 3 nM, or less than 2 nM, or less than 1 nM. In some embodiments, affinity is determined using a binding assay comprising a polyanionic inhibitor. In some embodiments, the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs. In one aspect, there is provided an aptamer that binds GDF11 with an affinity of less than 10 nM, wherein, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8, wherein the aptamer comprises the sequence 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3'(SEQ ID NO:110) or 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

each W is independently, and for each occurrence, A, T, or U;

each M is independently, and for each occurrence, A or C;

each S is independently, and for each occurrence, G or C;

each n is independently, and for each occurrence, 0 or 1; and

each m is independently, and for each occurrence, 0 or 1.

[009] In another aspect, an aptamer that binds GDF11 is provided, wherein the aptamer comprises the sequence 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO:110) or 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

each W is independently, and for each occurrence, A, T, or U;

each M is independently, and for each occurrence, A or C;

each S is independently, and for each occurrence, G or C;

each n is independently, and for each occurrence, 0 or 1; and

each m is independently, and for each occurrence, 0 or 1.

[010] In some embodiments, an aptamer that binds GDF11 comprises a sequence selected from:

a) 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 111);

b) 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 152);

c) 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 112);

d) 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 153);

e) 5'-$RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGC$-3' (SEQ ID NO: 113); and

f) $RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGS$-3' (SEQ ID NO: 154).

In some embodiments of the sequences above, if R is G, the first W may be A, and wherein if R is A, the first W may be C. In some embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 n are 0. In some embodiments, each n is 0. In some embodiments, at least 1, at least 2, at least 3 n are 1. In some embodiments, at least one m is 0. In some embodiments, at least one m is 1.

[011] In some embodiments, an aptamer that binds GDF11 comprises the sequence 5'-CPPGMPPP-3' (SEQ ID NO: 114), wherein each P is independently, and for each

occurrence, a C-5 modified pyrimidine, and wherein M is C or A. In some embodiments, an aptamer that binds GDF11 comprises the sequence 5'-PPPAGC-3'(SEQ ID NO: 115) or 5'-PPPAGG-3' (SEQ ID NO: 155), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine. In some embodiments, an aptamer that binds GDF11 comprises the sequence 5'-CPPGMPPPN$_x$PPPAGC-3' (SEQ ID NO: 116) or 5'-CPPGMPPPN$_x$PPPAGG-3' (SEQ ID NO: 160), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, wherein x is 2, 3, 4, or 5, wherein M is C or A, and wherein each N is independently, and for each occurrence, selected from A, C, G, T, and U. In some embodiments, x is 3 or 4. In some embodiments, N_x comprises the sequence 5'-AAG-3' or 5'-ACG-3' or 5'-AGG-3'.

[012] In some embodiments, an aptamer that binds GDF11 comprises the sequence 5'-NNCPPGRPPPAMGPPPAGS-3' (SEQ ID NO: 141), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, R is A or G; each N is independently, and for each occurrence, A, G, or C; M is A or C; and S is G or C.

[013] In some embodiments, each P is independently, and for each occurrence, selected from:

5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU),

5-(N-benzylcarboxyamide)-2'-O-methyluridine,

5-(N-benzylcarboxyamide)-2'-fluorouridine,

5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU),

5-(N-thiophenylmethylcarboxyamide)-2'-deoxyuridine (ThdU),

5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU),

5-(N-tyrosylcarboxyamide)-2'-deoxyuridine (TyrdU),

5-(N-3,4-methylenedioxybenzylcarboxyamide)-2'-deoxyuridine (MBndU),

5-(N-4-fluorobenzylcarboxyamide)-2'-deoxyuridine (FBndU),

5-(N-3-phenylpropylcarboxyamide)-2'-deoxyuridine (PPdU),

5-(N-imidizolylethylcarboxyamide)-2'-deoxyuridine (ImdU),

5-(N-isobutylcarboxyamide)-2'-O-methyluridine,

5-(N-isobutylcarboxyamide)-2'-fluorouridine,

5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU),

5-(N-*R*-threoninylcarboxyamide)-2'-deoxyuridine (ThrdU),

5-(N-tryptaminocarboxyamide)-2'-O-methyluridine,

5-(N-tryptaminocarboxyamide)-2'-fluorouridine,

5-(N-[1-(3-trimethylamonium) propyl]carboxyamide)-2'-deoxyuridine chloride,

5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU),

5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine),

5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU),

5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU),

5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU),

5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU),

5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU),

5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, and

5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine.

[014] In some embodiments, each P is independently, and for each occurrence, selected from:

5-(N-1-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU),

5-(N-1-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU),

5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU),

5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU),

5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU),

5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU),

5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, and

5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine.

In some embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 P are each independently selected from 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, and 5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine.

[015] In some embodiments, the aptamer comprises a sequence selected from SEQ ID NOs: 12, 13, 15 to 109, and 117 to 140. In some embodiments, the aptamer comprises a sequence selected from SEQ ID NOs: 12, 15, 26, 105 to 109, and 142 to 150.

[016] In any of the embodiments described herein, the aptamer may consist of 18 to 200 nucleotides, or 18 to 150 nucleotides, or 18 to 100 nucleotides, or 18 to 75 nucleotides, or 18 to 50 nucleotides, or 20 to 150 nucleotides, or 20 to 100 nucleotides, or 20 to 75 nucleotides, or 20 to 50 nucleotides, wherein each nucleotide may, independently, be a modified or unmodified nucleotide. In any of the embodiments described herein, the aptamer may comprise a detectable label.

[017] In some embodiments, GDF11 is human GDF11 and GDF8 is human GDF8. In some embodiments, GDF11 is mature human GDF11 comprising the sequence of SEQ ID NO: 118 and GDF8 is mature human GDF8 comprising the sequence of SEQ ID NO: 119.

[018] In one aspect, a method of detecting GDF11 in a sample is provided, comprising contacting proteins from the sample with an aptamer described herein. In some embodiments, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11. In some embodiments, the aptamer does not bind GDF8.

[019] In another aspect, a method of determining whether a sample comprises GDF11 is provided, comprising contacting proteins from the sample with an aptamer described herein. In some embodiments, the sample comprises GDF8. In some embodiments, the method comprises contacting the sample with the aptamer under stringent conditions. In some embodiments, the stringent conditions comprise a polyanionic inhibitor. In some embodiments, the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs.

[020] In some embodiments, the sample is a sample from a human. In some embodiments, the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample. In some embodiments, the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue.

[021] In some embodiments, the proteins have been separated from at least one other component of the sample. In some embodiments, the proteins have not been separated from other components of the sample.

[022] In one aspect, compositions are provided comprising an aptamer described herein and proteins from a sample. In some embodiments, the sample comprises GDF8. In some embodiments, the composition comprises a polyanionic inhibitor. In some embodiments, the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs. In some embodiments, the sample is a sample from a human. In some embodiments, the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample. In some embodiments, the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue. In some embodiments, the proteins have been separated from at least one other component of the sample. In some embodiments, the proteins have not been separated from other components of the sample.

[023] In another embodiment, the aptamer is at least 25 nucleotides in length. In another embodiment, the aptamer is at least 30 nucleotides in length. In another embodiment, the aptamer is at least 40 nucleotides in length. In another embodiment, the aptamer is from about 40 to about 100 nucleotides in length (or from 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, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 nucleotides in length).

[024] The present disclosure further provides a composition comprising an aptamer and a GDF11 protein, wherein the aptamer and the GDF11 protein are bound by a non-covalent interaction.

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

BRIEF DESCRIPTION OF THE DRAWINGS

[026] **FIG. 1** shows a comparison of the binding affinities of aptamer clone 12060-28_3 for human GDF11 protein and human myostatin (GDF8) protein in the presence or absence of a polyanionic inhibitor (Z-block). The x-axis shows the concentrations for the respective proteins and the y-axis shows the percent of the aptamer bound to the protein (1.0 is 100%).

[027] **FIG. 2** shows a comparison of the binding affinities of aptamer 12058-6_3 for human GDF11 protein and human myostatin (GDF8) protein in the presence or absence of a

polyanionic inhibitor (Z-block). The x-axis shows the concentrations for the respective proteins and the y-axis shows the percent of the aptamer clone bound to the protein (1.0 is 100%).

[028] **FIG. 3** shows a truncation analysis of aptamer clone 12060-28_3 (50-mer sequence) and the binding affinity of each sequence for GDF11.

DETAILED DESCRIPTION

I. Terms and Methods

[029] While the invention will be described in conjunction with certain representative embodiments, it will be understood that the invention is defined by the claims, and is not limited to those embodiments.

[030] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein may be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[031] Unless defined otherwise, technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8). Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice of the invention, certain methods, devices, and materials are described herein.

[032] All publications, published patent documents, and patent applications cited herein are hereby incorporated by reference to the same extent as though each individual publication, published patent document, or patent application was specifically and individually indicated as being incorporated by reference.

[033] As used in this application, including the appended claims, the singular forms "a," "an," and "the" include the plural, unless the context clearly dictates otherwise, and may be used interchangeably with "at least one" and "one or more." Thus, reference to "an aptamer" includes mixtures of aptamers, reference to "a probe" includes mixtures of probes, and the like.

[034] As used herein, the terms "comprises," "comprising," "includes," "including," "contains," "containing," and any variations thereof, are intended to cover a non-exclusive

inclusion, such that a process, method, product-by-process, or composition of matter that comprises, includes, or contains an element or list of elements may include other elements not expressly listed.

[035] It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

[036] In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

[037] **Aptamer:** As used herein, an "aptamer" refers to a nucleic acid that has a specific binding affinity for a target molecule. It is recognized that affinity interactions are a matter of degree; however, in this context, the "specific binding affinity" of an aptamer for its target means that the aptamer binds to its target generally with a much higher degree of affinity than it binds to other components in a test sample. An "aptamer" is a set of copies of one type or species of nucleic acid molecule that has a particular nucleotide sequence. An aptamer can include any suitable number of nucleotides, including any number of chemically modified nucleotides. "Aptamers" refers to more than one such set of molecules. Different aptamers can have either the same or different numbers of nucleotides. Aptamers can be DNA or RNA or chemically modified nucleic acids and can be single stranded, double stranded, or contain double stranded regions, and can include higher ordered structures. An aptamer can also be a photoaptamer, where a photoreactive or chemically reactive functional group is included in the aptamer to allow it to be covalently linked to its corresponding target. Any of the aptamer methods disclosed herein can include the use of two or more aptamers that specifically bind the same target molecule. As further described below, an aptamer may include a tag. If an aptamer includes a tag, all copies of the aptamer need not have the same tag. Moreover, if different aptamers each include a tag, these different aptamers can have either the same tag or a different tag.

[038] An aptamer can be identified using any known method, including the SELEX process. Once identified, an aptamer can be prepared or synthesized in accordance with any known method, including chemical synthetic methods and enzymatic synthetic methods.

[039] **GDF11 Aptamer:** GDF11 aptamer, as used herein, refers to an aptamer that is capable of binding to a mature GDF11 protein. A nonlimiting exemplary mature human GDF11 protein is shown below (amino acids 299 to 407 of UniProtKB/Swiss-Prot: O95390.1):

L GLDCDEHSSE SRCCRYPLTV DFEAFGWDWI IAPKRYKANY CSGQCEYMFM QKYPHTHLVQ QANPRGSAGP CCTPTKMSPI NMLYFNDKQQ IIYGKIPGMV VDRCGCS (SEQ ID NO: 118).

In some embodiments, a GDF11 aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11. In some embodiments, a GDF11 aptamer does not bind GDF8. A nonlimiting exemplary mature human GDF8 protein is shown below (amino acids 267 to 375 of UniProtKB/Swiss-Prot: O14793.1):

FGL DCDEHSTESR CCRYPLTVDF EAFGWDWIIA PKRYKANYCS GECEFVFLQK YPHTHLVHQA NPRGSAGPCC TPTKMSPINM LYFNGKEQII YGKIPAMVVD RCGCS (SEQ ID NO: 119).

[040] Inhibit: The term inhibit, as used herein, means to prevent or reduce the expression of a peptide or a polypeptide to an extent that the peptide or polypeptide no longer has measurable activity or bioactivity; or to reduce the stability and/or reduce or prevent the activity of a peptide or a polypeptide to an extent that the peptide or polypeptide no longer has measurable activity or bioactivity. As described herein, the protein that may be inhibited is GDF11.

[041] Nucleic acid: As used herein, "nucleic acid," "oligonucleotide," and "polynucleotide" are used interchangeably to refer to a polymer of nucleotides and include DNA, RNA, DNA/RNA hybrids and modifications of these kinds of nucleic acids, oligonucleotides and polynucleotides, wherein the attachment of various entities or moieties to the nucleotide units at any position are included. The terms "polynucleotide," "oligonucleotide," and "nucleic acid" include double- or single-stranded molecules as well as triple-helical molecules. Nucleic acid, oligonucleotide, and polynucleotide are broader terms than the term aptamer and, thus, the terms nucleic acid, oligonucleotide, and polynucleotide include polymers of nucleotides that are aptamers but the terms nucleic acid, oligonucleotide, and polynucleotide are not limited to aptamers.

[042] Modified: As used herein, the terms "modify", "modified", "modification", and any variations thereof, when used in reference to an oligonucleotide, means that at least one of the four constituent nucleotide bases (i.e., A, G, T/U, and C) of the oligonucleotide is an analog or ester of a naturally occurring nucleotide. In some embodiments, the modified nucleotide confers nuclease resistance to the oligonucleotide. In some embodiments, the modified nucleotides lead to predominantly hydrophobic interactions of aptamers with protein targets resulting in high binding efficiency and stable co-crystal complexes. A pyrimidine with a substitution at the C-5 position is an example of a modified nucleotide. Modifications can include backbone modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine, and the like. Modifications can also include 3' and 5' modifications, such as capping. Other modifications can include substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters,

phosphoamidates, carbamates, etc.) and those with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, and those with modified linkages (e.g., alpha anomeric nucleic acids, etc.). Further, any of the hydroxyl groups ordinarily present on the sugar of a nucleotide may be replaced by a phosphonate group or a phosphate group; protected by standard protecting groups; or activated to prepare additional linkages to additional nucleotides or to a solid support. The 5′ and 3′ terminal OH groups can be phosphorylated or substituted with amines, organic capping group moieties of from about 1 to about 20 carbon atoms, polyethylene glycol (PEG) polymers, in some embodiments, ranging from about 10 to about 80 kDa, PEG polymers, in some embodiments, ranging from about 20 to about 60 kDa, or other hydrophilic or hydrophobic biological or synthetic polymers. In one embodiment, modifications are of the C-5 position of pyrimidines. These modifications can be produced through an amide linkage directly at the C-5 position or by other types of linkages.

