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(54) Titre : APTAMERES DE PDGF ET VEGF PRESENTANT UNE STABILITE AMELIOREE ET LEUR UTILISATION DANS LE TRAITEMENT DE MALADIES ET DE TROUBLES MEDIES PAR PDGF ET VEGF

(54) Title: PDGF AND VEGF APTAMERS HAVING IMPROVED STABILITY AND THEIR USE IN TREATING PDGF AND VEGF MEDIATED DISEASES AND DISORDERS

(57) Abrégé/Abstract:
Aptamers having improved stability against nucleases that bind PDGF and aptamers that bind VEGF are provided. In addition, aptamer constructs comprising a PDGF aptamer and a VEGF aptamer are provided. Pharmaceutical compositions comprising the aptamers and aptamer constructs are provided, as well as methods of treating conditions using the aptamers and aptamer constructs.

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(54) Title: PDGF AND VEGF APTAMERS HAVING IMPROVED STABILITY AND THEIR USE IN TREATING PDGF AND VEGF MEDIATED DISEASES AND DISORDERS

(57) Abstract: Aptamers having improved stability against nucleases that bind PDGF and aptamers that bind VEGF are provided. In addition, aptamer constructs comprising a PDGF aptamer and a VEGF aptamer are provided. Pharmaceutical compositions comprising the aptamers and aptamer constructs are provided, as well as methods of treating conditions using the aptamers and aptamer constructs.

WO 2015/035305 A1

PDGF AND VEGF APTAMERS HAVING IMPROVED STABILITY AND THEIR USE IN TREATING PDGF AND VEGF MEDIATED DISEASES AND DISORDERS

[0001]

FIELD OF THE INVENTION

[0002] The present disclosure relates generally to the field of nucleic acids and more particularly to aptamers capable of binding to platelet-derived growth factor (PDGF) and aptamers capable of binding to vascular endothelial growth factor (VEGF). In some embodiments, such aptamers are useful as therapeutics for preventing, treating, and/or ameliorating proliferative disorders, including, but not limited to, atherosclerosis, macular degeneration, fibrosis, cancer, and other disorders in which PDGF and/or VEGF has been implicated. In some embodiments, the present disclosure relates to aptamer constructs that are capable of binding to VEGF and PDGF, either simultaneously or in a mutually exclusive manner, and are useful as therapeutics.

[0003]

BACKGROUND

[0004] The following description provides a summary, of information, and is not an admission that any of the information provided or publications referenced herein is prior art to the present disclosure.

[0005] Platelet-derived growth factors (PDGF-A, -B, -C and -D) are ubiquitous mitogens and chemotactic factors for many connective tissue cells (Fredriksson, L., *et al.* (2004) Cytokine Growth Factor Rev. 15(4):197). PDGFs occur as disulfide-linked dimers and contain a cysteine-knot-fold growth factor domain that functions through binding to PDGF receptors α and β on the surface of a cell (Claesson-Welsh, L., *et al.* (1989) Proc. Natl. Acad.

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Sci. U S A 86:4917). PDGF binding induces receptor dimerization, which leads to autophosphorylation at intracellular tyrosine residues (Claesson-Welsh, J. (1994) Biol. Chem. 269:32023). PDGF-BB is involved in several proliferative disorders, including atherosclerosis, fibrosis, macular degeneration, and cancer (Östman, A., *et al.* (2001) Adv. Cancer Res. 80:1; Appelmann, I., *et al.* (2010) Recent Results Cancer Res. 180:51; Trojanowska, M., *et al.* (2008) Rheumatology (Oxford) 47(Suppl 5):2; Rutherford *et al.* (1997) Atherosclerosis 130:45; Smits *et al.* (1992) Am. J. Pathol. 140:639; Heldin *et al.* (1991) Endocrinology 129:2187; Floege and Johnson (1995) Miner. Electrolyte Metab. 21:271; Raines *et al.* (1990) Experimental Pharmacology, Peptide Growth Factors and Their Receptors, Sporn & Roberts, pp. 173-262, Springer, Heidelberg.).

[0006] VEGF is a secreted disulfide-linked homodimer that selectively stimulates endothelial cells to proliferate, migrate and produce matrix-degrading enzymes, all of which are processes required for the formation of new blood vessels (Conn, G., *et al.* (1990) Proc. Natl. Acad. Sci. USA 87:1323; Ferrara, N. *et al.* (1989) Biochem. Biophys. Res. Commun. 161:851; Gospodarowicz, D., *et al.* (1989) Proc. Natl. Acad. Sci. USA 86:7311; Pepper, M.S., *et al.* (1991) Biochem. Biophys. Res. Commun. 181:902; Unemori, E.N., *et al.* (1992) J. Cell. Physiol. 153:557). In addition to being the only known endothelial cell-specific mitogen, VEGF is unique among angiogenic growth factors in its ability to induce a transient increase in blood vessel permeability to macromolecules (Dvorak, H.F., *et al.* (1979) J. Immunol. 122:166; Senger, D.R., *et al.* (1983) Science 219:983; Senger, D.R., *et al.* (1986) Cancer Res. 46:5629). Increased vascular permeability and the resulting deposition of plasma proteins in the extravascular space facilitate new vessel formation by providing a provisional matrix for the migration of endothelial cells. Hyperpermeability is indeed a characteristic feature of new vessels (Dvorak, H.F., *et al.* (1995) Am. J. Pathol. 146:1029). Furthermore, compensatory angiogenesis induced by tissue hypoxia is also mediated by VEGF (Levy, A.P., *et al.* (1996) J. Biol. Chem. 271:2746; Shweiki, D., *et al.* (1991) Nature 359:843). The identification of VEGF as a hypoxia-inducible protein, along with the complementary observation that hyperoxia causes suppression of VEGF expression, provides an appealing mechanism for matching oxygen demand with vascular supply (Benjamin, L.E., *et al.* (1999) J. Clin. Invest. 103:159; Alon, T., *et al.* (1995) Nat. Med. 1:1024).

[0007] Several isoforms of VEGF protein occur as a result of alternative splicing of the eight exons of the gene that encodes VEGF (Eming, S.A., *et al.* (2006) J. Invest. Dermatol. Symp. Proc.11:79). The most prevalent isoforms are VEGF-121, VEGF-165 and VEGF-189. Proteolytic processing of VEGF can generate additional isoforms. VEGF-165 can be cleaved

by plasmin between Arg-110 and Ala-111 to generate VEGF-110, which is functionally equivalent to VEGF-121 (Keyt, B.A., *et al.* (1996) J. Biol. Chem. 271:7788). VEGF-189 can be cleaved by urokinase within the exon 6 domain and then can be cleaved further by plasmin to generate VEGF-110 (Plouët, J., *et al.*, (1997) J. Biol. Chem. 272:13390). In addition, a subset of matrix metalloproteases (MMPs), including MMP-3, -7, -9 and -19, are capable of cleaving VEGF-165 and VEGF-189 in sequential steps to generate VEGF-113, which is functionally equivalent to VEGF-110. Therefore, the relative abundance of matrix-bound and diffusible forms of VEGF in a given tissue is determined by the combination of alternative splicing and proteolytic processing that occurs in the cells of the tissue (Ferrara, N., *et al.* (2006) Retina 26:859).

[0008] Age-related macular degeneration (AMD) remains the leading cause of blindness in people over 55 years of age. The disease is characterized by the formation of insoluble deposits called drusen within the macula, the part of the retina that has the highest density of photoreceptors and is involved in central vision. In the initial stages of AMD, the deposits are avascular and the disease generally progresses slowly. However, in about 10% of the patients, this so-called "dry" form of AMD becomes vascularized and turns into the "wet" form of AMD, during which the disease becomes more progressive and vision deteriorates at a faster rate. In many cases, the progression from blurriness of central vision to virtual blindness occurs in less than two years. In the advanced stage of the disease, the exudative or wet form of AMD, new blood vessels penetrate from the choriocapillaris into the central part of the retina (macula), occluding central vision. In the United States, the prevalence of wet AMD is about 1.8 million and is expected to increase to close to 3 million by 2020. The incidence of wet AMD in the United States is about 210,000 people each year.