[043] Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. As noted above, one or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include embodiments wherein phosphate is replaced by P(O)S (“thioate”), P(S)S (“dithioate”), (O)NR2 (“amidate”), P(O)R, P(O)OR', CO or CH2 (“formacetal”), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalky, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. Substitution of analogous forms of sugars, purines, and pyrimidines can be advantageous in designing a final product, as can alternative backbone structures like a polyamide backbone, for example.

[044] **Nuclease:** As used herein, the term “nuclease” refers to an enzyme capable of cleaving the phosphodiester bond between nucleotide subunits of an oligonucleotide. As used herein, the term “endonuclease” refers to an enzyme that cleaves phosphodiester bond(s) at a site internal to the oligonucleotide. As used herein, the term “exonuclease” refers to an enzyme which cleaves phosphodiester bond(s) linking the end nucleotides of an oligonucleotide. Biological fluids typically contain a mixture of both endonucleases and exonucleases.

[045] As used herein, the terms "nuclease resistant" and "nuclease resistance" refers to the reduced ability of an oligonucleotide to serve as a substrate for an endo- or exonuclease, such that, when contacted with such an enzyme, the oligonucleotide is either not degraded or is degraded more slowly than an oligonucleotide composed of unmodified nucleotides.

[046] **C-5 Modified Pyrimidine:** As used herein, the term "C-5 modified pyrimidine" refers to a pyrimidine with a modification at the C-5 position including, but not limited to, those moieties illustrated herein. Examples of a C-5 modified pyrimidine include those described in U.S. Pat. Nos. 5,719,273 and 5,945,527. Examples of a C-5 modification include substitution of deoxyuridine at the C-5 position with a substituent independently selected from: benzylcarboxyamide (alternatively benzylaminocarbonyl) (Bn), naphthylmethylcarboxyamide (alternatively naphthylmethylaminocarbonyl) (Nap), tryptaminocarboxyamide (alternatively tryptaminocarbonyl) (Trp), phenethylcarboxyamide (alternatively phenethylamino carbonyl) (Pe), thiophenylmethylcarboxyamide (alternatively thiophenylmethylaminocarbonyl) (Th) and isobutylcarboxyamide (alternatively isobutylaminocarbonyl) (iBu) as illustrated immediately below.

[047] Chemical modifications of a C-5 modified pyrimidine can also be combined with, singly or in any combination, 2'-position sugar modifications, modifications at exocyclic amines, and substitution of 4-thiouridine and the like.

[048] Representative C-5 modified pyrimidines include: 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamide)-2'-O-methyluridine, 5-(N-benzylcarboxyamide)-2'-fluorouridine, 5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU), 5-(N-isobutylcarboxyamide)-2'-O-methyluridine, 5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PedU), 5-(N-thiophenylmethylcarboxyamide)-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxyamide)-2'-fluorouridine, 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-(N-tryptaminocarboxyamide)-2'-O-methyluridine, 5-(N-tryptaminocarboxyamide)-2'-fluorouridine, 5-(N-[1-(3-trimethylamonium) propyl]carboxyamide)-2'-deoxyuridine chloride, 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine or 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine).

[049] Nucleotides can be modified either before or after synthesis of an oligonucleotide. A sequence of nucleotides in an oligonucleotide may be interrupted by one or more non-nucleotide components. A modified oligonucleotide may be further modified after polymerization, such as, for example, by conjugation with any suitable labeling component.

[050] As used herein, the term "at least one pyrimidine," when referring to modifications of a nucleic acid, refers to one, several, or all pyrimidines in the nucleic acid,

indicating that any or all occurrences of any or all of C, T, or U in a nucleic acid may be modified or not.

[051] Modulate: The term modulate, as used herein, means to alter the expression level of a peptide, protein or polypeptide by increasing or decreasing its expression level relative to a reference expression level, and/or alter the stability and/or activity of a peptide, protein or polypeptide by increasing or decreasing its stability and/or activity level relative to a reference stability and/or activity level.

[052] Pharmaceutically Acceptable: Pharmaceutically acceptable, as used herein, means approved by a regulatory agency of a federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals and, more particularly, in humans.

[053] Pharmaceutically Acceptable Salt: Pharmaceutically acceptable salt or salt of a compound (e.g., aptamer), as used herein, refers to a product that contains an ionic bond and is typically produced by reacting the compound with either an acid or a base, suitable for administering to an individual. A pharmaceutically acceptable salt can include, but is not limited to, acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, arylalkylsulfonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates, and tartrates; alkali metal cations such as Li, Na, K, alkali earth metal salts such as Mg or Ca, or organic amine salts.

[054] Pharmaceutical Composition: Pharmaceutical composition, as used herein, refers to formulation comprising a GDF11 aptamer in a form suitable for administration to an individual. A pharmaceutical composition is typically formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, oral and parenteral, e.g., intravenous, intradermal, subcutaneous, inhalation, topical, transdermal, transmucosal, and rectal administration.

[055] SELEX: The terms "SELEX" and "SELEX process" are used interchangeably herein to refer generally to a combination of (1) the selection of aptamers that interact with a target molecule in a desirable manner, for example binding with high affinity to a protein, with (2) the amplification of those selected nucleic acids. The SELEX process can be used to identify aptamers with high affinity to a specific target or biomarker.

[056] Sequence Identity: Sequence identity, as used herein, in the context of two or more nucleic acid sequences is a function of the number of identical nucleotide positions shared by the sequences (i.e., % identity=number of identical positions/total number of positions×100), taking into account the number of gaps, and the length of each gap that needs to be introduced to optimize alignment of two or more sequences. The comparison of

sequences and determination of percent identity between two or more sequences can be accomplished using a mathematical algorithm, such as BLAST and Gapped BLAST programs at their default parameters (e.g., Altschul et al., *J. Mol. Biol.* 215:403, 1990; see also BLASTN at www.ncbi.nlm.nih.gov/BLAST). For sequence comparisons, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, Adv. Appl. Math., 2:482, 1981, by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol., 48:443, 1970, by the search for similarity method of Pearson and Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally, Ausubel, F. M. et al., Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987)). As used herein, when describing the percent identity of a nucleic acid, such as a GDF11 aptamer, the sequence of which is at least, for example, about 95% identical to a reference nucleotide sequence, it is intended that the nucleic acid sequence is identical to the reference sequence except that the nucleic acid sequence may include up to five point mutations per each 100 nucleotides of the reference nucleic acid sequence. In other words, to obtain a desired nucleic acid sequence, the sequence of which is at least about 95% identical to a reference nucleic acid sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or some number of nucleotides up to 5% of the total number of nucleotides in the reference sequence may be inserted into the reference sequence (referred to herein as an insertion). These mutations of the reference sequence to generate the desired sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[057] SOMAmer: The term SOMAmer, as used herein, refers to an aptamer having improved off-rate characteristics. SOMAmers are alternatively referred to as Slow Off-Rate Modified Aptamers, and may be selected via the improved SELEX methods described in U.S. Publication No. 20090004667, entitled "Method for Generating Aptamers with Improved Off-

Rates", which is incorporated by reference in its entirety. In some embodiments, a slow off-rate aptamer (including an aptamers comprising at least one nucleotide with a hydrophobic modification) has an off-rate (t½) of ≥ 2 minutes, ≥ 4 minutes, ≥ 5 minutes, ≥ 8 minutes, ≥ 10 minutes, ≥ 15 minutes ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

[058] Target Molecule: "Target", "target molecule", and "analyte" are used interchangeably herein to refer to any molecule of interest that may be present in a sample. The term includes any minor variation of a particular molecule, such as, in the case of a protein, for example, minor variations in amino acid sequence, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component, which does not substantially alter the identity of the molecule. A "target molecule", "target", or "analyte" refers to a set of copies of one type or species of molecule or multi-molecular structure. "Target molecules", "targets", and "analytes" refer to more than one type or species of molecule or multi-molecular structure. Exemplary target molecules include proteins, polypeptides, nucleic acids, carbohydrates, lipids, polysaccharides, glycoproteins, hormones, receptors, antigens, antibodies, affybodies, antibody mimics, viruses, pathogens, toxic substances, substrates, metabolites, transition state analogs, cofactors, inhibitors, drugs, dyes, nutrients, growth factors, cells, tissues, and any fragment or portion of any of the foregoing. In some embodiments, a target molecule is a protein, in which case the target molecule may be referred to as a "target protein."

[059] Polyanionic inhibitor: A polyanionic inhibitor is an inhibitor molecule that comprises or mimics the poyanionic phosphate backbone of a nucleic acid molecule. In some embodiments, a polyanionic inhibitor is included in a binding reaction with an aptamer to increase the stringency of the binding conditions. Nonlimiting exemplary polyanionic inhibitors include dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, dNTPs, and the like.

[060] Z-Block: Z-block as used herein is a single-stranded oligonucleotide of sequence 5′- (AC-BndU-BndU)$_7$-AC-3′, where BndU indicates a benzyl-substituted deoxyuridine residue. Z-block may be synthesized using conventional phosphoramidite chemistry

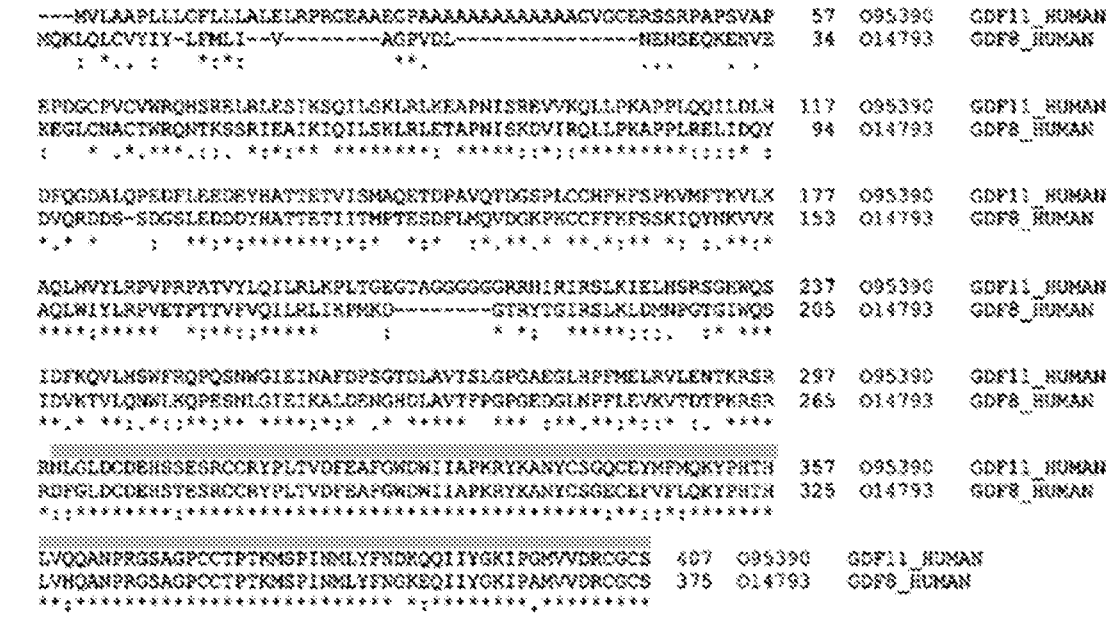
[061] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41,

42, 43, 44, 45, 46, 47, 48, 49, or 50 (as well as fractions thereof unless the context clearly dictates otherwise). Any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, "about" or "consisting essentially of mean ± 20% of the indicated range, value, or structure, unless otherwise indicated. As used herein, the terms "include" and "comprise" are open ended and are used synonymously. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (e.g. , "or") should be understood to mean either one, both, or any combination thereof of the alternatives.

II. Overview

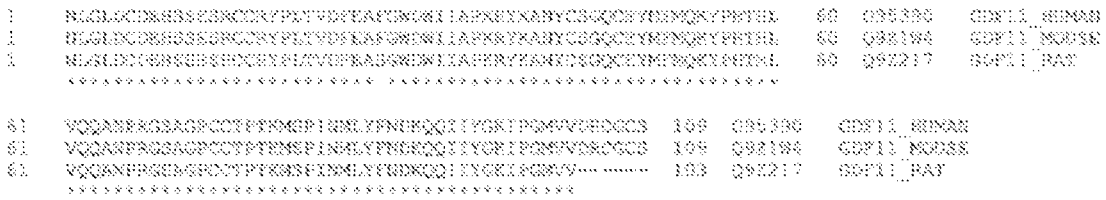
A. Growth differentiation factor 11 (GDF11) and Myostatin (GDF8) Proteins

[062] The native mature forms of GDF11 and myostatin are homodimers and are about 90% identical by sequence alignment, with 98 of 109 matching amino acid residues. *See* amino acid sequence alignment for GDF11 (Protein ID 095390; amino acids 1 to 407; SEQ ID NO: 4) and myostatin (Protein ID 014793; amino acids 1 to 375; SEQ ID NO: 5), mature form indicated by gray bar. The alignment of the amino acid sequences of GDF11 and myostatin prepropeptides are shown below. The mature protein portions are highlighted with the gray bar.

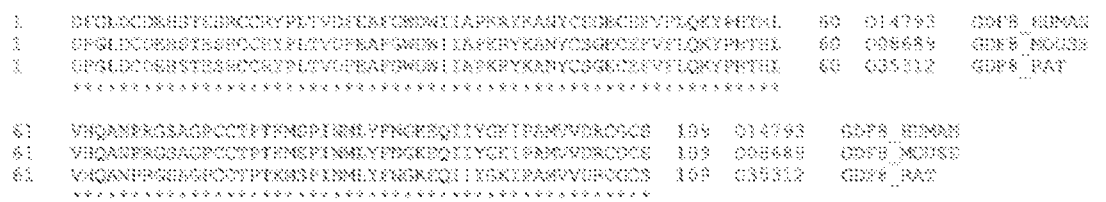


[063] GDF11 and GDF8 are thought to share common ancestry and common regulatory mechanism, yet have diverged with regard to their tissue specificity (primarily heart and muscle, respectively).

[064] Mature GDF11 is 100% identical between human and mouse, and 99% identical in rat. Amino acid sequence alignment of human, mouse and rat GDF11 protein (mature) is show below. Human GDF11 Protein ID is O95390; an exemplary human mature GDF11 is shown below; SEQ ID NO: 6). Mouse GDF11 Protein ID is Q9Z1W4; an exemplary mouse mature GDF8 is shown below; SEQ ID NO: 7). Rat GDF11 Protein ID is Q9Z217; an exemplary rat mature GDF8 is shown below; SEQ ID NO: 8).



[065] Myostatin (GDF8) is 100% identical between human, mouse, and rat. Amino acid sequence alignment of human, mouse and rat myostatin protein (mature) is show below. Human myostatin (GDF8) Protein ID is O14793; an exemplary human mature GDF8 is shown below; SEQ ID NO: 9). Mouse GDF8 Protein ID is O08689; an exemplary mouse mature GDF8 is shown below; SEQ ID NO: 10). Rat GDF11 Protein ID is O35312; an exemplary rat mature GDF8 is shown below; SEQ ID NO: 11).



B. SELEX

[066] SELEX generally includes preparing a candidate mixture of nucleic acids, binding of the candidate mixture to the desired target molecule to form an affinity complex, separating the affinity complexes from the unbound candidate nucleic acids, separating and isolating the nucleic acid from the affinity complex, purifying the nucleic acid, and identifying a specific aptamer sequence. The process may include multiple rounds to further refine the affinity of the selected aptamer. The process can include amplification steps at one or more points in the process. See, e.g., U.S. Pat. No. 5,475,096, entitled "Nucleic Acid Ligands". The SELEX process can be used to generate an aptamer that covalently binds its target as well as

an aptamer that non-covalently binds its target. See, e.g., U.S. Pat. No. 5,705,337 entitled "Systematic Evolution of Nucleic Acid Ligands by Exponential Enrichment: Chemi-SELEX."

[067] The SELEX process can be used to identify high-affinity aptamers containing modified nucleotides that confer improved characteristics on the aptamer, such as, for example, improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX process-identified aptamers containing modified nucleotides are described in U.S. Pat. No. 5,660,985, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides", which describes oligonucleotides containing nucleotide derivatives chemically modified at the 5′- and 2′-positions of pyrimidines. U.S. Pat. No. 5,580,737, see supra, describes highly specific aptamers containing one or more nucleotides modified with 2′-amino (2′—NH2), 2′-fluoro (2′-F), and/or 2′-O-methyl (2′-OMe). See also, U.S. Patent Application Publication 20090098549, entitled "SELEX and PHOTOSELEX", which describes nucleic acid libraries having expanded physical and chemical properties and their use in SELEX and photoSELEX.

[068] SELEX can also be used to identify aptamers that have desirable off-rate characteristics. See U.S. Patent Application Publication 20090004667, entitled "Method for Generating Aptamers with Improved Off-Rates", which describes improved SELEX methods for generating aptamers that can bind to target molecules. As mentioned above, these slow off-rate aptamers are known as "SOMAmers." Methods for producing aptamers or SOMAmers and photoaptamers or SOMAmers having slower rates of dissociation from their respective target molecules are described. The methods involve contacting the candidate mixture with the target molecule, allowing the formation of nucleic acid-target complexes to occur, and performing a slow off-rate enrichment process wherein nucleic acid-target complexes with fast dissociation rates will dissociate and not reform, while complexes with slow dissociation rates will remain intact. Additionally, the methods include the use of modified nucleotides in the production of candidate nucleic acid mixtures to generate aptamers or SOMAmers with improved off-rate performance.

[069] A variation of this assay employs aptamers that include photoreactive functional groups that enable the aptamers to covalently bind or "photocrosslink" their target molecules. See, e.g., U.S. Pat. No. 6,544,776 entitled "Nucleic Acid Ligand Diagnostic Biochip". These photoreactive aptamers are also referred to as photoaptamers. See, e.g., U.S. Pat. No. 5,763,177, U.S. Pat. No. 6,001,577, and U.S. Pat. No. 6,291,184, each of which is entitled "Systematic Evolution of Nucleic Acid Ligands by Exponential Enrichment: Photoselection of Nucleic Acid Ligands and Solution SELEX"; see also, e.g., U.S. Pat. No.

6,458,539, entitled "Photoselection of Nucleic Acid Ligands". After the microarray is contacted with the sample and the photoaptamers have had an opportunity to bind to their target molecules, the photoaptamers are photoactivated, and the solid support is washed to remove any non-specifically bound molecules. Harsh wash conditions may be used, since target molecules that are bound to the photoaptamers are generally not removed, due to the covalent bonds created by the photoactivated functional group(s) on the photoaptamers.

[070] In both of these assay formats, the aptamers or SOMAmers are immobilized on the solid support prior to being contacted with the sample. Under certain circumstances, however, immobilization of the aptamers or SOMAmers prior to contact with the sample may not provide an optimal assay. For example, pre-immobilization of the aptamers or SOMAmers may result in inefficient mixing of the aptamers or SOMAmers with the target molecules on the surface of the solid support, perhaps leading to lengthy reaction times and, therefore, extended incubation periods to permit efficient binding of the aptamers or SOMAmers to their target molecules. Further, when photoaptamers or photoSOMAmers are employed in the assay and depending upon the material utilized as a solid support, the solid support may tend to scatter or absorb the light used to effect the formation of covalent bonds between the photoaptamers or photoSOMAmers and their target molecules. Moreover, depending upon the method employed, detection of target molecules bound to their aptamers or photoSOMAmers can be subject to imprecision, since the surface of the solid support may also be exposed to and affected by any labeling agents that are used. Finally, immobilization of the aptamers or SOMAmers on the solid support generally involves an aptamer or SOMAmer-preparation step (i.e., the immobilization) prior to exposure of the aptamers or SOMAmers to the sample, and this preparation step may affect the activity or functionality of the aptamers or SOMAmers.

[071] SOMAmer assays that permit a SOMAmer to capture its target in solution and then employ separation steps that are designed to remove specific components of the SOMAmer-target mixture prior to detection have also been described (see U.S. Patent Application Publication 20090042206, entitled "Multiplexed Analyses of Test Samples"). The described SOMAmer assay methods enable the detection and quantification of a non-nucleic acid target (e.g., a protein target) in a test sample by detecting and quantifying a nucleic acid (i.e., a SOMAmer). The described methods create a nucleic acid surrogate (i.e., the SOMAmer) for detecting and quantifying a non-nucleic acid target, thus allowing the wide variety of nucleic acid technologies, including amplification, to be applied to a broader range of desired targets, including protein targets.

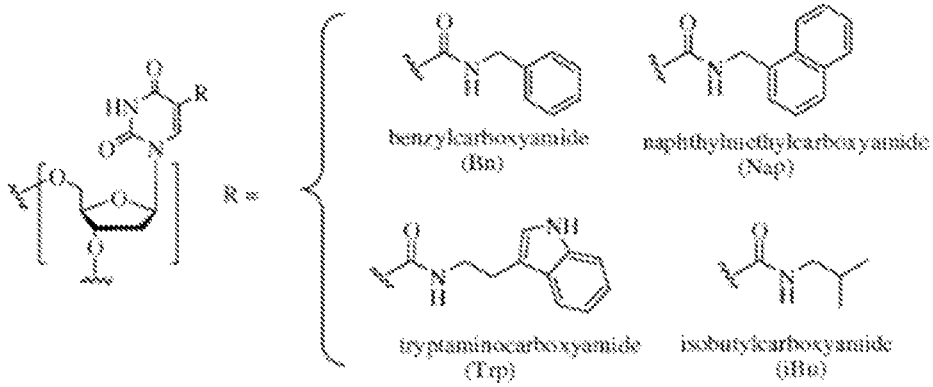
[072] Embodiments of the SELEX process in which the target is a peptide are described in U.S. Pat. No. 6,376,190, entitled "Modified SELEX Processes Without Purified Protein." In the instant case, the target is the GDF11 protein.

C. Chemically Modified Aptamers

[073] Aptamers may contain modified nucleotides that improve it properties and characteristics. Non-limiting examples of such improvements include, *in vivo* stability, stability against degradation, binding affinity for its target, and/or improved delivery characteristics.

[074] Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions of a nucleotide. SELEX process-identified aptamers containing modified nucleotides are described in U.S. Pat. No. 5,660,985, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," which describes oligonucleotides containing nucleotide derivatives chemically modified at the 5′- and 2′-positions of pyrimidines. U.S. Pat. No. 5,580,737, see supra, describes highly specific aptamers containing one or more nucleotides modified with 2′-amino (2′—NH_2), 2′-fluoro (2′-F), and/or 2′-O-methyl (2′-OMe). See also, U.S. Patent Application Publication No. 20090098549, entitled "SELEX and PHOTOSELEX," which describes nucleic acid libraries having expanded physical and chemical properties and their use in SELEX and photoSELEX.

[075] Specific examples of a C-5 modification include substitution of deoxyuridine at the C-5 position with a substituent independently selected from: benzylcarboxyamide (alternatively benzylaminocarbonyl) (Bn), naphthylmethylcarboxyamide (alternatively naphthylmethylaminocarbonyl) (Nap), tryptaminocarboxyamide (alternatively tryptaminocarbonyl) (Trp), and isobutylcarboxyamide (alternatively isobutylaminocarbonyl) (iBu) as illustrated immediately below.

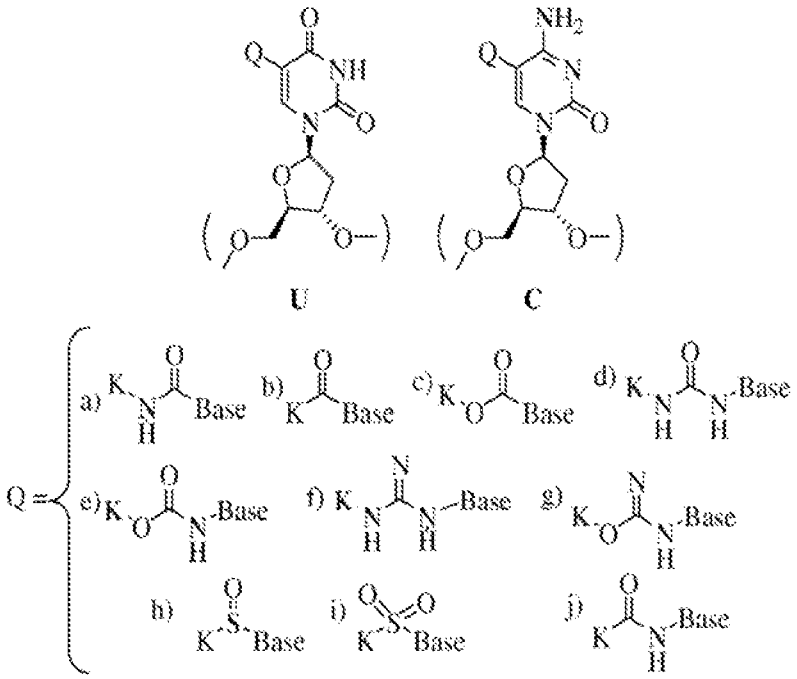


[076] Chemical modifications of a C-5 modified pyrimidine can also be combined with, singly or in any combination, 2'-position sugar modifications, modifications at exocyclic amines, and substitution of 4-thiouridine and the like.

[077] Representative C-5 modified pyrimidines include: 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamide)-2'-0-methyluridine, 5-(N-benzylcarboxyamide)-2'-fluorouridine, 5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU), 5-(N-isobutylcarboxyamide)-2'-0-methyluridine, 5-(N-isobutylcarboxyamide)-2'-fluorouridine, 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-(N-tryptaminocarboxyamide)-2'-0-methyluridine, 5-(N-tryptaminocarboxyamide)-2'-fluorouridine, 5- (N- [1 - (3 -trimethylamonium) propyl] carboxyamide)-2'-deoxyuridine chloride, 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-naphthylmethylcarboxyamide)-2'-0-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine or 5-(N-[l-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine).

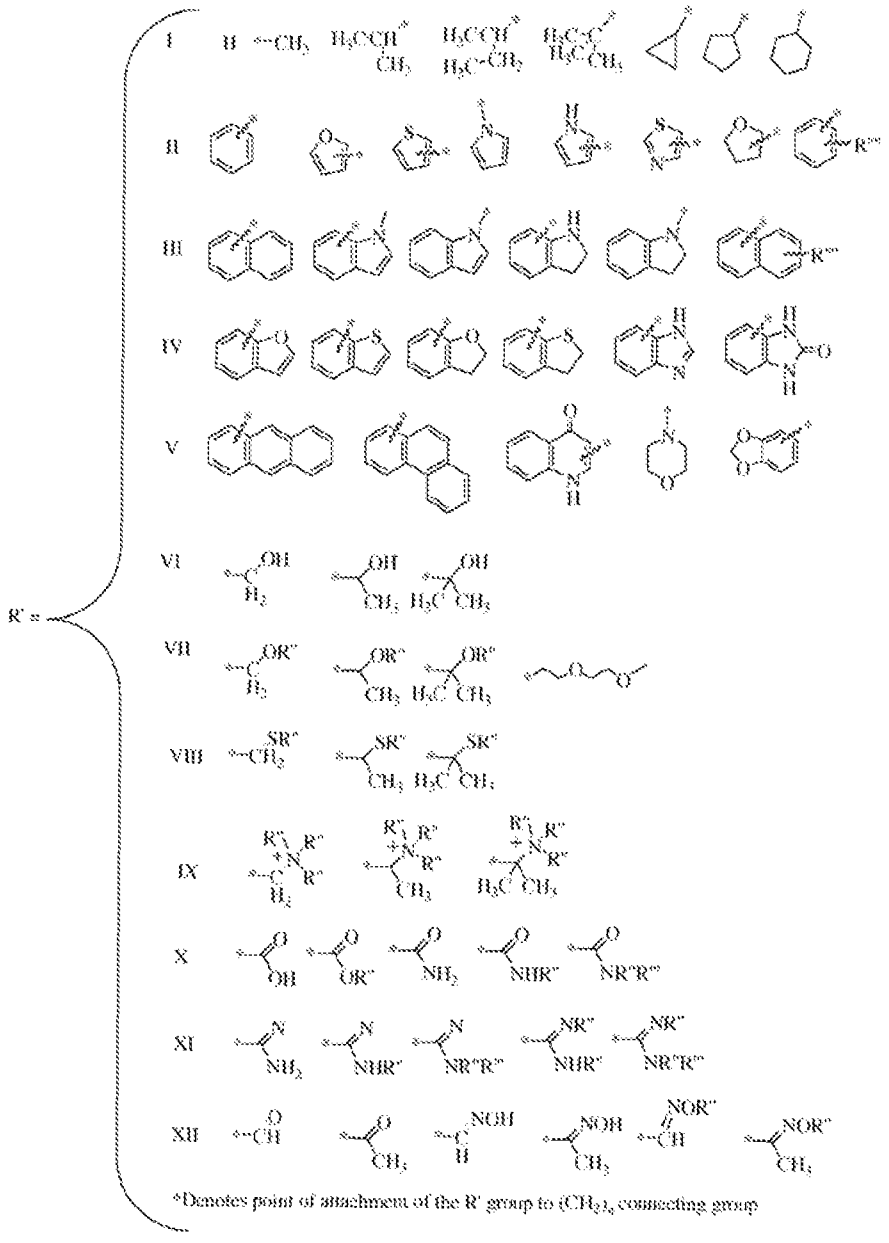
[078] If present, a modification to the nucleotide structure can be imparted before or after assembly of the polynucleotide. A sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component.