[0009] Recently, AMD has been treated by blocking VEGF-mediated induction of angiogenesis and blood vessels leakiness by direct injection into the eye of high-affinity antagonists that bind to VEGF, preventing interaction of VEGF with its cell-surface receptors on endothelial cells.

[0010] There is considerable evidence that dual inhibition of VEGF and PDGF-B signaling leads to more efficient blocking of angiogenesis coupled with regression of new blood vessels. For example, clinical evidence suggests that dual inhibition of VEGF and PDGF-B can achieve a more complete inhibition of ocular angiogenesis in AMD patients. An aptamer inhibitor of PDGF-B (E10030), originally discovered at NeXstar Pharmaceuticals (Green, L.S., *et al.* (1996) Biochemistry 35:14413; U.S. Patent Nos. 6,207,816; 5,731,144; 5,731,424; and 6,124,449), is being developed by Ophthotech Corporation as a treatment for AMD.

E10030 (Fovista®) is a DNA-based modified aptamer that binds to PDGF-AB or PDGF-BB with a K_d of approximately 100 pM and inhibits the functions of PDGF-B both *in vitro* and *in vivo*.

[0011] In a Phase 1 study, anti-PDGF therapy with E10030 tested in combination with Lucentis® anti-VEGF therapy resulted in vision gain of three lines in 59% of treated patients after 12 weeks of therapy. This is a considerably higher percentage of patients with improved visual acuity compared to the 34-40% observed historically with Lucentis alone. In addition, the combination treatment was accompanied with marked neovascular regression in all study participants. Enhanced efficacy with combination treatment was recently corroborated in a phase 2 study of 449 patients with wet AMD. Patients receiving the combination of Fovista (1.5 mg) and Lucentis gained a mean of 10.6 letters of vision at 24 weeks, compared to 6.5 letters for patients receiving Lucentis monotherapy (p=0.019), representing a 62% additional vidual acuity benefit.

SUMMARY

[0012] The present disclosure provides aptamers that bind to platelet-derived growth factor B (PDGF-B, including PDGF-BB and PDGF-AB), aptamers that bind vascular endothelial growth factor (VEGF, including VEGF-121 and VEGF-165), and aptamer constructs comprising an aptamer that binds PDGF-B and an aptamer that binds VEGF. The disclosed aptamers and aptamer constructs are useful as therapeutics for preventing, treating, and/or ameliorating proliferating diseases or conditions, including but not limited to, atherosclerosis, macular degeneration, fibrosis, diabetic retinopathy, and cancer, and/or other diseases or conditions in which PDGF and/or VEGF is implicated. In various embodiments, the aptamer constructs are capable of binding to each of VEGF and PDGF-B independently and/or VEGF and PDGF-B simultaneously. Included are pharmaceutical compositions or formulations comprising a PDGF aptamer, a VEGF aptamer, or a VEGF/PDGF-B aptamer construct, or a pharmaceutically acceptable salt of any of the foregoing, and at least one pharmaceutically acceptable carrier. Such compositions can be prepared in any suitable pharmaceutically acceptable dosage form.

[0013] In one aspect, the disclosure provides for a nucleic acid molecule comprising the nucleic sequence: 5' - C-G-A-C-A-G-C-A-Z-G-Z-A-Z-G-C-A-C-A-Z-C-Z - 3' (SEQ ID NO: 830), wherein Z is a C-5 modified pyrimidine, and at least one of positions 4, 9, 10, 11 and 12 of the nucleic acid molecule comprise a phosphorothioate linkage or moiety, wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule. In a

related aspect, the at least two, three, four or five of positions 4, 9, 10, 11 and 12 of the nucleic acid molecule comprise a phosphorothioate linkage or moiety, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule.

[0014] In another aspect, the nucleic acid molecule comprises at least one, two, three, four, five, six, seven, eight, nine or ten 2'-O-methyl nucleosides. In a related aspect, at least one of positions 1,4, 6, 7, 14, 15, 16, 17, 18, 20 and 21 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule. In yet another related aspect, the at least two, three, four, five, six, seven, eight, nine, ten or eleven of positions 1, 4, 6, 7, 14, 15, 16, 17, 18, 20 and 21 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule.

[0015] In another aspect, the C-5 modified pyrimidine independently, and for each occurrence, is selected from the group consisting a 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-isobutylcarboxyamide)-2'-O-methyluridine, 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, and 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine). In a related aspect, the C-5 modified pyrimidine is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU).

[0016] In another aspect, the nucleic acid molecule comprises DNA, RNA or a combination thereof.

[0017] In another aspect, the nucleic acid molecule binds a PDGF protein with a binding affinity of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.

[0018] In another apsect, the nucleic acid molecule further comprises an inverted deoxynucleotide at the 3' terminus of the nucleic acid molecule. In a related aspect, the deoxynucleotide is a deoxythymidine.

[0019] In another apsect, the nucleic acid molecule is less sensitive to a nuclease compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety. In a related aspect, the nuclease is a DNase enzyme. In yet another related aspect, the DNase enzyme is a DNase I or DNase II enzyme.

[0020] In another aspect, the nucleic acid molecule is more stable in viterous humor from New Zealand White Rabbits compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety.

[0021] In another aspect, the nucleic acid molecule is selected from the group consisting of the 5169 aptamers having the following aptamer ID number 146, 150-159, 172-182 and 188.

[0022] In a related aspect, the nucleic acid molecule comprises eleven 2'-O-methyl nucleosides and four or five phosphorothioate linkages or moieties.

[0023] In a related aspect, the nucleic acid molecule comprises at least one nucleotide having a phosphorothioate linkage or moiety and a 2'-O-methyl group.

[0024] In another aspect, this disclosure provides for a nucleic acid molecule comprising the nucleic sequence: 5' - C-C-G-Z-Z-C-A-A-G-Z-G-C-Z-Z-G-Z-A-G-G-A-Z-Z-Z-A-A-A-Z-G-G - 3' (SEQ ID NO: 831), wherein Z is a C-5 modified pyrimidine, and at least one of positions 24, 25 and 26 of the nucleic acid molecule comprise a phosphorothioate linkage or moiety, wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule. In a related aspect, at least two or three of positions 24, 25 and 26 comprise a phosphorothioate linkage or moiety, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule.

[0025] In a related aspect, the nucleic acid molecule comprises at least one, two, three, four, five, six, seven, eight or nine 2'-O-methyl nucleosides.

[0026] In another aspect, at least one of positions 1, 6, 9, 12, 15, 17, 18, 22, 28 and 29 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule. In a related aspect, at least two, three, four, five, six, seven, eight or nine of positions 1, 6, 9, 12, 15, 17, 18, 22, 28 and 29 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule.

[0027] In another aspect, the C-5 modified pyrimidine independently, and for each occurrence, is selected from the group consisting a 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-isobutylcarboxyamide)-2'-O-methyluridine, 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, and 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine). In a related aspect, the C-5 modified pyrimidine is a 5-(N-naphthylmethylcarboxyamide)-2′-deoxyuridine (NapdU).

[0028] In another aspect, the nucleic acid molecule comprises DNA, RNA or a combination thereof.

[0029] In another aspect, the nucleic acid molecule binds a VEGF protein with a binding affinity of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM.

[0030] In another aspect, the nucleic acid molecule further comprises an inverted deoxynucleotide at the 3' terminus of the nucleic acid molecule. In a related aspect, the deoxynucleotide is a deoxythymidine.

[0031] In another aspect, the nucleic acid molecule is less sensitive to a nuclease compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety. In a related aspect, the nuclease is a DNase enzyme. In yet another related aspect, the DNase enzyme is a DNase I or DNase II enzyme.

[0032] In another aspect, the nucleic acid molecule is more stable in viterous humor from New Zealand White Rabbits compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety.

[0033] In another aspect, the nucleic acid molecule is selected from the group consisting of the 4867-31 aptamers having the following aptamer ID number 192, 409-417, 419-429 ,438-445 and 475-483.