[079] Additional non-limiting examples of modified nucleotides (e.g., C-5 modified pyrimidine) that may be incorporated into the nucleic acid sequences of the present disclosure include the following:



Base = Uridine (U) or Cytidine(C) (attachment is to the 5-position)
K = R' group plus $(CH_2)_n$ connecting group, where n = 0-3

R' is defined as follows:



And, R", R"' and R"" are defined as follows:

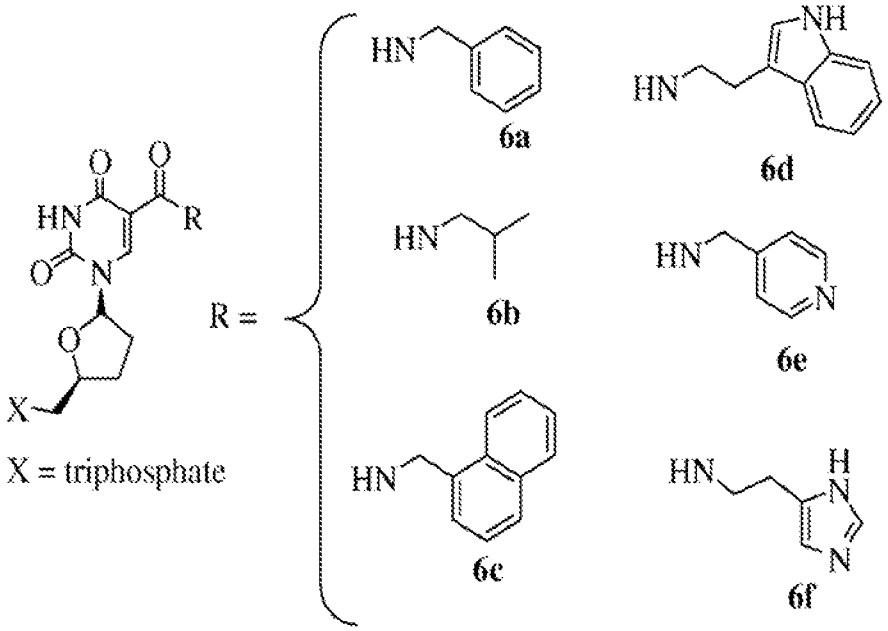
wherein

R"" is selected from the group consisting of a branched or linear lower alkyl (C1-C20); halogen (F, Cl, Br, I); nitrile (CN); boronic acid (BO_2H_2); carboxylic acid (COOH); carboxylic acid ester (COOR"); primary amide ($CONH_2$); secondary amide (CONHR"); tertiary amide (CONR"R"'); sulfonamide (SO_2NH_2); N-alkylsulfonamide (SONHR").

wherein

R", R"' are independently selected from a group consisting of a branched or linear lower alkyl (C1-C2)); phenyl (C_6H_5); an R"" substituted phenyl ring (R""C_6H_4); wherein R"" is defined above; a carboxylic acid (COOH); a carboxylic acid ester (COOR""); wherein R"" is a branched or linear lower alkyl (C1-C20); and cycloalkyl; wherein R" = R"' = $(CH_2)_n$; wherein n =2-10.

[080] Further C-5 modified pyrimidine nucleotides include the following:



[081] In some embodiments, the modified nucleotide confers nuclease resistance to the aptamer. A pyrimidine with a substitution at the C-5 position is an example of a modified nucleotide. Modifications can include backbone modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine, and the like. Modifications can also include 3' and 5' modifications, such as capping. Other modifications

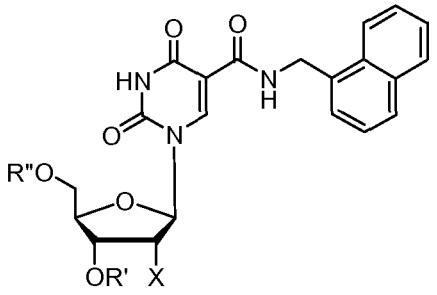
can include substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and those with charged linkages (e.g. , phosphorothioates, phosphorodithioates, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, and those with modified linkages (e.g., alpha anomeric nucleic acids, etc.). Further, any of the hydroxyl groups ordinarily present on the sugar of a nucleotide may be replaced by a phosphonate group or a phosphate group; protected by standard protecting groups; or activated to prepare additional linkages to additional nucleotides or to a solid support. The 5' and 3' terminal OH groups can be phosphorylated or substituted with amines, organic capping group moieties of from about 1 to about 20 carbon atoms, polyethylene glycol (PEG) polymers in one embodiment ranging from about 10 to about 80 kDa, PEG polymers in another embodiment ranging from about 20 to about 60 kDa, or other hydrophilic or hydrophobic biological or synthetic polymers. In one embodiment, modifications are of the C-5 position of pyrimidines. These modifications can be produced through an amide linkage directly at the C-5 position or by other types of linkages.

[082] Aptamers can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including 2'-0-methyl-, 2'-0-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, a-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. As noted above, one or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include embodiments wherein phosphate is replaced by P(0)S ("thioate"), P(S)S ("dithioate"), (0)NR_2 ("amidate"), P(0)R, P(0)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-0-) linkage, aryl, alkenyl, cycloalky, cycloalkenyl or araldyl. Not all linkages in an aptamer need be identical. Substitution of analogous forms of sugars, purines, and pyrimidines can be advantageous in designing a final product, as can alternative backbone structures like a polyamide backbone, for example.

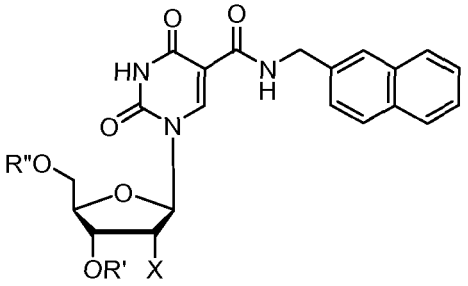
[083] Specific examples of C-5 modified pyrimidines that may be included or incorporated into an aptamer include, but are not limited to the structures herein. While the naming convention used for the structure assumes X is -H (i.e., DNA), they also encompass structures where X may be -OH (RNA), or other substituents described herein (e.g., -OCH_3; -O-allyl; -F, -OEt; -OPr, -NH_2; -azido or -$OCH_2CH_2OCH_3$), R' may -H; -OAc; -OBz; -

$OCH_2CH_2OCH_3$ and -$OSiMe_2tBu$ and R" may be -H; DMT and triphosphate (-P(O)(OH)-O-P(O)(OH)-O-P(O)$(OH)_2$), and salts thereof.

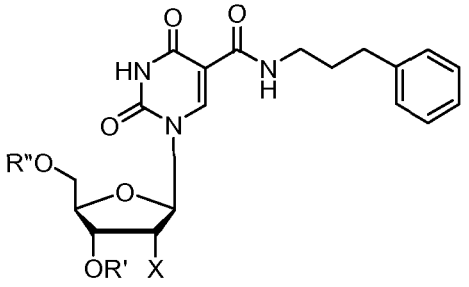
[084] Exemplary NapdU Structure (5-[N-(1-naphthylmethyl)carboxamide]-2'-deoxyuridine):



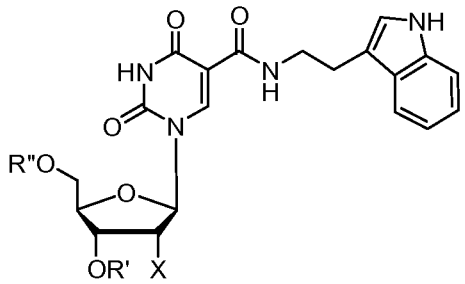
[085] Exemplary 2NapdU structure (5-[N-(2-naphthylmethyl)carboxamide]-2'-deoxyuridine):



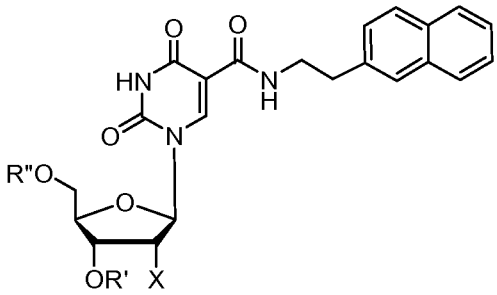
[086] Exemplary PPdU structure (5-[N-(phenyl-3-propyl)carboxamide]-2'-deoxyuridine):



[087] Exemplary TrpdU structure (5-[N-(3-indole-2-ethyl)carboxamide]-2'-deoxyuridine):



[088] Exemplary 2NEdU structure 5-[N-(2-naphthyl-2-ethyl)carboxamide]-2'-deoxyuridine):



[089] The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

D. Exemplary GDF11 Aptamers

[090] Provided herein are aptamers that bind GDF11. In some embodiments, an aptamer binds GDF11 with an affinity of less than 10 nM. In some such embodiments, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11, when both affinities are measured under the same binding conditions. In some embodiments, the aptamer binds GDF11 with an affinity of between 0.5 nM and 10 nM and binds GDF8 with an affinity of greater than 50 nM, or greater than 100 nM, or greater than 150 nM, or greater than 200 nM, or greater than 300 nM. In some embodiments, the aptamer does not bind GDF8 under the same conditions under which the aptamer binds GDF11 with an affinity of less than 10 nM. In some embodiments, the aptamer binds GDF11 with an affinity of less than 8 nM, or less than 7 nM, or less than 6 nM, or less than 5 nM, or less than 4 nM, or less than 3 nM, or less than 2 nM, or less than 1 nM; or between 0.1 nM and 10 nM, between 0.1 nM and 8 nM, or between 0.1 nM and 5 nM.

[091] In some embodiments, affinity determined using an assay described herein. In some embodiments, affinity is determined in the presence of a polyanionic inhibitor. Nonlimiting exemplary polyanionic inhibitors are described herein. In some embodiments, affinity is determined in the presence of Z block.

In some embodiments, an aptamer that binds GDF11 comprises the sequence:

5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3'(SEQ ID NO:110),

wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

each W is independently, and for each occurrence, A, T, or U;

each M is independently, and for each occurrence, A or C;

S is G or C;

each n is independently, and for each occurrence, 0 or 1; and

each m is independently, and for each occurrence, 0 or 1.

[092] In some embodiments, an aptamer that binds GDF11 comprises a sequence selected from:

a) 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 111);

b) 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 112); and

c) 5'-$RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGC$-3' (SEQ ID NO: 113);

wherein R, W, P, M, S, n, and m are defined as above.

[093] In some embodiments, if R is G, the first W is A; or if R is A, the first W is C. In some embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 n are 0. In some embodiments, each n is 0. In some embodiments, at least 1, at least 2, at least 3 n are 1. In some embodiments, at least 1 m is 1. In some embodiments, at least 1 m is 0.

[094] In some embodiments, a GDF11 aptamer is provided, wherein the aptamer comprises the sequence 5'-CPPGMPPP-3' (SEQ ID NO: 114), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, and wherein M is C or A. In some embodiments, a GDF11 aptamer is provided, wherein the aptamer comprises the sequence 5'-PPPAGC-3'(SEQ ID NO: 115), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine. In some embodiments, the aptamer comprises the sequence 5'-$CPPGMPPPN_xPPPAGC$-3' (SEQ ID NO: 116), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, wherein x is 2, 3, 4, or 5, wherein M is C or A, and wherein each N is independently, and for each occurrence, selected from A, C, G, T, and U. In some such embodiments, x is 3 or 4. In some embodiments, N_x comprises the sequence 5'-AAG-3' or 5'-ACG-3' or 5'-AGG-3'.

[095] In some embodiments, an aptamer that binds GDF11 comprises the sequence 5'-NNCPPGRPPPAMGPPPAGS-3' (SEQ ID NO: 141), wherein each P is independently, and

for each occurrence, a C-5 modified pyrimidine, R is A or G; each N is independently, and for each occurrence, A, G, or C; M is A or C; and S is G or C.

[096] In any of the embodiments described herein, each P is independently selected from the C-5 modified pyrimidines described herein. In some embodiments, each P is selected from 5-(N-1-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU),

5-(N-1-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU),

5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU),

5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU),

5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU),

5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU),

5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, and

5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine.

In some embodiments, at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 P are each independently selected from 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, and 5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine.

[097] In some embodiments, a GDF11 aptamer comprises a sequence selected from SEQ ID NOs: 12, 13, 15 to 109, and 117 to 140. In some embodiments, a GDF11 aptamer comprises a sequence selected from SEQ ID NOs: 12, 15, 26, 105 to 109, and 142 to 150.