[0034] In another aspect, the nucleic acid molecule comprises nine or ten 2'-O-methyl nucleosides and one, two or three phosphorothioate linkages or moieties.

[0035] In another aspect, the nucleic acid molecule comprises at least one nucleotide having a phosphorothioate linkage or moiety and a 2'-O-methyl group.

[0036] In another aspect, the disclosure provides a nucleic acid molecule comprising the nucleic sequence: 5' - Z-Z"-A-C-H-G-Z-Z-A-C-V-C-G-C-G-Z'-Z-Z-A-Z-A-G-C-G - 3' (SEQ ID NO: 832), wherein Z, Z' and Z" are independently, for each occurrence selected from the

group consisting of 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-isobutylcarboxyamide)-2'-O-methyluridine, 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-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine, 5-(N-3,4-methylenedioxybenzylcarboxyamide)-2'-deoxyuridine (MBndU), and 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine);; V is a three carbon linker and H is hexaethylene glycol linker; and at least one of positions 8, 9, 15, 16 and 17 of the nucleic acid molecule comprise a phosphorothioate linkage or moiety, wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule, and H and V are considered a single position for purposes of counting positions of the nucleic acid molecule. In a related aspect, Z and Z' are indpendently, for each occurance, selected from is selected from the group consisting a 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-isobutylcarboxyamide)-2'-O-methyluridine, 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, and 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine). In another related aspect, Z is indepedently, for each occurance, selected from a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) or methylenedioxybenzyl-dU. In another related aspect, Z' is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU).

[0037] In another apsect, at least two or three of positions 8, 9, 15, 16 and 17 comprise a phosphorothioate linkage or moiety, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule, and H and V are considered a single position for purposes of counting positions of the nucleic acid molecule.

[0038] In another aspect, the nucleic acid molecule comprises at least one, two, three, four, five, six, seven or eight 2'-O-methyl nucleosides.

[0039] In another aspect, at least one of positions 3, 7, 12, 13, 18, 19, 21 and 24 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule, and H and V are considered a single position for purposes of counting positions of the nucleic acid molecule. In a related aspect, at least two, three, four, five, six, seven or eight of positions 3, 7, 12, 13, 18, 19, 21 and 24 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of the nucleic acid molecule, and H and V are considered a single position for purposes of counting positions of the nucleic acid molecule.

[0040] In another aspect, the Z is a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU).

[0041] In another aspect, the nucleic acid molecule comprises DNA, RNA or a combination thereof.

[0042] In another aspect, the nucleic acid molecule binds a PDGF protein with a binding affinity of less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM. In another aspect, the nucleic acid molecule inhibits PDGF-mediated phosphorylation of a PDGF receptor.

[0043] In another aspect, the nucleic acid molecule further comprises an inverted deoxynucleotide at the 3' terminus of the nucleic acid molecule. In a related aspect, the deoxynucleotide is a deoxythymidine.

[0044] In another aspect, the nucleic acid molecule is less sensitive to a nuclease compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety. In a related aspect, the nuclease is a DNase enzyme. In yet another related aspect, the DNase enzyme is a DNase I or DNase II enzyme.

[0045] In another aspect, the nucleic acid molecule is more stable in viterous humor from New Zealand White Rabbits compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety.

[0046] In another aspect, the nucleic acid molecule is selected from the group consisting of the 4149-8 aptamers having the following aptamer ID number 379, 391, 418-426, 431-437 and 453-459.

[0047] In another aspect, the nucleic acid molecule comprises seven or eight 2'-O-methyl nucleosides and four, five, six, seven or eight phosphorothioate linkages or moieties.

[0048] In another aspect, the nucleic acid molecule comprises at least one nucleotide having a phosphorothioate linkage or moiety and a 2'-O-methyl group.

[0049] In another aspect the disclosure provides for a composition comprising two nucleic acid molecules selected from the nucleic acid molecules described herein.

[0050] In another aspect, the composition is in the form selected from the group selected from a solution, a powder, a gel and an emulsion.

[0051] In another aspect, the two nucleic acid molecules are covalently linked or non-covalently linked.

[0052] In another aspect, this disclosure provides for a pharmaceutical composition comprising at least one nucleic acid molecule of any of the nucleic acid molecules describered herein, and a pharmaceutically acceptable carrier.

[0053] In another aspect, the pharmaceutical composition is for intravitreal injection.

[0054] In another aspect, this disclosure provides for a method for treating macular degeneration comprising administering to a subject with macular degeneration a therapeutically effective amount of the pharmaceutical composition as described herein.

[0055] In another aspect, this disclosure provides for a method for preventing macular degeneration comprising administering to a subject at risk for developing macular degeneration a therapeutically effective amount of the pharmaceutical composition as described herein.

[0056] In another aspect, this disclosure provides for a method for treating an ophthalmic condition comprising administering to a subject with an ophthalmic condition a therapeutically effective amount of the pharmaceutical composition as described herein. In a related aspect, the ophthalmic condition is selected from the group consisting of retinitis, macular degeneration, choroiditis, retinopathy, hypertensive retinopathy, diabetic retinopathy, chronic dry eye, AIDS-related vision loss, amblyopia, hemianopia, retinal vein occlusions, trachoma, keratoconus, chorioretinal inflammation, central serous retinopathy, uveitis, retinal dystrophy, edema, glaucoma, and cataract.

[0057] In another aspect, the macular degeneration is age-related macular degeneration. In a related aspect, the macular degeneration is dry age-related macular degeneration or wet age-related macular degeneration.

[0058] In another aspect, this disclosure provides for a method for treating fibrosis comprising administering to a subject with fibrosis a therapeutically effective amount of the pharmaceutical composition as described herein. In a related aspect, the fibrosis is selected from pulmonary fibrosis, renal fibrosis, and cystic fibrosis.

[0059] In another aspect, this disclosure provides for a method for treating a cardiovascular disease comprising administering to a subject with the cardiovascular disease a therapeutically effective amount of the pharmaceutical composition as described herein. In a related aspect, the cardiovascular disease is selected from atherosclerosis, restenosis, a cardiac hypertrophy related condition, and a vascular disorder.

[0060] In another aspect, this disclosure provides for a method for treating cancer comprising administering to a subject with cancer a therapeutically effective amount of the pharmaceutical composition as described herein. In a related aspect, the cancer is selected from bladder cancer, lung cancer, breast cancer, melanoma, colon and rectal cancer, lymphoma, endometrial cancer, pancreatic cancer, liver cancer, renal cancer, prostate cancer, leukemia, and thyroid cancer.

[0061] In another aspect, this disclosure provides for a nucleic acid molecule comprising the nucleic sequence: 5' - C^1-G-A-$C^{1,2}$-A-G^1-C^1-A-Z^2-G^2-Z^2-A^2-Z-G^1-C^1-A^1-C^1-A^1-Z-C^1-Z^1 - 3' (SEQ ID NO: 833), wherein, C^1 is independently, for each occurrence, selected from the group consisting of a cytidine, a deoxycytidine and a 2'-O-methylcytidine; $C^{1,2}$ is independently, for each occurrence, selected from the group consisting of a cytidine, a cytidine comprising a phosphorothioate linkage, a deoxycytidine, a deoxycytidine comprising a phosphorothioate linkage, a 2'-O-methylcytidine and a 2'-O-methylcytidine comprising a phosphorothioate linkage; G^1 is independently, for each occurrence, selected from the group consisting of a guanosine, a deoxyguanosine and a 2'-O-methylguanosine; G^2 is independently, for each occurrence, selected from the group consisting of a guanosine, a guanosine comprising a phosphorothioate linkage, a deoxyguanosine and a deoxyguanosine comprising a phosphorothioate linkage; Z is independently, for each occurrence, selected from the group consisting of a uridine, a deoxyuridine and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU); Z^1 is independently, for each occurrence, selected from the group consisted of a uridine, a deoxyuridine, a 2'-O-methyluridine, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) and a 5-(N-

naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group; Z^2 is independently, for each occurrence, selected from the group consisted of a uridine, a uridine comprising a phosphorothioate linkage, a deoxyuridine, a deoxyuridine comprising a phosphorothioate linkage, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU)comprising a phosphorothioate linkage; A^1 is independently, for each occurrence, selected from the group consisting of an adenosine, a deoxyadenosine and a 2'-O-methyladenosine; A^2 is independently, for each occurrence, selected from the group consisting of an adenosine, an adenosine comprising a phosphorothioate linkage, a deoxyadenosine and a deoxyadenosine comprising a phosphorothioate linkage; and wherein, the nucleic acid molecule comprises at least one, two, three, four or five phosphorothioate linkages.