[098] In some embodiments, a GDF11 aptamer may include up to about 100 nucleotides, up to about 95 nucleotides, up to about 90 nucleotides, up to about 85 nucleotides, up to about 80 nucleotides, up to about 75 nucleotides, up to about 70 nucleotides, up to about 65 nucleotides, up to about 60 nucleotides, up to about 55 nucleotides, up to about 50 nucleotides, up to about 45 nucleotides, up to about 40 nucleotides, up to about 35 nucleotides, up to about 30 nucleotides, up to about 25 nucleotides, and up to about 20 nucleotides.

[099] In another aspect this disclosure, the GDF11 aptamer may be at least about 95% identical, at least about 90% identical, at least about 85% identical, at least about 80% identical, or at least about 75% identical to any of SEQ ID NO:1 or 2. In another embodiment, the GDF11 aptamer includes a sequence from any of SEQ ID NOs:1 or 2, and fragments thereof. In a related aspect, the fragments thereof are from about 25 to 49 nucleotides in length (or from about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or 49 nucleotides in length).

[0100] In another aspect this disclosure, the GDF11 aptamer may a dissociation constant (K_d) for GDF11of about 10 nM or less. In another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein of about 15 nM or less. In yet another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein of about 20 nM or less. In yet another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein of about 25 nM or less. In yet another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein of about 30 nM or less. In yet another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein of about 35 nM or less. In yet another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein of about 40 nM or less. In yet another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein of about 45 nM or less. In yet another exemplary embodiment, the GDF11aptamer has a dissociation constant (K_d) for the GDF11 protein of about 50 nM or less. In yet another exemplary embodiment, the GDF11 aptamer has a dissociation constant (K_d) for the GDF11 protein in a range of about 3-10 nM (or 3, 4, 5, 6, 7, 8, 9 or 10 nM. A suitable dissociation constant can be determined with a binding assay using a multi-point titration and fitting the equation y = (max - min)(Protein)/(K_d + Protein) + min as described herein. In other embodiments, the GDF11 aptamer is an aptamer with a K_d that is less than or equal to the K_d of an aptamer comprising the sequence of SEQ ID NO: 12.

[0101] In some embodiments, an aptamer comprises a detectable label.

Methods of Detecting GDF11

[0102] In some embodiments, methods of detecting GDF11 in a sample are provided, comprising contacting the sample with an aptamer described herein. In some embodiments, methods of detecting or quantifying GDF11 in the presence of GDF8 are provided, comprising contacting the sample suspected of containing both GDF11 and GDF8 with an aptamer described herein. In some embodiments, methods of distinguishing GDF11 from GDF8 in a sample are provided, comprising contacting the sample with an aptamer described herein. In

some embodiments, the method comprises contacting the sample with a GDF11 aptamer described herein in the presence of a polyanionic inhibitor.

[0103] Detecting and/or quantifying GDF11 bound by the GDF11 aptamer can be accomplished using methods in the art and/or methods described herein. In some embodiments, the GDF11 aptamer comprises a detectable label. In some embodiments, the GDF11 aptamer is bound to a solid support, or comprises a member of a binding pair that may be captured on a solid support (for example, a biotinylated aptamer may be bound to a solid support comprising streptavidin).

Kits Comprising GDF11 Aptamer Compositions

[0104] The present disclosure provides kits comprising any of the GDF11 aptamers described herein. Such kits can comprise, for example, (1) at least one GDF11 aptamer; and (2) at least one pharmaceutically acceptable carrier, such as a solvent or solution. Additional kit components can optionally include, for example: (1) any of the pharmaceutically acceptable excipients identified herein, such as stabilizers, buffers, etc., (2) at least one container, vial or similar apparatus for holding and/or mixing the kit components; and (3) delivery apparatus.

EXAMPLES

Example 1: Selection and Identification of Aptamers Having GDF11 Binding Specificity

[0105] This example provides the representative method for the selection and production of aptamers that bind the mature GDF11 protein.

[0106] Previous attempts to select aptamers having specificity to GDF11 have been challenging. A comparison of protein binding affinity for GDF11 and myostatin (GDF8) of several aptamers selected using the SELEX process are shown in Table 1 below.

Table 1.

Aptamer Identifier	Affinity K_d (nM)		K_d Ratio
	GDF11	GDF-8	GDF11 / GDF-8
1	1.3	8.9	0.14
12	0.8	0.8	0.92
19	1.6	3.3	0.49
47	343	54.5	6.29
8	253	41.2	6.14
9	78.2	20.7	3.78
4	0.9	0.5	1.65

[0107] As shown in Table 1, the aptamers selected to bind GDF11 generally also had affinity for myostatin. The ratio of binding affinity for GDF11 to myostatin for each aptamer is also shown in Table 1. The ratios vary from below one (1) (i.e., the aptamer had greater affinity for myostatin compared to GDF11), to ratios of from about 1.6 to about 6.3 (i.e., the aptamer had a greater affinity for GDF11 compared to myostatin). However, these affinities differences are not sufficient to discriminate between GDF11 and myostatin in a protein binding assay. As a result, the presence and/or levels of GDF11 and GDF8 cannot be distinguished. In light of the challenges of identifying a GDF11 aptamer that can discriminate GDF11 from myostatin, a counter-selection strategy was introduced into the SELEX process.

Aptamer Selection with Slow Off-Rate Enrichment Process

[0108] SELEX with GDF11 was performed using the SELEX methods described herein. In addition, protocol modifications involving two different types of counter-selections were applied to select GDF11 aptamers that do not bind myostatin or have a greater affinity for GDF11 than myostatin: passive counter-selection and active count-selection.

[0109] Passive counter-selection was done with the unwanted target (myostatin) in untagged form by adding it to PCB (protein competitor buffer containing prothrombin, casein and albumin) during selection, removing sequences that lack preference for GDF11 over myostatin. The ratio of myostatin to GDF11 was 1:1 in the first round (100 pmol each), 2:1 in the second round (20 pmol myostatin and 10 pmol GDF11), and increased in subsequent rounds of SELEX as the GDF11 target concentration was reduced (20 pmol myostatin and 0.3 pmol GDF11 or about 67:1).

[0110] Active counter-selection was done with biotinylated myostatin immobilized on streptavadin (SA) beads. Prior to each round of SELEX, the library was incubated with the counter-selection beads for 10 min with shaking at 37°C. Then the supernatant was recovered and moved into selection with GDF11, leaving sequences that bind myostatin behind on the beads. The first round used 100 pmol myostatin to counter-select 1000 pmol library, each subsequent round used 20 pmol myostatin to counter-select approximately 20-50 pmol library.

Preparation of Candidate Mixture

[0111] A candidate mixture of partially randomized ssDNA oligonucleotides was prepared by polymerase extension of a DNA primer annealed to a biotinylated ssDNA template (shown in Table 2 below). The candidate mixture contained a 40 nucleotide randomized cassette containing dATP, dGTP, dCTP and 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine triphosphate (NapdUTP).

Table 2. Sequences of Template and Primers

Oligo Designation	Sequence (5′→ 3′)	SEQ ID NO:
Template 1	AB'AB'TTTTTTTTGTCTCTTGTTCTTGTCTTGTG-$(N)_{40}$-CAGGGCATCAGAGCGTTTCG	1
Primer 1	ATATATATCGAAACGCTCTGATGCCCTG	2
Primer 2	AB'AB'TTTTTTTTGTCTCTTGTTCTTGTCTTGTG	3

B' = biotin

[0112] Five milliliters of a 50% slurry of Streptavidin Plus UltraLink Resin (PIERCE) was washed once with 2.5 mL of 20 mM NaOH, twice with 2.5 mL of SB18T0.05 (40 mM HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid) buffer adjusted to pH 7.5 with NaOH, 102 mM NaCl, 5 mM KCl, 5 mM MgCl2 and 0.05% TWEEN 20) and twice with 2.5 mL of 16 mM NaCl. Eighty nanomoles of template 1 (SEQ ID NO: 1) possessing two biotin residues (designated as B' in the sequence) and 40 randomized positions (designated as N_{40} in the sequence) were added to the washed UltraLink SA beads and rotated at 37°C for 2.5 hours. The beads were then washed three times with 16 mM NaCl. Between each wash, the beads were recovered by centrifugation. The beads, now containing the captured template, were suspended in 3.75 mL of extension reaction buffer [containing 80 nmol of primer (SEQ ID NO: 2), 1X SQ20 buffer (120 mM Tris-HCl, pH7.8, 10 mM KCl, 7 mM $MgSO_4$, 6 mM $(NH_4)_2SO_4$, 0.001% BSA and 0.1% Triton X-100), 470 units of KOD XL DNA Polymerase (EMD MILLIPORE), and 1 mM each of dATP, dCTP, dGTP and NapdUTP. The beads were allowed to incubate with rotation at 68°C for 2 hours. The beads were then washed once with SB18T0.01 (SB18 as above, but with 0.01% TWEEN-20) and twice with 16 mM NaCl. The aptamer library was eluted from the beads with 2.5 mL of 40 mM NaOH. The eluted library and immediately neutralized with 50 µL of neutralizer (700 mM HCl, 180 mM HEPES, 0.45% TWEEN-20). Elution was repeated twice more, and the eluates were pooled. The library was concentrated with an AMICON Ultracel YM-10 filter to approximately 0.4 mL and the concentration of library determined by ultraviolet absorbance spectroscopy.

Biotin labeling of human GDF11

[0113] Untagged recombinant human GDF11-Protein purified in homodimeric form after over-expression in *E. coli* (Peprotech, catalog number 120-11) was biotinylated by covalent coupling of NHS-PEO4-biotin (PIERCE, EZ-Link NHS-PEG4-Biotin) to residues containing primary amines. Protein (1600 pmol in 80 µL) was mixed with a 4-fold molar excess of NHS-PEG4-biotin and the reaction was allowed to incubate at 20°C for 1 hour.

After the reaction was completed, unreacted NHS-PEG4-biotin was removed using a Zeba™ spin desalting column (PIERCE) where the buffer had been exchanged to SB18T0.05.

Biotin labeling of human myostatin

[0114] Untagged recombinant human myostatin-Protein purified in homodimeric form after over-expression in *E. coli* (Peprotech, catalog number 120-00) was biotinylated as described above.

Immobilization of myostatin

[0115] MYONE-SA paramagnetic beads (MYONE SA, INVITROGEN, or hereinafter referred to as SA beads) were prepared by washing the beads once with 14 mL of 20 mM NaOH and twice with 14 mL of SB18T0.05. Finally, the SA beads were suspended at 10 mg/mL in SB18T0.05 and stored at 4°C until use. Biotin labeled myostatin-protein (500 pmol) was immobilized on for all rounds of SELEX. This was accomplished by mixing SA beads (5 mgs) with biotin labeled myostatin (500 pmol) for 30 min with shaking, followed by washing three times with SB18T0.05. The SA-myostatin beads were resuspended in 0.5 mL SB18T0.05 (10 mgs/mL beads with 1 μM myostatin attached) and stored at 4°C until use.

Aptamer Selection with Slow Off-Rate Enrichment Process

[0116] A total of 11 rounds of the SELEX process were completed with selection for affinity and slow off-rate. Prior to each round a counter selection was performed to reduce background and to reduce the likelihood of obtaining aptamers with nonspecific binding to protein. In addition, separate types of counter-selections were applied to force selection of GDF11 aptamers that do not bind the closely related, 90% identical protein myostatin. Counter selections were performed as follows.

[0117] For round 1, 100 μL of the DNA candidate mixture containing approximately 1 nmole of DNA in SB18T0.05 was heated at 95°C for 5 minutes and then cooled to 70°C for 5 minutes, then to 48°C for 5 minutes and then transferred to a 37°C block for 5 minutes. The sample was then combined with 10 μL of protein competitor mixture and 1 mg (100 μL) of counter-selection beads and incubated at 37°C for 10 minutes with mixing. Beads were removed by magnetic separation. The protein competitor mixture and the counter-selection beads were composed as follows. Standard SELEX used 10 μL of standard protein competitor mixture (0.1% HSA, 10 μM casein, and 10 μM prothrombin in SB18T0.05), and 1 mg (100 μL) of SA beads. SELEX with passive counter-selection used 10 μL of a modified protein competitor mixture (0.1% HSA, 10 μM casein, 10 μM prothrombin, and 10 μM unlabeled myostatin in SB18T0.05) and 1 mg (100 μL) of SA beads. SELEX with active counter-selection used 10 μL of standard protein competitor mixture (0.1% HSA, 10 μM

casein, and 10 μM prothrombin in SB18T0.05), and 1 mg (100 μL) of SA-myostatin beads (SA beads with 1 μM myostatin attached).

[0118] For Rounds 2-11, a 68 μL aliquot of the DNA candidate mixture obtained from the previous round (68% of eDNA obtained from previous round) was mixed with 13 μL of 5x SB18T0.05. The sample was heated to 95°C for 3 minutes and cooled to 37°C at a rate of 0.1°C /second. The sample was then combined with 9 μL of protein competitor mixture (0.1% HSA, 10 μM casein, and 10 μM prothrombin in SB18T0.05), and 0.1 mg (10 μL) of SA beads and incubated at 37°C for 10 minutes with mixing (standard SELEX). As in round 1, the protein competitor mixture was supplemented with 10 μM unlabeled myostatin (SELEX with passive counter-selection), and the SA-myostatin beads (SA beads with 1 μM myostatin attached) were used instead of SA beads (SELEX with active counter-selection). Beads were removed by magnetic separation.

[0119] Following the first counter selection the target protein was pre-immobilized on SA beads for the Round 1 selection process. To accomplish this, 0.5 mg of SA beads was mixed with 50 pmoles of biotin labeled target protein (GDF11 homodimer) and incubated with shaking for 30 minutes at 37°C. Unbound target was removed by washing the beads twice with SB18T0.05. The counter-selected-DNA candidate mixture (100 μL) was added to the beads and incubated at 37°C for 60 minutes with mixing. No slow off-rate enrichment process was employed in the first round and beads were simply washed 5 times for 2 min each with 100 μL SB18T0.05. Following the washes, the bound aptamer was eluted from the beads by adding 45 μL of 8 mM NaOH, and incubating at 37°C for 5 minutes with mixing. The aptamer-containing-eluate (40 μL) was transferred to a new tube after magnetic separation of the beads. Elution was repeated once more with 45 μL of 8 mM NaOH, and incubating at 37°C for 5 minutes with mixing. The eluates were combined (80 uL) and the solution neutralized by addition of 20 μL of 32 mM HCl and 2 μL Tris-HCl pH 7.5.