[0062] In a related aspect, C^1 is a 2'-O-methylcytidine. In a related aspect, $C^{1,2}$ is a 2'-O-methylcytidine comprising a phosphorothioate linkage. In a related aspect, G^1 is a 2'-O-methylguanosine. In a related aspect, G^2 is a deoxyguanosine comprising a phosphorothioate linkage. In a related aspect, Z^1 is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group. In a related aspect, Z^2 is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a phosphorothioate linkage. In a related aspect, A^1 is a 2'-O-methyladenosine. In a related aspect, A^2 is a deoxyadenosine comprising a phosphorothioate linkage.

[0063] In another aspect, this disclosure provides for a nucleic acid molecule comprising the nucleic sequence: 5' - C^1-C-G-Z-Z-C^1-A-A-G^1-Z-G-C^1-Z-Z-G^1-Z-A^1-G^1-G-A-Z-Z^1-Z-A^2-A^2-A^2-Z-G^1-G^1 -3' (SEQ ID NO: 834) wherein, C^1 is independently, for each occurrence, selected from the group consisting of a cytidine, a deoxycytidine and a 2'-O-methylcytidine;G^1 is independently, for each occurrence, selected from the group consisting of a guanosine, a deoxyguanosine and a 2'-O-methylguanosine; G^1 is independently, for each occurrence, selected from the group consisting of a guanosine, a deoxyguanosine and a 2'-O-methylguanosine; Z is independently, for each occurrence, selected from the group consisted of a uridine, a deoxyuridine and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU); Z^1 is independently, for each occurrence, selected from the group consisted of a uridine, a deoxyuridine, a 2'-O-methyluridine, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU)and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group; A^1 is independently, for each occurrence, selected from the group consisting of an adenosine, a deoxyadenosine and a 2'-O-methyladenosine; A^2 is independently, for each occurrence, selected from the group consisting of an adenosine, an

adenosine comprising a phosphorothioate linkage, a deoxyadenosine and a deoxyadenosine comprising a phosphorothioate linkage; and wherein, the nucleic acid molecule comprises at least one, two, three, four or five phosphorothioate linkages.

[0064] In a related aspect, C^1 is a 2'-O-methylcytidine. In a related aspect, G^1 is a 2'-O-methylguanosine. In a related aspect, Z is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU). In a related aspect, Z^1 is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group. In a related aspect, A^1 is a 2'-O-methyladenosine. In a related aspect, A^2 is a deoxyadenosine comprising a phosphorothioate linkage.

In another aspect, this disclosure provides for a nucleic acid molecule comprising the nucleic sequence: 5'-Z-Z"-A^1-C-H-G-Z^1-Z^2-A^2-C-V-C^1-G^1-C-G^2-Z'^2-Z^2-Z^1-A^1-Z-A^1-G-C-G^1 -3' (SEQ ID NO: 835) wherein, C^1 is independently, for each occurrence, selected from the group consisting of a cytidine, a deoxycytidine and a 2'-O-methylcytidine; G^1 is independently, for each occurrence, selected from the group consisting of a guanosine, a deoxyguanosine and a 2'-O-methylguanosine; G^2 is independently, for each occurrence, selected from the group consisting of a guanosine, a guanosine comprising a phosphorothioate linkage, a deoxyguanosine and a deoxyguanosine comprising a phosphorothioate linkage; Z is independently, for each occurrence, selected from the group consisting of a uridine, a deoxyuridine, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU), a 5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU), a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) and a 5-(N-3,4-methylenedioxybenzylcarboxyamide)-2'-deoxyuridine (MBndU); Z" is independently, for each occurrence, selected from the group consisting of a uridine, a deoxyuridine, a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) and a 5-(N-3,4-methylenedioxybenzylcarboxyamide)-2'-deoxyuridine (MBndU); Z'^2 is independently, for each occurrence, selected from the group consisted of a uridine, a uridine comprising a phosphorothioate linkage, a deoxyuridine, a deoxyuridine comprising a phosphorothioate linkage, a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU), a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) comprising a phosphorothioate linkage, a 5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU), a 5-(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU) comprising a phosphorothioate linkage, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU)comprising a phosphorothioate linkage; Z^1 is independently, for each occurrence, selected from the group consisting of a uridine, a deoxyuridine, a 2'-O-methyluridine, a 5-(N naphthylmethylcarboxyamide)-2'-

deoxyuridine (NapdU), a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group, a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU), a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) comprising a 2'-O-methyl group, a methylenedioxybenzyl-dU and a methylenedioxybenzyl-dU comprising a 2'-O-methyl group; Z^2 is independently, for each occurrence, selected from the group consisting of a uridine, a uridine comprising a phosphorothioate linkage, a deoxyuridine, a deoxyuridine comprising a phosphorothioate linkage, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a phosphorothioate linkage, a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU), a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) comprising a phosphorothioate linkage, a methylenedioxybenzyl-dU and a methylenedioxybenzyl-dU comprising a phosphorothioate linkage; A^1 is independently, for each occurrence, selected from the group consisting of an adenosine, a deoxyadenosine and a 2'-O-methyladenosine; A^2 is independently, for each occurrence, selected from the group consisting of an adenosine, an adenosine comprising a phosphorothioate linkage, a deoxyadenosine and a deoxyadenosine comprising a phosphorothioate linkage; V is a three carbon linker; H is hexaethylene glycol linker; and wherein, the nucleic acid molecule comprises at least one, two, three, four or five phosphorothioate linkages.

[0065] In a related aspect, C^1 is a 2'-O-methylcytidine. In a related aspect, G^1 is a 2'-O-methylguanosine. In a related aspect, G^2 is a deoxyguanosine comprising a phosphorothioate linkage. In a related aspect, Z is a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU). In a related aspect, Z'^2 is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a phosphorothioate linkage. In a related aspect, In a related aspect, Z^1 is a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) comprising a 2'-O-methyl group. In a related aspect, Z^2 is a 5-(N-benzylcarboxyamide)-2′-deoxyuridine (BndU) comprising a phosphorothioate linkage. In a related aspect, A^1 is a 2'-O-methyladenosine. In a related aspect, A^2 is a deoxyadenosine comprising a phosphorothioate linkage.

[0066] In another aspect, this disclosure provides for a composition comprising two nucleic acid molecules selected from the group consisting of any one of the nucleic acid molecules described herein.

[0067] In a related aspect, the composition is in the form selected from the group selected from a solution, a powder, a gel and an emulsion.

[0068] In a related aspect, the two nucleic acid molecules are covalently linked or non-covalently linked.

[0069] In another aspect, the nucleic acid molecule inhibits PDGF-mediated phosphorylation of a PDGF receptor.

[0070] In another aspect, this disclosure provides for a pharmaceutical composition comprising at least one nucleic acid molecule described herein and a pharmaceutically acceptable carrier.

[0071] In a related aspect, the pharmaceutical composition is for intravitreal injection.

[0072] In another aspect, this disclosure provides for a method for treating macular degeneration comprising administering to a subject with macular degeneration a therapeutically effective amount of the pharmaceutical composition comprising at least one nucleic acid molecule described herein and a pharmaceutically acceptable carrier.

[0073] In another aspect, this disclosure provides for a method for preventing macular degeneration comprising administering to a subject at risk for developing macular degeneration a therapeutically effective amount of the pharmaceutical composition comprising at least one nucleic acid molecule described herein and a pharmaceutically acceptable carrier.

[0074] In another aspect, this disclosure provides for a method for treating an ophthalmic condition comprising administering to a subject with an ophthalmic condition a therapeutically effective amount of the pharmaceutical composition comprising at least one nucleic acid molecule described herein and a pharmaceutically acceptable carrier.

[0075] In a related aspect, the ophthalmic condition is selected from the group consisting of retinitis, macular degeneration, choroiditis, retinopathy, hypertensive retinopathy, diabetic retinopathy, chronic dry eye, AIDS-related vision loss, amblyopia, hemianopia, retinal vein occlusions, trachoma, keratoconus, chorioretinal inflammation, central serous retinopathy, uveitis, retinal dystrophy, edema, glaucoma, and cataract.

[0076] In a related aspect, the macular degeneration is age-related macular degeneration.

[0077] In a related aspect, the macular degeneration is dry age-related macular degeneration or wet age-related macular degeneration.

[0078] In another aspect, this disclosure provides for a method for treating fibrosis comprising administering to a subject with fibrosis a therapeutically effective amount of the pharmaceutical composition comprising at least one nucleic acid molecule described herein and a pharmaceutically acceptable carrier.

[0079] In a related aspect, the fibrosis is selected from pulmonary fibrosis, renal fibrosis, and cystic fibrosis.

[0080] In another aspect, this disclosure provides for a method for treating a cardiovascular disease comprising administering to a subject with the cardiovascular disease a therapeutically effective amount of the pharmaceutical composition comprising at least one nucleic acid molecule described herein and a pharmaceutically acceptable carrier.

[0081] In a related aspect, the cardiovascular disease is selected from atherosclerosis, restenosis, a cardiac hypertrophy related condition, and a vascular disorder.

[0082] In another aspect, this disclosure provides for a method for treating cancer comprising administering to a subject with cancer a therapeutically effective amount of the pharmaceutical composition comprising at least one nucleic acid molecule described herein and a pharmaceutically acceptable carrier.

[0083] In a related aspect, the cancer is selected from bladder cancer, lung cancer, breast cancer, melanoma, colon and rectal cancer, lymphoma, endometrial cancer, pancreatic cancer, liver cancer, renal cancer, prostate cancer, leukemia, and thyroid cancer.

[0084] In a related aspect, anyone of the nucleic acid molecule described herein is an aptamer.

[0085] In another aspect, the present disclosure provides methods for preventing, treating, and/or ameliorating a disease or condition mediated by PDGF and/or VEGF. In some embodiments, a method comprises administering a PDGF aptamer, a VEGF aptamer, and/or a VEGF/PDGF-B aptamer construct, or pharmaceutical compositions comprising any of these, to a subject, such as a mammal. In some embodiments, the subject is a human. Specifically, methods for treating, preventing, and/or ameliorating fibrosis, atherosclerosis, macular degeneration, diabetic retinopathy, and/or cancer are provided. In some embodiments, a disease or condition mediated by PDGF and/or VEGF is one in which PDGF and/or VEGF activity may directly or indirectly contribute to the disease or condition. Such diseases or conditions include, but are not limited to, fibrosis, atherosclerosis, macular degeneration, diabetic retinopathy, and cancer. In some embodiments, the disease or condition to be treated, prevented, and/or ameliorated is age-related macular degeneration (AMD), diabetic retinopathy, or other ocular diseases, such as glaucoma, chronic dry eye, AIDS-related vision loss, amblyopia, hemianopia, retinal vein occlusions, trachoma, keratoconus, chorioretinal inflammation, central serous retinopathy, uveitis, retinitis, hypertensive retinopathy, retinal dystrophy, etc. In some embodiments, the disease or condition to be treated, prevented, and/or ameliorated is renal fibrosis or renal cancer.

[0086] In some embodiments, aptamers and aptamer constructs disclosed herein have potential applications ranging from biomarker discovery and diagnostics (Ostroff, R.M., *et al.*

(2010) PLoS One 5:e15003; Mehan, M., *et al.* (2012) PLoS One 7:e35157) to histochemistry and imaging (Gupta, S., *et al.* (2011) Appl. Immunohistochem. Mol. Morphol. 19:273).

[0087] In some embodiments, a therapeutic effect (*e.g.*, treating, preventing, and/or ameliorating fibrosis, atherosclerosis, macular degeneration, or cancer, etc.) may be achieved by administering a PDGF aptamer, a VEGF aptamer, and/or a PDGF/VEGF aptamer construct such that the aptamer or aptamer construct is exposed to, and can bind to, PDGF and/or VEGF. In some embodiments, such binding occurs regardless of the method of delivery of the aptamer to the subject being treated. In some embodiments, the therapeutic effect may be achieved by administering the PDGF aptamer, VEGF aptamer, or PDGF/VEGF aptamer construct such that it is exposed to, and binds to, PDGF and/or VEGF and prevents or reduces the binding of PDGF and/or VEGF to one or more cell receptors.

[0088] In some embodiments, the binding of a PDGF aptamer to PDGF-BB or PDGF-AB interferes with the binding of PDGF-BB or PDGF-AB to the PDGF-α receptor. In some embodiments, the binding of a PDGF aptamer to PDGF-BB or PDGF-AB interferes with the binding of PDGF-BB or PDGF-AB to the PDGF-β receptor. In some embodiments, a PDGF aptamer to PDGF-BB or PDGF-AB reduces phosphorylation of a PDGF receptor (such as PDGF-α receptor and/or PDGF-β receptor).

[0089] In some embodiments, the binding of a VEGF aptamer to VEGF-121, VEGF-110, VEGF-165, VEGF-189, or another alternatively spliced or functionally-active proteolytic fragment of VEGF interferes with the binding of the growth factor to VEGFR-1 (Flt-1). In some embodiments, the binding of a VEGF aptamer to VEGF-121, VEGF-110, VEGF-165, VEGF-189, or another alternatively spliced or functionally-active proteolytic fragment of VEGF, interferes with the binding of the growth factor to VEGFR-2 (KDR). In some embodiments, a VEGF aptamer reduces phosphorylation of a VEGF receptor (such as VEGF-1 receptor and/or VEGF-1 receptor).

[0090] In some embodiments, a PDGF/VEGF aptamer construct reduces the level of phosphorylation of a PDGF receptor (such as PDGF-α receptor and/or PDGF-β receptor) and reduces the level of phosphorylation of a VEGF receptor (such as VEGFR-1 and/or VEGFR-2). In some embodiments, a PDGF aptamer, a VEGF aptamer, or a PDGF/VEGF aptamer construct reduces signaling along the signal transduction pathway of a PDGF receptor and/or a VEGF receptor.

[0091] In some embodiments, a PDGF aptamer, a VEGF aptamer, or a PDGF/VEGF aptamer construct is administered with one or more additional active agents. Such administration may be sequential or in combination.

[0092] In some embodiments, an *in vitro* diagnostic method comprises contacting a PDGF aptamer with a sample suspected of comprising PDGF. In some embodiments, an *in vivo* diagnostic method comprises administering a suitably labeled PDGF aptamer to an individual suspected of having a PDGF-mediated disease or disorder, wherein the labeled PDGF aptamer is detected for the purpose of diagnosing or evaluating the health status of the individual. The label used may be selected in accordance with the imaging modality to be used.

[0093] In some embodiments, an *in vitro* diagnostic method comprises contacting a VEGF aptamer with a sample suspected of comprising VEGF. In some embodiments, an *in vivo* diagnostic method comprises administering a suitably labeled VEGF aptamer to an individual suspected of having VEGF-mediated disease or disorder, wherein the labeled VEGF aptamer is detected for the purpose of diagnosing or evaluating the health status of the individual. The label used may be selected in accordance with the imaging modality to be used.

[0094] In some embodiments, an *in vitro* diagnostic method comprises contacting a PDGF/VEGF aptamer construct with a sample suspected of comprising PDGF and/or VEGF. In another aspect, the present disclosure provides an *in vivo* diagnostic method comprising obtaining a suitably labeled PDGF/VEGF aptamer construct, injecting the labeled PDGF/VEGF aptamer construct into an individual suspected of having a PDGF/VEGF-mediated disease or disorder, and detecting the labeled PDGF/VEGF aptamer construct for the purpose of diagnosing or evaluating the health status of the individual. The label used may be selected in accordance with the imaging modality to be used.