[0120] For Rounds 2-11, selections were performed with the DNA candidate mixture and target protein as described below while, in parallel, an identical selection was performed with the DNA candidate mixture, but without the target protein. Comparison of the Ct values obtained from PCR for the sample with target protein (signal S) and sample without target protein (background B) were used as a guide to reduce the target concentration in the next round. If the delta Ct value was greater than 4, but less than 8, the target protein was reduced three fold in the next round. If the delta Ct value was greater than 8, the target was reduced 10-fold in the next round.

[0121] Following these scheme, reductions in GDF11 concentrations after the rounds of standard SELEX were 3-fold (Rounds 5-6), 10-fold (Rounds 7-8), 30-fold (Rounds 9-10), and 100-fold (Round 11). For selections with passive counter-selection, reductions in target concentration were 3-fold (Rounds 5-6), 10-fold (Rounds 7-10), and 30-fold (Round 11). For selections with active counter-selection, reductions in target concentration were 3-fold (Rounds 5-6), 10-fold (Round 7), 30-fold (Round 8-10), and 100-fold (Round 11). The "RX", where X is a number, represents the round number for SELEX (e.g., R3 indicates Round 3 for SELEX).

		Reduction of Target Concentration after Round of SELEX (based on delta Ct)								
Target	Counter-selection	R3	R4	R5	R6	R7	R8	R9	R10	R11
GDF-11	standard	1	1	3	3	10	10	30	30	100
GDF-11	passive	1	1	3	3	10	10	10	10	30
GDF-11	active	1	1	3	3	10	30	30	30	100

[0122] For Round 2, labeled target protein (5 pmoles of GDF11 homodimer in 10 µL) was mixed with 40 µL of counter selected DNA candidate mixture and incubated at 37°C for 15 minutes. A slow off-rate enrichment process was begun by adding 50 µL of 10 mM dextran sulfate followed by the immediate addition of 0.1 mg of SA beads. This was allowed to incubate for 15 minutes at 37°C with mixing. Beads were then washed 5 times with 100 µL of SB18T0.05. The aptamer strand was eluted from the beads by adding 105 µL of sodium perchlorate elution buffer (1.8 M NaClO4, 40 mM PIPES pH 6.8, 1 mM EDTA, 0.05% Triton X-100), and incubating at 37°C for 10 minutes with mixing. Beads were removed by magnetic separation and 100 µL of aptamer eluate was transferred to a new tube containing primer capture beads (25 µL of 2.5 mg/mL SA beads with 12.5 pmol primer 2 (SEQ ID NO: 2) attached) and allowed to incubate for 30 minutes at 50°C with mixing. Beads were then washed three times with 100 µL of SB18T0.05. The aptamer strand was eluted from the beads by adding 85 µL of 40 mM NaOH, and the eluate (80 µL) was neutralized with 20 µL of 160 mM HCl and 2 µL of 500 mM Tris-HCl pH 7.5.

[0123] Rounds 3 through 11 selections were performed as described for Round 2 except dextran sulfate was added 15 minutes (rounds 3-4), 30 minutes (round 5), 45 minutes (round 6), 60 minutes (round 7), 75 min (round 8), or 90 minutes (rounds 9-11) prior to the addition of beads. Partitioning was done with 0.1 mg Neutravidin Coated Sera-Mag SpeedBeads (Fisher Scientific, Catalog No. 09-981-155, or hereinafter referred to as NA beads, rounds 3, 5, 7, 9, 11) or with 0.1 mg of SA beads (rounds 4, 6, 8, 10).

[0124] Pools obtained after 11 rounds of SELEX showed good activity to warrant Ion Torrent ePCR and sequencing (Table 3). From each pool, 384 sequences were obtained for comparative sequence analysis followed by binding assays of the lead candidates.

Table 3. Affinity data for GDF11 aptamer pools obtained with standard SELEX, and with passive or active counter-selection with myostatin.

Target Protein	SELEX method	Counter-selection		NapdU		TrpdU	
		Passive	Active (beads)	Pool	K_d (nM)	Pool	K_d (nM)
GDF11	Standard	PCB	SA	12058	0.66	12076	0.29
GDF11	Modified	PCB + myostatin	SA	12060	0.63	12078	0.37
GDF11	Modified	PCB	SA/myostatin	12061	0.54	12080	0.58

Aptamer Amplification and Purification

[0125] Selected aptamer DNA from each round was amplified and quantified by QPCR. 48 µL DNA was added to 12 µL QPCR Mix (10X KOD DNA Polymerase Buffer; Novagen #71157, diluted to 5X, 25 mM $MgCl_2$, 10 µM forward PCR primer (Primer 1, SEQ ID NO:2), 10 µM biotinylated reverse PCR primer (Primer 2, SEQ ID NO:3), 5X SYBR Green I, 0.075 U/µL KOD XL DNA Polymerase, and 1 mM each dATP, dCTP, dGTP, and dTTP) and thermal cycled in A BIO-RAD MyIQ QPCR instrument with the following protocol: 1 cycle of 96°C for 15 seconds, 55°C for 10 seconds, and 68°C for 30 minutes; followed by 30 cycles of 96°C for 15 seconds, 68°C for 1 minute. Quantification was done with the instrument software and the number of copies of DNA selected, with and without target protein, was compared to determine signal/background ratios.

[0126] Following amplification, the PCR product was captured on SA beads via the biotinylated antisense strand. 1.25 mL SA beads (10 mg/mL) were washed once with 14 mL 20 mM NaOH, twice with 14 mL SB18T0.05, resuspended in 1.25 mL 3 M NaCl + 0.05% TWEEN, and stored at 4°C. 25 µL SA beads (10 mg/mL in 3 M NaCl) were added to 50 µL double-stranded QPCR products and incubated at 25°C for 5 minutes with mixing. The "sense" strand was eluted from the beads by adding 100 µL of 40 mM NaOH, and incubating at 37°C for 5 minutes with mixing. The eluted strand was discarded and the beads were washed twice with SB18T and once with 16 mM NaCl.

[0127] Aptamer sense strand containing NapdUTP was prepared by primer extension from the immobilized antisense strand. The beads were suspended in 40 µL primer extension reaction mixture (1X Primer Extension Buffer (120 mM Tris-HCl pH 7.8, 10 mM KCl, 7 mM $MgSO_4$, 6 mM $(NH_4)_2SO_4$, 0.1% TRITON X-100 and 0.001% bovine serum albumin), 2.5 µM forward primer (Primer 1, SEQ ID NO: 2), 0.5 mM each dATP, dCTP, dGTP, and NapdUTP, and 0.015 U/µL KOD XL DNA Polymerase) and incubated at 68°C for 60 minutes with mixing. The beads were washed 3 times with SB18T0.05, and the aptamer strand was eluted from the beads by adding 45 µL of 40 mM NaOH, and incubating at 37°C for 5 minutes with mixing. 40 µL aptamer eluate was transferred to a new tube after magnetic separation, and elution was repeated once more with 45 µL of 40 mM NaOH, and incubating at 37°C for 5 minutes with mixing. The eluates were combined (80 uL) and the solution neutralized with 20 µL of 160 mM HCl and buffered with 10 µL of 0.1 M HEPES, pH 7.5.

Selection Stringency and Feedback

[0128] The relative target protein concentration of the selection step was lowered each round in response to the QPCR signal (Δ Ct) following the rule below:

If $\Delta Ct < 4$, $[P]_{(i+1)} = [P]_{(i)}$

If $4 \leq \Delta Ct < 8$, $[P]_{(i+1)} = [P]_{(i)} / 3.2$

If $\Delta Ct \geq 8$, $[P]_{(i+1)} = [P]_{(i)} / 10$

Where [P] = protein concentration and i = current round number.

[0129] After each selection round, the convergence state of the enriched DNA mixture was determined. 10 µL double-stranded QPCR product was diluted to 200 µL with 4 mM $MgCl_2$ containing 1X SYBR Green I. Samples were analyzed for convergence using a Cot analysis which measures the hybridization time for complex mixtures of double stranded oligonucleotides. Samples were thermal cycled with the following protocol: 3 cycles of 98°C for 1 minute, 85°C for 1 minute; 2 cycles of 98°C for 1 minute, then 85°C for 30 minutes. During the 30 minutes at 85°C, fluorescent images were measured at 5-second intervals. The fluorescence intensity was plotted as a function of the logarithm of time, and an increased rate of hybridization with each SELEX round was observed, indicating sequence convergence.

Enriched Pool Sequencing & Aptamer Identification

[0130] After 11 rounds of SELEX, the converged pool was sequenced. Sequence preparation was performed as follows. The pool was amplified by PCR using SELEX library-specific primers containing a unique barcode/index sequence (unique sequence identifier for each pool). Individual PCR products were quantified using a Quant-iT™ PicoGreen® dsDNA Reagent (LIFE TECHNOLOGIES) assay, combined at equimolar concentrations, and

concentrated/buffer exchanged using an AMICON Ultra-0.5 Centrifugal Filter Device (MILLIPORE). The mixture was then purified by SDS-polyacrylamide gel electrophoresis (PAGE), and the eluate concentrated using an Amicon Ultra-0.5 Centrifugal Filter Device and visualized by PAGE to confirm the size, purity and yield of the final mix. The sample was submitted to SeqWright Genomic Services (GE HEALTHCARE, Houston, TX) for Ion Torrent PGM sequencing. From a sequence pool containing over 40,000 sequences, 384 were randomly selected and analyzed for convergence using custom software that determines sequence counts/copy number and identifies common convergence patterns using a local-alignment algorithm. Sequences with the greatest representation/copy number in the pool and at least one sequence from every convergence pattern were chosen for further characterization. Of note, those sequence reads from the SELEX aptamer pools that had a sequence quality score that was "low" (i.e., a Phred quality score of 20 or below) were removed from the sequence analysis and motif identification.

Aptamer Identification by Functional Assay and by Subtractive Sequence Analysis

[0131] Convergence pattern 1 was originally identified from sequences 12060-28_3 (SEQ ID NO:12) and 12060-16_3 (SEQ ID NO:13) via comparative binding assays with GDF11 and myostatin (see Example 2). The letter "P" in the sequences indicate a NapdU.

Aptamer ID 12060-28_3:

5' - ccctgCGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAPcacaa - 3' (SEQ ID NO: 12)

Aptamer ID 12060-16_3:

5' - ccctgPGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGGAACCAcacaa - 3' (SEQ ID NO: 13)

[0132] In parallel, Convergence pattern 1 was identified *in silico* via comparative and subtractive sequence analysis of sequences from pools obtained with different SELEX variations. These included 768 sequences from pool 12058 (GDF11 standard SELEX) , 384 sequences from pool 12060 (GDF11 SELEX with passive counter-selection), 384 sequences from pool 12061 (GDF11 SELEX with active counter-selection), and 384 sequences from pool 12059 (myostatin standard SELEX). Analysis and alignments of all sequences in these combined pools was used to de-prioritize sequences with common patterns present in all pools, indicative of non-discriminating binding of GDF11 and myostatin (see Table 4). Convergence pattern 1 was highly enriched in pool 12060 from GDF11 SELEX with passive counter-selection (15%) and had lower abundance in pool 12058 from GDF11 standard SELEX (3%)

and was absent in pool 12059 from myostatin standard SELEX, indicative of GDF11 specificity.

Table 4.

Pattern	Pool 12058 (GDF11, standard SELEX)		Pool 12060 (GDF11, passive counter-selection)		Pool 12061 (GDF11, active counter-selection)		Pool 12059 (Myostatin, standard SELEX)	
	# seq	% seq	# seq	% seq	# seq	% seq	# seq	% seq
A	195	25.4	18	4.7	29	7.6	15	3.9
B	137	17.8	17	4.4	82	21.4	54	14.1
C	57	7.4	14	3.6	3	0.8	11	2.9
D	49	6.4	14	3.6	5	1.3	0	0.0
Pattern 1	24	3.1	57	14.8	0	0.0	0	0.0
E	21	2.7	0	0.0	1	0.3	1	0.3
F	15	2.0	0	0.0	0	0.0	6	1.6
G	1	0.1	0	0.0	6	1.6	4	1.0

Example 2: Sandwich SELEX for Aptamers the bind GDF11

[0133] A sandwich SELEX assay was used to identify additional GDF11-specific aptamers, using a 2NEdU-containing aptamer that binds GDF11. Sandwich SELEX is described in WO 2015/048084. Briefly, SELEX is performed using a target-aptamer complex (i.e., GDF11 complexed with the 2NEdU-containing aptamer). It was found that some of the aptamers identified in the NapdU pool screened using sandwich SELEX are similar to aptamer 12060-28. Those clones are discussed further below.

Example 3: Equilibrium Binding Constant (K_d) for Aptamers to GDF11 Protein Versus Myostatin

[0134] This example provides the protein binding affinities (K_d) for aptamer-GDF11 and aptamer-Myostatin protein, and the identification of aptamers that preferentially bind GDF11 over myostatin.

[0135] Several aptamer clones selected via the counter-selection SELEX method and via the sandwich SELEX method described herein were further characterized and selected for preferential binding to GDF11 over myostatin (see Table 5). Binding assays were done in the absence and in the presence of 1 μM Z-block (random oligonucleotide sequence) as a non-specific competitor. An aptamer having BndU as the C-5 modified pyrimidine (BndU GDF11 aptamer), which was previously selected via SELEX without counter-selection steps and was used as a control for binding to both GDF11 and myostatin. Previous binding experiments with the BndU GDF11 aptamer showed that it is generally non-discriminatory for GDF11 and myostatin.