[0095] In some embodiments, the present invention provides aptamer constructs comprising a PDGF aptamer and a VEGF aptamer.

[0096] In some embodiments, the present disclosure provides an aptamer that efficiently binds to a protein predominantly through hydrophobic interactions.

[0097] In some embodiments, the present disclosure provides an aptamer-protein complex, wherein the aptamer binds to the protein substantially through hydrophobic interactions.

[0098] In some embodiments, the present disclosure provides an aptamer capable of forming a co-crystal complex with a protein target, wherein the complex comprises fewer than 7 hydrogen bonds.

[0099] In some embodiments, the present disclosure provides an aptamer capable of forming a co-crystal complex with a protein target, wherein the complex comprises a pseudoknot domain involving 16 nucleotides or less.

[00100] In some embodiments, the present disclosure provides an aptamer capable of binding to a protein target, wherein the aptamer binds to the protein target with less than or equal to 1 polar contact per 100 $Å^2$ of interface area, wherein the polar contact comprises one or more hydrogen bonds and one or more charge-charge interactions, and wherein the interface area is a fraction of the protein surface area occupied by the aptamer.

[00101] In another aspect, this disclosure provides for a nucleic acid molecule comprising the nucleic sequence: 5' - C-C-G-Z-Z-C^1-A-A-G^1-Z-G-C^1-Z-Z-G^1-Z-A^1-G^1-G-A-Z-Z^1-Z-A^2-A^2-A^2-Z-G^1-G^1 -3' (SEQ ID NO: 836) wherein, C^1 is independently, for each occurrence, selected from the group consisting of a cytidine, a deoxycytidine and a 2'-O-methylcytidine;G^1 is independently, for each occurrence, selected from the group consisting of a guanosine, a deoxyguanosine and a 2'-O-methylguanosine; Z is independently, for each occurrence, selected from the group consisted of a uridine, a deoxyuridine and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU); Z^1 is independently, for each occurrence, selected from the group consisted of a uridine, a deoxyuridine, a 2'-O-methyluridine, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU)and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group; A^1 is independently, for each occurrence, selected from the group consisting of an adenosine, a deoxyadenosine and a 2'-O-methyladenosine; A^2 is independently, for each occurrence, selected from the group consisting of an adenosine, an adenosine comprising a phosphorothioate linkage, a deoxyadenosine and a deoxyadenosine comprising a phosphorothioate linkage; and wherein, the nucleic acid molecule comprises at least one, two, three, four or five phosphorothioate linkages.

[00102] In a related aspect, C^1 is a 2'-O-methylcytidine. In a related aspect, G^1 is a 2'-O-methylguanosine. In a related aspect, Z is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU). In a related aspect, Z^1 is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group. In a related aspect, A^1 is a 2'-O-methyladenosine. In a related aspect, A^2 is a deoxyadenosine comprising a phosphorothioate linkage.

BRIEF DESCRIPTION OF THE DRAWINGS

[00103] **Figure 1** shows (A) determination of K_d values for three slow off rate modified aptamers and aptamer E10030; (B) inhibition of PDGF-BB-stimulated phosphorylation of PDGFRβ by three PDGF aptamers described herein and a scrambled control oligonucleotide.; (C) the K_d ratios for modified aptamers versus the parent aptamer , 4149-8_130, in which a

particular Bn-dU nucleobase has been replaced by another modified dU nucleobase, relative to the parent aptamer (a value of 1 indicates the modified aptamer inhibits phosphorylation of PDGFRβ equally as well as the parent, while a value >1 indicates the modified aptamer has less potent inhibitory activity compared to the parent).

[00104] Figure 2 shows (A) a table of aptamer 4149-8_260 intramolecular contacts and aptamer-PDGF contacts; (B) a representation of nonpolar intramolecular and aptamer-PDGF contacts; and (C) a representation of polar intramolecular and aptamer-PDGF contacts; as described in Example 2. Hydrophobic (nonpolar) interactions include π- π interactions (both face-to-face and edge-to-face aromatic interactions) and van der Waals contact (vW). Polar interactions include hydrogen bonds (dashed lines) and charge-charge interactions (solid lines. Certain aptamer residues (e.g., dC4, dG6, dA9, dC10, dC12, dG13, dC14, dG15, dG22, dC23 and 2'-O-methyl G24) participate in canonical base pairing and base stacking, as shown in B and C, and 2'-O-methyl A11 is extruded.

[00105] Figure 3 shows (A) the consensus sequence for a set of clones from the SELEX pool as determined by 454 pyrosequencing and nucleotide frequency at each position, and the sequences for six of the clones; and (B) K_d values for modified aptamers based on parent aptamer 4149-8, which were modified as shown, as described in Example 1.

[00106] Figure 4 illustrates certain stereoviews of a PDGF-BB: 4149-8_260 complex, as described in Example 2.

[00107] Figure 5 shows (A) binding affinity of various aptamers for different PDGF dimeric isoforms, in the presence and absence of 200 nM tRNA; and (B) an alignment of the amino acid sequences for the mature forms of PDGF-A, -B, -C and -D; as described in Example 3. Amino acid residues for PDGF-A that are shown in bold are involved in propeptide binding, and amino acid residues for PDGF-B that are shown in bold are involved in PDGFRβ binding (Shim *et al*, (2010) Proc. Natl. Acad. Sci. USA 107(25):11307). Box shading indicates residues involved in aptamer 4149-8_260 binding to PDGF-B chain 1 (dark shading), and PDGF-B chain 2 (light shading).

[00108] Figure 6 shows (A) the K_d ratio for modified aptamers made by substituting each indicated position in aptamer 4149-8_38 (SED ID NO: 38) with a three-carbon C-3 linker, and K_d values for PDGF-BB binding, PDGF-AB binding, as well as cellular IC_{50} for five modified aptamers based on parent aptamer 4149-8; and (B) K_d ratios for modified aptamers based on parent aptamer 4149-8_130 (SEQ ID NO: 130), in which a particular Bn-dU nucleobase has been replaced by another modified dU nucleobase, relative to the parent

aptamer (numbers <1 indicate the modified aptamer has greater affinity than the parent aptamer, and numbers >1 indicate the modified aptamer has lower affinity than the parent aptamer); as described in Example 1.

[00109] **Figure 7** shows (A) a ribbon diagram of the PDGF-BB homodimer bound to aptamer 4149-8_260 (SEQ ID NO: 211); and (B) a schematic representation and structural represent ation of the aptamer conformation when bound to a PDGF-B subunit; as described in Example 2. Non-canonical base pairs are coded based on the nomenclature of Leontis and Westhof (Leontis N.B. *et al.* (2003) Curr. Opin. Struct. Biol. 13(3):300). Dark gray = PDGF-B Chain 1; Light gray = PDGF-B Chain 2; Bn = Bn-dU, Pe = Pe-dU, Th = Th-dU.

[00110] **Figures 8A-L** illustrate certain structural features of the PDGF aptamer from the crystal structure of the PDGF-BB: 4149-8_260 aptamer complex, as described in Example 2. **Figure 8A** illustrates the aptamer structure, showing domains, basepairing and stacking interactions. The stems of the miniknot deviate significantly from B-form DNA due to substantial buckling and propeller angles as well as helical underwinding. Bn20 is the hinge that interfaces with the 5' stem through stacking with U8. **Figure 8B** illustrates Stem 1 (S1) end view. **Figure 8C** illustrates S1 and L2 side views. The modified nucleotides form a hydrophobic cluster with Bn2, Bn7 and Bn8 from the 5' stem interacting with Bn16, Pe17, Th18 and Bn20 from the miniknot. Bn8 makes edge-to-face π-π interactions with Bn16 and Bn20. The non-canonical dU-dU base pair utilizes H-bonding to the amide linker of Bn20. **Figure 8D** illustrates the details of the non-canonical base pair between Pe-dU17 and Bn-dU20. **Figure 8E** illustrates aromatic interactions stabilizing the base of miniknot S1. **Figure 8F** illustrates a base triple which is formed between the C10-G15 Watson-Crick pair in the S1 and L 2 nucleotide, A21. The Leontis-Westhof classification of this base triple is *cis* Watson-Crick/Watson-Crick, *trans* Sugar edge/ Hoogsteen (Leontis N.B. *et al.* (2003) Curr. Opin. Struct. Biol. 13:300). The base triple is not planar as there is a 34° propeller twist angle between A21 and G15 as well as considerable buckling and propeller twisting between the Watson-Crick base pair (Table 5). **Figure 8G** illustrates residue mA11, the single extruded base in L1 and the backbone turn. **Figure 8H** illustrates an axial view of S2. **Figure 8I** illustrates an axial view of the 5' stem motif which highlights the significant deviation from B-form DNA. The global C1'-C1' helical parameters indicate the dU-dU pair is overtwisted (40°) resulting in a ~124° bend in the backbone which flattens to near linear (~172°) between Bn-dU1 and Bn-dU2. The significant radial displacement between Bn-dU7-Bn-dU8 and near zero displacement between Bn-dU2-dA3 results in greater stacking overlap between bases 2-4 and 6-7. **Figure 8J** which illustrates the non-canonical base pair between Bn-dU2 and Bn-

dU8. **Figure 8K** illustrates the interdomain junction formed by modified nucleotides. **Figure 8L** illustrates Bn8 making edge-to-face π-π interactions with Bn16 and Bn20 that define the topology of the interdomain junction. The deleterious impact of substituting Bn-dU at position 8 of aptamer 4149-8_260 (SEQ ID NO: 211) with an iB-dU (SEQ ID NO: 255) is evident in the space-filling images shown in **Figure 8M** and **Figure 8N**. Bn8 (**8M**) is capable of making energetically favorable π-π interactions with neighboring aromatic groups and allows the SOMAmer to pack more tightly. In contrast, iB8 (**8N**) is not aromatic and thus lacks the ability for π-stacking interactions with neighboring aromatic groups. Additionally, the iB group is not as large and leaves a hole in the middle of the hydrophobic cluster.

[00111] **Figure 9** illustrates certain protein-aptamer interactions, as described in Example 2. In **Figure 9A**, Bn-dU1 occupies a pocket under a salt bridge at the homodimer interface. The U1 base makes hydrogen bonds to the protein backbone at Val39 while the benzyl ring is sandwiched between the aliphatic side chain of Arg 56 and the disulfide bond of Cys43-Cys52. In **Figure 9B**, Bn2 has an edgewise interaction with Trp40 and is nestled between the methylene side chains of Asn55 and Leu38. **Figure 9C** illustrates that the aromatic ring of Bn7 tucks up against the aliphatic portions of Asn54 and Asn55 side chains. **Figure 9D** illustrates that the Leu38 and Ile75 side chains present a hydrophobic surface for the benzyl ring of Bn-dU8 to contact the protein. **Figure 9E** illustrates that Bn16 has edge-to-face π-stacking with Trp40 and van der Waals contact with the aliphatic region of Arg73. **Figure 9F** illustrates that Pe17 is surrounded by the hydrophobic side chains of Leu38, Trp40, Arg73 and Ile75. Arg73 makes a hydrogen bond to the amide linker of Pe-dU17 and a charge-charge interaction with the aptamer backbone. **Figure 9G** illustrates Th18 encircled by the hydrophobic side chains of Arg73, Ile75 and Phe84. **Figure 9H** illustrates that stacking interactions between the protein and the aptamer are present between Pro82 and Bn20 and U8, and Phe84 makes edge-to-face contact with U20. Bn20 makes additional hydrophobic contact to Ile77 and Lys80.

[00112] **Figure 10** shows the six co-crystal structures of traditional aptamers (PDB IDs: vWF, 3HXO; Thrombin, 3QLP; GlnRs tRNA, 1EXD; Human IgG, 3AGV; MS2 coat protein, 6MSF; NF-kB, 1OOA)that were analyzed for the number of polar contacts (hydrogen bonds plus charge-charge interactions), and the contact surface area. The results are plotted *versus* the reported binding affinities for these six aptamer-target complexes (dark gray bars) and for three SOMAmers (light gray bars) including the PDGF-SL5 (4149-8_260) complex and two unpublished SOMAmer-target structures. The relationship between the number of polar

contacts and the contact surface area for the six traditional aptamers was analyzed by linear regression, and the 99% confidence interval is indicated by the gray shading on the floor of the figure. **Figure 10B** illustrates shape complementarity of the PDGF-SOMAmer complex as exhibited by Pe-dU17 and Th-dU18. Left, PDGF chain 1 is shown as a dark gray surface, Pe-dU17 and Th-dU18 are shown as space-filling representations. Right, same view as left panel, except Pe-dU17 is shown as stick representation. **Figure 10C** illustrates detail of Bn-dU1 interaction with PDGF, with PDGF chain 1 shown as a dark gray surface, PDGF chain 2 shown as a light gray surface, and Bn-dU1 shown as a space-filling representation.

[00113] Figure 11 shows a comparison of SL5 and PDGFRβ binding to PDGF-BB. (*A*) Receptor co-crystal showing PDGF homodimer (chain 1, medium gray; chain 2, light gray) and receptor extracellular domain colored dark gray (from Shim, A.H., *et al.* (2010) Proc. Natl. Acad. Sci. USA 107(25):11307). (*B*) Complex of PDGF homodimer (chain 1, medium gray; chain 2, light gray) and SL5 (dark gray).(*C*) Amino acid sequence of the PDGF-B mature form. Box shading indicates contact residues to 4149-8_260, PDGFRβ or both. PDGF residues that make a 4 Å contact with 4149-8_260, but do not contact PDGFRβ are boxed with no shading. PDGF residues that make 4 Å contact with PDGFRβ, but do not contact 4149-8_260 are boxed with dark gray shading. PDGF residues that make 4 Å contact with both 4149-8_260 and PDGFRβ are boxed with medium gray shading.

[00114] Figure 12 illustrates certain exemplary C-5 pyrimidine modifications that may be incorporated into aptamers, such as slow off-rate aptamers wherein R"" is selected from the group consisting of a branched or linear lower alkyl (C1-C20); hydroxyl (OH), halogen (F, CL, Br, I); nitrile (CN); boronic acid (BO_2NH_2); carboxylic acid (COOH); carboxylic acid ester (COOR"); primary amide ($CONH_2$); N-alkylsulfonamide (SONHR"); wherein R", R"' are independently selected from a group consisting of a branched or linear lower alkyl (C1-C2)); phenyl (C6H5); an R"" substituted phenyl ring (R""C6H4); 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"' = (CH2)n; wherein n= 2-10.

[00115] Figure 13 shows a representative plot of the inhibition of PDGF-BB-induced PDGF Rβ phosphorylation in Hs27 fibroblasts with SOMAmers 4149-8_379 (labeled as OH-4149-8_379) or 5' amino-linker modified SOMAmer 4149-8_379 (labeled as N-4149-8_379), as described in Example 4.

Date Reçue/Date Received 2021-04-30

[00116] Figure 14 shows the consensus sequence for a set of PDGF-binding clones from the SELEX pool as determined by 454 pyrosequencing and nucleotide frequency at each position, as described in Example 5. Nucleotide position and consensus sequence for the 5169-4 aptamer family is shown in the top two rows. Rows A, C, G and P (where P is Nap-dU) indicate the frequency at which these nucleotides are observed in the 5169-4 aptamer family at each of the 21 positions that define the minimal sequence required for high affinity binding. In the consensus sequence, multiple nucleotide consensus is indicated with the following single letter code: R = A or G; M = A or C; K = G or P; V = A, C or G; N = any nucleotide.