Table 5. Aptamer Binding Affinity Comparison: GDF11 and Myostatin

Aptamer ID	Counter-selection SELEX Method	Mod. base	GDF11	Myostatin	K_d ratio
			K_d (nM)	K_d (nM)	
BndU GDF11 Aptamer	Standard	BndU	0.05	0.11	2.29
12058-6	Standard	NapdU	0.09	0.12	1.32
12060-15	Passive (myostatin in buffer)	NapdU	0.20	0.85	4.22
12060-41		NapdU	0.37	1.81	4.96
12060-105		NapdU	0.19	0.32	1.74
12060-28		NapdU	4.17	>320	>100
12060-16	Active (myostatin on beads)	NapdU	6.52	>320	>50
12061-46		NapdU	0.22	0.20	0.88
12061-5		NapdU	0.43	0.30	0.70
12061-12		NapdU	0.14	0.11	0.81
12076-80	Standard	TrpdU	0.90	0.77	0.85
12076-15		TrpdU	0.26	0.36	1.36
12076-349		TrpdU	0.78	1.22	1.57
12078-15	Passive	TrpdU	0.18	0.62	3.54
12078-44		TrpdU	0.50	0.24	0.49
12080-1	Active	TrpdU	0.32	0.55	1.71
16139-122	Sandwich SELEX	NapdU	2.23	>320	>100
16139-12		NapdU	1.7	>320	>100
16139-178		NapdU	1.08	>320	>100
16139-198		NapdU	0.62	>320	>100
16139-19		NapdU	1.7	>320	>100
16139-30		NapdU	0.92	>320	>100

16139-37		NapdU	1.21	>320	>100
16139-3		NapdU	1.58	>320	>100

[0136] Aptamer pool 12060 (NapdU aptamers), which had been obtained by SELEX with passive counter-selection, contained several nucleotide sequences with affinity, and a varying degree of selectivity, for GDF11 (see K_d ratio where a larger number indicates greater specificity for GDF11 over myostatin). In particular, aptamer clones 12060-16_3 (NapdU) showed about a 50-fold differential K_d for GDF11 over myostatin (i.e., K_d of 6.52 nM *vs.* >320 nM) when tested in the presence of 1 μM Z-block , and 12060-28_3 (NapdU), which made up 9% of the 12060 aptamer pool of nucleotide sequences showed about a 100-fold differential K_d for GDF11 over myostatin (i.e., K_d of 1.74 nM *vs.* >320 nM) when tested in the presence of 1 μM Z-block (see Figure 1). Aptamer pool 16139 (NapdU), which was obtained by sandwich SELEX, also contained several nucleotide sequences with high affinity and specificity for GDF11.

[0137] The other aptamer clones of Table 5 showed affinity for GDF11 with a range of affinity for myostatin. For example, aptamer pool 12058 (NapdU) from standard SELEX was dominated by sequence 12058-6 (17%). This aptamer clone had a K_d of 0.09 nM for GDF11, but also had a K_d of 0.12 nM for myostatin (Figure 2).

Example 4: Post-SELEX Truncation and Analysis of GDF11 Aptamer 12060-28

[0138] This example provides post-SELEX the GDF11 binding affinities for truncated sequences of the 12060-28_3 aptamer and the identification of a core minimal sequence length that is capable of binding to GDF11.

[0139] Aptamer 12060-28 (NapdU) truncations indicated that the 20-mer sequence 5' - GACPPGCPPPAAGPPPAGCC - 3' (Aptamer 12060-28_37; SEQ ID NO: 156) was capable of binding GDF11. However, the activity of the 21-mer sequence 5'-GGACPPGCPPPAAGPPPAGCC-3' (Aptamer clone 12060-28_36; SEQ ID NO: 157) showed relatively better binding to GDF11 (K_d of 4.43E-10) (see Figure 3). This is consistent with the conserved motif CPPGCPPPANGPPPAGC (SEQ ID NO:105) found in a family of active clones related to 12060-28, *i.e.* only the NapdU residues within positions 12-24 of the 40-mer central region (motif sequence) appear to be needed for binding.

Example 5: Specific GDF11 Aptamer Sequences

[0140] This example provides a sequence alignment of a lead GDF11 selective aptamer binder (12060-28_3) with other aptamer sequences that were selected via the SELEX process. This resulted in the identification of the motif sequence in multiple sequences in the pool of

sequences selected via SELEX. Affinity data is further provided for select sequences. See Table 6 for the alignment of sequences (50-mers) and binding affinity. Table 7 provides additional sequences from the aptamer pools 12060 and 12058 and their alignment. Sequences in Table 7 are shown with five (5) nucleotides removed on each of the 5' and 3' ends of the sequence (40-mers). Both Tables 6 and 7 provide the basis for a "core sequence" motif shared among the sequences. This sequence may be GACPPGAPPPACGPPPAGC (SEQ ID NO: 106); GACPPGAPPPAAGPPPAGC (SEQ ID NO: 107); GACPPGCPPPAAGPPPAGC (SEQ ID NO: 108); GACPPGCPPPACGPPPAGC (SEQ ID NO: 109); GCCPPGAPPPACGPPPAGC (SEQ ID NO: 142); CACPPGAPPPACGPPPAGG (SEQ ID NO: 143); GACPPGGPPPACGPPPAGC (SEQ ID NO: 144); GCCPPGCPPPACGPPPAGC (SEQ ID NO: 145); GCAPPGAPPPACGPPPAGC (SEQ ID NO: 146); GGCPPGCPPPACGPPPAGA (SEQ ID NO: 147); CGCPPGAPPPAAGPPPAGG (SEQ ID NO: 148); AACPPGAPPPAAGPPPAGG (SEQ ID NO: 149); or GACPPGAPPPAGGPPPAGC (SEQ ID NO: 150); where in each instance P is a NapdU. In some embodiments, the conserved motif is NNCPPGRPPPAMGPPPAGS (SEQ ID NO: 141), where in each instance P is a NapdU, R is A or G; each N is independently, and for each occurrence, A, G, or C; M is A or C; and S is G or C. It was found that sequence 12060-209_3 in Table 6, which contains a cytosine in place of one of a conserved NapdU in 12060-28, does not bind GDF11.

Table 6. Sequence Alignment and affinity for GDF-11 specific aptamer 12060-28 (Napd U identified by "P" in the sequences), and related clones. Affinity was determined in filter binding assays in the presence of 0.1 μM Z-block. Sequences are shown full-length (50-mers). The "x" of the motif sequence may be an A or C nucleotide.

Aptamer ID	Nucleotide Sequence (5' to 3') (P is a NapdU)	SEQ ID NO:	GDF11 (K_d)	Myostatin (K_d)
12060-28_3	ccctgCGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAPcacaa	12	1.7 nM	>320 nM
12060-209_3	ccctgCGCCPPCGGACPPGCPPCAAGPPPAGCCGCPPGCPCACAPcacaa	14	>320 nM	>320 nM
12060-300_3	ccctgCGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAAcacaa	15	0.99 nM	>320 nM
12060-32_3	ccctgPGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAPcacaa	16	1.45 nM	>320 nM
12060-153_3	ccctgPGCCPPCAGACPPGCPPPAAGPPPAGCCGCPPGCPCACAPcacaa	17	ND	ND
12060-43_3	ccctgGGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAPcacaa	18	ND	ND
12060-130_3	ccctgCGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACGPcacaa	19	0.61 nM	>320 nM
12060-116_3	ccctgCGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPPACAPcacaa	20	1.14 nM	>320 nM
12060-238_3	ccctgCGCCPPCGGACPPGCPPPACGPPPAGCCGCPPGCPCACAPcacaa	21	3.4 nM	>320 nM
12060-204_3	ccctgCGCCPPCGGACPPGCPPPAAGPPPAGCCGCPCGCPCACAPcacaa	22	0.94 nM	>320 nM
12060-4_3	ccctgCGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGPPCACAPcacaa	23	0.90 nM	>320 nM
12060-16_3	ccctgPGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGGAACCAcacaa	13	6.5 nM	>320 nM
12058-386_3	ccctgGPPGACAAGGPACPGGACPPGAPPPACGPPPAGCCPCAAGcacaa	24	6.7 nM	>320 nM
12058-130_3	ccctgAPGACCPAGGACPPGCPPPACGPPPAGCACGPCGAACAGGcacaa	25	ND	ND
16139-288_3	ctgtgCGPACPGACPPGAPPPACGPPPAGCGPGCCGAGGCcacaa	117	ND	ND
16139-102_3	ctgtgGAGPCPCCCGACPPGAPPPACGPPPAGCGPGCGGAGGCcacaa	158	ND	ND
16139-85_3	ctgtgPCCACPCAGCCGACPPGAPPPACGPPPAGCGPGCGGAGGCcacaa	159	ND	ND
16139-12_3	ctgtgACPAAPGPGACCGGAGACPPGCPPPACGPPPAGCGACCPGcacaa	120	1.7 nM	>320 nM
16139-45_3	ctgtgAPGCCGPGGACPPGCPPPACGPPPAGCCPCGCGGGGAGCPcacaa	121	ND	ND
16139-258_3	ctgtgAGAGGAAAPACGCGCCPPGAPPPACGPPPAGCPGCGAPAAcacaa	122	ND	ND
16139-227_3	ctgtgAGCGPACCPCGCACPPGAPPPACGPPPAGCGPGCGGAGGCcacaa	123	ND	ND
16139-178_3	ctgtgACCPPCACCCGPGACPPGGPPPACGPPPAGCGGGPAGGPcacaa	124	1.08 nM	>320 nM
16139-3_3	ctgtgGACPGAGPPGGAPGGGACPPGAPPPAAGPPPAGCCCGCAAcacaa	125	1.58 nM	>320 nM
16139-20_3	ctgtgGAAPGGCGACPPGAPPPAAGPPPAGCGCPACCCCCAAPPGcacaa	126	ND	ND
16139-21_3	ctgtgPPAGGPGCCPPGCPPPACGPPPAGCCCPACGAAACGCCPGcacaa	127	ND	ND
16139-126_3	ctgtgAACPPCGAPAPCGCACPPGAPPPACGPPPAGGCGGPGPCAcacaa	128	ND	ND
16139-365_3	ctgtgAGAGPAAAPACGCGCAPPGAPPPACGPPPAGCGPGCGGAGGCcacaa	129	ND	ND
16139-119_3	ctgtgPCPAGGAGCGGCPPGCPPPACGPPPAGACGGACCCCACGAcacaa	130	ND	ND

16139-30_3	ctgtgAGPGPACCPCGCGCPPGAPPPACGPPPAGGCGAGGCAGGCcacaa	131	0.92 nM	>320 nM
16139-198_3	ctgtgAACPPGAPPPAAGPPPAGGCACAPACACCACPCCGGACGA	132	0.62 nM	>320 nM
16139-19_3	ctgtgGPPAACGACGACPPGAPPPACGPPPAGCGGCACCPPACACCaacaa	133	1.70 nM	>320 nM
16139-195_3	ctgtgPCCACPCAGCCGACPPGAPPPACGPPPAGCGGCACCPPACACCacaa	134	ND	ND
16139-360_3	ctgtgCGPACPGACCPPGAPPPACGPPPAGCGGCACCPPACACCacaa	135	ND	ND
16139-122_3	ctgtgCGPACPGACCPPGAPPPACGPPPPAGCGAPGCAPGCPPGAGCcacaa	136	2.23 nM	>320 nM
16139-37_3	ctgtgCPAAGACPGCGGACCPPGAPPPAGGPPPAGCCGCAAGAAGPcacaa	137	1.21 nM	>320 nM
12060-28_38	ccctgCGCCPPCGGACPPGAPPPAAGPPPAGCCGCPPGCPCACAPcacaa	138	1.1 nM	>320 nM
12060-28_39	ccctgCGCCPPCGGACPPGGPPPAAGPPPAGCCGCPPGCPCACAPcacaa	139	1.2 nM	>320 nM
12060-28_40	ccctgCGCCPPCGGACPPGCPPPAGGPPPAGCCGCPPGCPCACAPcacaa	140	1.4 nM	>320 nM
Motif	GACPPGxPPPAxGPPPAGC	26		

Table 7. Sequence Alignment for 12060-28 (Napd U identified by "P" in the sequences), and additional related clones. Sequences are shown truncated (40-mers). The "x" of the motif sequence may be an A or C nucleotide.

Aptamer ID	Nucleotide Sequence (5' to 3') (P is a NapdU)	SEQ ID NO:
12060-28	-CGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAP-----	27
12060-209	-CGCCPPCGGACPPGCPPCAAGPPPAGCCGCPPGCPCACAP-----	28
12060-4	-CGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGPPCACAP-----	29
12060-130	-CGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACGP-----	30
12060-204	-CGCCPPCGGACPPGCPPPAAGPPPAGCCGCPCGCPCACAP-----	31
12060-116	-CGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPPACAP-----	32
12060-300	-CGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAA-----	33
12060-32	-PGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAP-----	34
12060-43	-GGCCPPCGGACPPGCPPPAAGPPPAGCCGCPPGCPCACAP-----	35
12060-153	-PGCCPPCAGACPPGCPPPAAGPPPAGCCGCPPGCPCACAP-----	36
12060-238	-CGCCPPCGGACPPGCPPPACGPPPAGCCGCPPGCPCACAP-----	37
12058-111	PPGACCPAGGACPPGCPPPACGPPPAGCACGPCGAACAGG------	38
12058-130	APGACCPAGGACPPGCPPPACGPPPAGCACGPCGAACAGG------	39
12058-714	APGACCPAGGAPPPGCPPPACGPPCAGCACGPCGPACAGG------	40
12058-284	APGACCPAGGACPPGCPPPACGPPPAGCACGPCGAAPCGG------	41
12058-215	APGACCPGGGACPPGCPPPACGPPPAGCACGPCGAACAGG------	42
12058-35	APGAPCPGGGACPPGCPPPACGPPPAGCACGPCGAACAGG------	43
12058-59	PPGACCPAGGACPPGCPPPACGPPPAGCAPGPGGAACAGG------	44
12058-546	APGACCPAGGACPPGCPPPACGPPPAGCAGGPCGAACAGG------	45
12060-313	------PGAGACPPGACPPACGPPPAGCPGCPAACAPGGGGAACCA	46
12060-16	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGGAACCA	47
12060-133	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGGPACCA	48
12060-90	------PGAGACPPGAPPPACGPPPAGCPGCPA-CAPGGGGAACCA	49
12060-259	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGPAACCA	50
12060-9	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGAAACCA	51
12060-273	------PGAGACPPGAPPPACGPPPAGCPACPAACAPGGGAACCC-	52
12060-136	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGAACCC-	53
12060-71	------PGCGACPPGAPPPACGPPPAGCPGCPAACAPGGGAACCC-	54
12060-12	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGGAACCC	55
12060-186	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGGAPCCC	56
12060-114	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGPGAACPA	57
12060-382	------PGAGACPPGAPPPACGPPPAGCPGCPAACAPGGGGAACPC	58
12060-330	------PGAGACPPGAPPPACGPPPAGPPGCPAACAPGGGGAACCA	59
Motif	GACPPGxPPPAxGPPPAGC	26

Example 6: GDF11 Aptamer Sequences

[0141] This example provides the results of a BLAST analysis of the SELEX aptamer pool using a lead GDF11 selective aptamer binder sequence (12060-28_3). This analysis was less stringent than the alignment performed in Example 5, and resulted in the identification of additional aptamer sequences that also contained a common or core motif. See Table 8 for the alignment of sequences.

[0142] From the alignment of the sequences in Table 8, several motif sequences may be derived, where each motif maintains overlap with the motif (or common sequence) identified from the alignment of the 102060-28_3 aptamer sequence in Examples 3 and 4.

[0143] A first motif may be defined as follows: 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 110), where each P is independently, and for each occurrence, a C-5 modified pyrimidine; R is A or G; each W is independently, and for each occurrence, A, T, or U; each M is independently, and for each occurrence, A or C; S is G or C; each n is independently, and for each occurrence, 0 or 1; and each m is independently, and for each occurrence, 0 or 1.

[0144] A second motif may be defined as follows: 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 111). A third motif may be as follows: 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 112). A fourth motif may be as follows: 5'-$RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGC$-3' (SEQ ID NO: 113). In each of the second, third and fourth motifs, each P is independently, and for each occurrence, a C-5 modified pyrimidine; R is A or G; each W is independently, and for each occurrence, A, T, or U; each M is independently, and for each occurrence, A or C; S is G or C; each n is independently, and for each occurrence, 0 or 1; and each m is independently, and for each occurrence, 0 or 1.

[0145] A first motif may be defined as follows: 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), where each P is independently, and for each occurrence, a C-5 modified pyrimidine; R is A or G; each W is independently, and for each occurrence, A, T, or U; each M is independently, and for each occurrence, A or C; each S is independently, and for each occurrence, G or C; each n is independently, and for each occurrence, 0 or 1; and each m is independently, and for each occurrence, 0 or 1.

[0146] A second motif may be defined as follows: 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 152). A third motif may be as follows: 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 153). A fourth motif may be as follows: 5'-$RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGS$-3' (SEQ ID NO: 154). In each of the second, third and fourth motifs, each P is independently, and for each occurrence, a C-5

modified pyrimidine; R is A or G; each W is independently, and for each occurrence, A, T, or U; each M is independently, and for each occurrence, A or C; each S is independently, and for each occurrence, G or C; each n is independently, and for each occurrence, 0 or 1; and each m is independently, and for each occurrence, 0 or 1.

Table 8. Alignment of Aptamer Nucleotide Sequences with GDF11 Binder Aptamer 12060-28 (SEQ ID NO: 27)

SEQ ID NO:	Nucleotide Sequence (5' to 3') (P is a NapdU)			GDF11 (K_d)
27	GCGCCPPCG	G-A-CPPG-CPPP-A-A-G-PP-PA-GCC	GCPPGCPCACAP	1.7 nM
60	GPCGCCPCG	G-A-C-PG-CPPP-A-A-G--P-PA-GCC	GPCCPACPACCA	>320 nM
61	GCCGCCPPCG	G-A-CPPGACPPP-A-A-G-PP-PACGCPC	GACPPGACPCCAPCAP	>320 nM
62	GPGCAPAAGPPCCGGGP	G-A-CPPG-APPP-A-A-G-PP-PA-GCC	CCCA	ND
63	GPGCCPPCCG	G-A-CPPGCCPPP-A-ACG-PP-PA-GCC	PAGCPPGCPCACAP	>320 nM
64	GCCGCCPPCG	GPA-CPPGACPPP-A-A-GPPPGPAGGCC	AGCGPPGCPCACAP	>320 nM
65	GPGA	G-A-CPPG-CPPP-A-A-G-PP-PA-GCC	GCPPGCPCAPAP	ND
66	GCGCCPPCG	G-A-CPPG-CPPP-A-A-G-PP-PA-GCC	GCPPGAPCAPCPGCA	ND
67	GPCGCPACG	G-A-CPPG-APPP-A-ACGPPP-PA-GCC	GCPPACAPGGGAACCA	>320 nM
68	GPGPA	G-A-CPPG-APPP-A-C-G-PP-PA-GCC	GPAPGCPCACAP	ND
69	GCGCCPPCG	G-A-CPPG-CPPA-A-A-G-PP-PA-GCC	GCPPGPP	>320 nM
70	GPCGCCPPCG	GPA-CPPG-CPPP-ACC-G-PP-PA-GCC	ACPPAACPCACAP	ND
71	GACGGCCPPCCG	G-A-CPPGACPPP-A-C-G-PP-PA-GCC	GCCPGCPCACAP	ND
72	GPGCPAG	G-A-CPPGACPPP-A-ACG-PP-PA-G-C	PGCPPACAPGGAACPC	>320 nM
73	GCGCCPCG	GAACCPPG-CPPP-A-A-G-PP-PA-GCC	GCPGCPPCACAPA	ND
74	GPCGCCPCG	G-A-CPPG-CPPP-A-A-GAPP-PA-GCC	PGCPGCAACACAP	ND
75	GCGCPPACG	G-A-CPPGACPPPAA-A-G--P-PA-GCC	GCPPGCPCACCAP	ND

LISTING OF CLAIMS

1. An aptamer that binds GDF11 with an affinity of less than 10 nM, wherein, under the same conditions, the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8, wherein the aptamer comprises the sequence 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3'(SEQ ID NO:110) or 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

each W is independently, and for each occurrence, A, T, or U;

each M is independently, and for each occurrence, A or C;

each S is independently, and for each occurrence, G or C;

each n is independently, and for each occurrence, 0 or 1; and

each m is independently, and for each occurrence, 0 or 1.

2. An aptamer that binds GDF11, wherein the aptamer comprises the sequence 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO:110) or 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 151), wherein:

each P is independently, and for each occurrence, a C-5 modified pyrimidine;

R is A or G;

each W is independently, and for each occurrence, A, T, or U;

each M is independently, and for each occurrence, A or C;

each S is independently, and for each occurrence, G or C;

each n is independently, and for each occurrence, 0 or 1; and

each m is independently, and for each occurrence, 0 or 1.

3. The aptamer of claim 1 or claim 2, wherein the aptamer comprises a sequence selected from:

a) 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 111);

b) 5'-$RW_nAC_nCPPGM_mMPPPA_nAC_nMC_mRW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 152);

c) 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGC$-3' (SEQ ID NO: 112);

d) 5'-$RW_nMC_nCPPGM_mMPPPA_nAC_nMC_mGW_nPPG_nPAS_nGS$-3' (SEQ ID NO: 153);

e) 5'-$RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGC$-3' (SEQ ID NO: 113); and

f) $RMCPPGM_mMPPPA_nAC_nMC_mRW_nPPPAS_nGS$-3' (SEQ ID NO: 154).

4. The aptamer of any one of claims 1 to 3, wherein if R is G, the first W is A, and wherein if R is A, the first W is C.

5. The aptamer of any one of claims 1 to 4, wherein at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 n are 0.

6. The aptamer of any one of claims 1 to 4, wherein each n is 0.

7. The aptamer of any one of claims 1 to 4, wherein at least 1, at least 2, at least 3 n are 1.

8. The aptamer of any one of claims 1 to 7, wherein at least 1 m is 0.

9. The aptamer of any one of claims 1 to 7, wherein at least 1 m is 1.

10. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-CPPGMPPP-3' (SEQ ID NO: 114), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, and wherein M is C or A.

11. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-PPPAGC-3'(SEQ ID NO: 115) or 5'-PPPAGG-3' (SEQ ID NO: 155), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine.

12. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-CPPGMPPPN$_x$PPPAGC-3' (SEQ ID NO: 160) or 5'-CPPGMPPPN$_x$PPPAGG-3' (SEQ ID NO: 116), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, wherein x is 2, 3, 4, or 5, wherein M is C or A, and wherein each N is independently, and for each occurrence, selected from A, C, G, T, and U.

13. The aptamer of claim 9, wherein x is 3 or 4.

14. The aptamer of claim 12 or claim 13, wherein N_x comprises the sequence 5'-AAG-3' or 5'-ACG-3' or 5'-AGG-3'.

15. The aptamer of any one of the preceding claims, wherein the aptamer comprises the sequence 5'-NNCPPGRPPPAMGPPPAGS-3' (SEQ ID NO: 141), wherein each P is independently, and for each occurrence, a C-5 modified pyrimidine, R is A or G; each N is independently, and for each occurrence, A, G, or C; M is A or C; and S is G or C.

16. The aptamer of any one of claims 1 to 15, wherein each P is independently, and for each occurrence, selected from:

5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU),

5-(N-benzylcarboxyamide)-2'-O-methyluridine,

5-(N-benzylcarboxyamide)-2'-fluorouridine,

5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU),

5-(N-thiophenylmethylcarboxyamide)-2'-deoxyuridine (ThdU),

5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU),

5-(N-tyrosylcarboxyamide)-2'-deoxyuridine (TyrdU),

5-(N-3,4-methylenedioxybenzylcarboxyamide)-2'-deoxyuridine (MBndU),

5-(N-4-fluorobenzylcarboxyamide)-2'-deoxyuridine (FBndU),

5-(N-3-phenylpropylcarboxyamide)-2'-deoxyuridine (PPdU),

5-(N-imidizolylethylcarboxyamide)-2'-deoxyuridine (ImdU),

5-(N-isobutylcarboxyamide)-2'-O-methyluridine,

5-(N-isobutylcarboxyamide)-2'-fluorouridine,

5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU),

5-(N-*R*-threoninylcarboxyamide)-2'-deoxyuridine (ThrdU),

5-(N-tryptaminocarboxyamide)-2'-O-methyluridine,

5-(N-tryptaminocarboxyamide)-2'-fluorouridine,

5-(N-[1-(3-trimethylamonium) propyl]carboxyamide)-2'-deoxyuridine chloride,

5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU),

5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine),

5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU),

5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU),

5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU),

5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU),

5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU),

5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, and

5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine.

17. The aptamer of any one of claims 1 to 15, wherein each P is independently, and for each occurrence, selected from:

5-(N-1-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU),

5-(N-1-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU),

5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine,

5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU),

5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU),

5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine,

5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU),

5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine,

5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine,

5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU),

5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, and

5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine.

18. The aptamer of any one of claims 1 to 15, wherein at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 P are each independently selected from 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, and 5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine.

19. The aptamer of any one of claims 1 to 15, wherein each P is independently selected from 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, and 5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine.

20. The aptamer of any one of the preceding claims, wherein the aptamer comprises a sequence selected from SEQ ID NOs: 12, 13, 15 to 109, and 117 to 140.

21. The aptamer of any one of the preceding claims, wherein the aptamer comprises a sequence selected from SEQ ID NOs: 12, 15, 26, 105 to 109, and 142 to 150.

22. The aptamer of any one of the preceding claims, wherein the aptamer consists of 18 to 200 nucleotides, or 18 to 150 nucleotides, or 18 to 100 nucleotides, or 18 to 75 nucleotides, or 18 to 50 nucleotides, or 20 to 150 nucleotides, or 20 to 100 nucleotides, or 20 to 75 nucleotides, or 20 to 50 nucleotides, wherein each nucleotide may, independently, be a modified or unmodified nucleotide.

23. The aptamer of any one of the preceding claims, wherein the aptamer comprises a detectable label.

24. The aptamer of any one of the preceding claims, wherein GDF11 is human GDF11 and GDF8 is human GDF8.

25. The aptamer of any one of the preceding claims, wherein GDF11 is mature human GDF11 comprising the sequence of SEQ ID NO: 118 and GDF8 is mature human GDF8 comprising the sequence of SEQ ID NO: 119.

26. A method of detecting GDF11 in a sample, comprising contacting proteins from the sample with an aptamer of any one of claims 1 to 25.

27. The method of claim 26, wherein the aptamer binds GDF8 with an affinity that is at least 10-fold weaker than the affinity for GDF11 or does not bind GDF8.

28. A method of determining whether a sample comprises GDF11, comprising contacting proteins from the sample with an aptamer of any one of claims 1 to 25.

29. The method of claim 28, wherein the sample comprises GDF8.

30. The method of any one of claims 26 to 29, wherein the method comprises contacting the sample with the aptamer under stringent conditions.

31. The method of claim 30, wherein the stringent conditions comprise a polyanionic inhibitor.

32. The method of claim 31, wherein the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs.

33. The method of any one of claims 26 to 32, wherein the sample is a sample from a human.

34. The method of claim 33, wherein the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample.

35. The method of claim 34, wherein the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue.

36. The method of any one of claims 26 to 35, wherein the proteins have been separated from at least one other component of the sample.

37. The method of any one of claims 26 to 35, wherein the proteins have not been separated from other components of the sample.

38. A composition comprising an aptamer of any one of claims 1 to 25 and proteins from a sample.

39. The composition of claim 38, wherein the sample comprises GDF8.

40. The composition of claim 38 or claim 39, comprising a polyanionic inhibitor.

41. The composition of claim 40, wherein the polyanionic inhibitor is selected from dextran sulfate, heparin, Z-block, poly-dI/dC, sonicated or sheared salmon sperm DNA, calf thymus DNA, and dNTPs.

42. The composition of any one of claims 38 to 41, wherein the sample is a sample from a human.

43. The composition of claim 42, wherein the sample is selected from blood, serum, plasma, saliva, urine, and a tissue sample.

44. The composition of claim 43, wherein the tissue sample is selected from heart muscle tissue, skeletal muscle tissue, pancreatic tissue, cartilage tissue and neural tissue.

45. The composition of any one of claims 38 to 44, wherein the proteins have been separated from at least one other component of the sample.

46. The composition of any one of claims 38 to 44, wherein the proteins have not been separated from other components of the sample.