[00117] Figure 15 shows K_d ratios for modified aptamers based on parent aptamer 4867-31_143, in which a particular Nap-dU nucleobase has been replaced by another modified dU nucleobase, relative to the parent aptamer (numbers <1 indicate the modified aptamer has greater affinity than the parent aptamer, and numbers >1 indicate the modified aptamer has lower affinity than the parent aptamer); as described in Example 7.

[00118] Figure 16 shows the consensus sequence for a set of VEGF-binding clones from the SELEX pool as determined by 454 pyrosequencing and nucleotide frequency at each position, as described in Example 7. Nucleotide position and consensus sequence for the 4867-31 aptamer family is shown in the top two rows. Rows A, C, G and T/Z indicate the frequency at which these nucleotides are observed in the 4867-31 aptamer family at each of the 29 conserved positions.

Date Reçue/Date Received 2021-04-30

[00119] **Figure 17** shows percent VEGFR2 phosphorylation in human umbilical vein endothelial cells (HUVECs) stimulated with VEGF-121 or VEGF-165 and VEGF aptamers 4867-31_43 and 4867-31_192, as described in Example 9.

[00120] **Figure 18** shows (A) inhibition of PDGF-induced PDGF Rβ phosphorylation in Hs27 fibroblasts with PDGF aptamer 4149-8_379 (open circles) and PDGF/VEGF aptamer construct 4149-8_401 (closed circles); and (B) inhibition of VEGF-induced VEGF R2 phosphorylation in HUVECs with VEGF aptamer 4867-31_192 (open circles) and PDGF/VEGF aptamer construct 4149-8_401 (closed circles); as described in Example 11.

[00121] **Figure 19** shows simultaneous binding of PDGF and VEGF by PDGF/VEGF aptamer construct SL1012 (20 kDa PEG-N-4149-8_401) on (A) microtiter plates coated with the VEGF with addition of biotinylated PDGF, and (B) microtiter plates coated with PDGF with the addition of biotinylated VEGF, as described in Example 12.

[00122] **Figure 20** shows simultaneous binding of PDGF and VEGF by PDGF/VEGF aptamer constructs (A) SL1012 (20 kDa PEG-N-4149-8_401) and (B) SL1013 (40 kDA PEG-N-4149-8-401), on microtiter plates that were coated with PDGF with the addition of biotinylated VEGF, as described in Example 12.

[00123] **Figure 21** shows the simultaneous binding of PDGF and VEGF by various PDGF/VEGF aptamer constructs on (A) microtiter plates coated with VEGF with the addition of biotinylated PDGF, and (B) microtiter plates coated with PDGF with the addition of biotinylated VEGF, as described in Example 12.

[00124] **Figure 22** shows (A) a plot of a number of polar contacts (defined as the sum of hydrogen bonds and charge-charge interactions) versus interface area for traditional aptamers (diamonds) and SOMAmers (circles) (the linear regression fit has an $R^2 = 0.91$ with a slope of 0.016; dashed lines represent the 99% confidence intervals of this trend, with the SOMAmers falling outside those boundaries), (B) a plot of free energy binding versus polar contacts for traditional aptamers (diamonds) and SOMAmers (circles) (the linear regression fit has an $R^2 = 0.64$ with a slope of 0.073), and (C) a table showing various thermodynamic properties and contact characteristics of six previous aptamer-protein crystal structures and three SOMAmer-protein crystal structures, including PDGF-BB: 4149-8_260, as described in Example 2. (C) Interaction features for aptamers and SOMAmers bound to protein targets (figure references: (a) Convery *et al.* (1998) Nat. Struct. Biol. 5(2):133; (b) Nomura *et al.*

Date Reçue/Date Received 2021-04-30

(2010) Nucleic Acids Res. 38(21): 7822; (c) Pagano *et al.* (2008) Biophys. J. 94(2):562; (d) Huang *et al.* (2003) Proc. Natl. Acad. Sci. USA 100(16):9268; (e) Huang *et al.* (2009) Structure 17(11):1476; (f) Bullock *et al.* (2000) Nat. Struct. Biol. 7(6):497. Free energy calculations were determined from the measured binding affinities for SOMAmers, or the published K_d values using the following temperatures: MS2, thrombin, NFkB, vWF, and GlnRs, room temperature (296 K); IgG, 298 K; SOMAmers, 310 K. SOMAmers show a trend toward higher binding affinities; average free energy of binding, or $-\Delta G$ value, is 11.4 ± 1.3 kcal/mol for the six aptamers and 14.3 kcal/mol ± 0.8 kcal/mol for the three SOMAmers. Protein contact atoms within 4 Å of each ligand were determined in PyMOL. Interface area calculations were made with PISA (aptamers) (Krissinel *et al.* (2007) J. Mol. Biol. 372(3):774) or PyMOL (SOMAmers) (DeLano (2002) The Pymol Molecular Graphics System, Delano Scientific, San Carlos, CA). Within this relatively small data set of crystallographically evaluated interactions, aptamers engage their targets with an average ligand efficiency of 0.21 ± 0.14 kcal/mol per nonhydrogen contact atom, compared to 0.16 ± 0.04 kcal/mol per non-hydrogen contact atom for SOMAmers. Free energies of binding per interface area are also similar, with an average value of 0.017 ± 0.009 $kcal \cdot mol^{-1} \cdot Å^{-2}$ for aptamers and 0.012 ± 0.001 $kcal \cdot mol^{-1} \cdot Å^{-2}$ for SOMAmers. The value of free energy of binding per polar contact, calculated from values in the table, is about twice as large for SOMAmers (average of 1.75 ± 0.36 kcal/mol per polar contact) as for aptamers (0.89 ± 0.56 kcal/mol per polar contact).

[00125] Figure 23 shows plots of the percent of full-length aptamer 4867-31_192 (250 nM starting concentration) remaining verus time when digested at 37°C for the indicated number of hours with 0.002 units/μL DNase I (A) or 0.24 units/μL DNase II (B) as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer band determined by desitometry.

[00126] Figure 24 shows plots of the percent full-length aptamer remaining (250 nM starting concentration) versus time when incubated at 37°C for the indicated number of hours with 0.24 units/μL DNase II as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer remaining, as indicated by aptamer identification no. (Aptamer ID No.), was determined by desitometry. The plot for aptamer ID no. 4867-31_192 is the same plot as shown in Figure 23B.

[00127] **Figure 25** shows plots of the percent full-length aptamer remaining (500 nM starting concentration) versus time when incubated at 37°C for the indicated number of hours in 90% vitreous humor obtained from New Zealand White rabbits as described in Example 14. (Aptamers were resolved by polyacrylamide gel electrophoresis and stained with SYBR Gold. Percent full-length aptamer remaining, as indicated by aptamer identification no. (Aptamer ID No.), was determined by desitometry.

[00128] **Figure 26** shows plots of the percent of full-length aptamer 5169-4_146 (250 nM starting concentration) remaining verus time when digested at 37°C for the indicated number of hours with 0.01 units/μL DNase I (A) or 0.12 units/μL DNase II (B) as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer was determined by desitometry.

[00129] **Figure 27** shows a plot of the percent full-length aptamer 5169-4_182 remaining (250 nM starting concentration) versus time when incubated at 37°C for the indicated number of hours with 0.01 units/μL DNase I as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer determined by desitometry.

Figure 28 shows plots of the percent of full-length aptamer remaining (500 nM starting concentration) verus time when incubated at 37°C for the indicated number of hours in 90% vitreous humor obtained from New Zealand White rabbits as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer, as indicated by aptamer identification no. (Aptamer ID No.), was determined by desitometry.

[00130] **Figure 29** shows plots of the percent full-length aptamer remaining (250 nM starting concentration) versus time when incubated at 37°C for the indicated number of hours with 0.01 units/μL DNase I as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer remaining, as indicated by aptamer identification no. (Aptamer ID No.), was determined by desitometry.

[00131] **Figure 30** shows a plot of the percent full-length aptamer 4149-8_379 remaining (250 nM starting concentration) versus time when incubated at 37°C for the indicated number of hours with 0.12 units/μL DNase II as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer determined by desitometry.

[00132] **Figure 31** shows plots of the percent of full-length aptamer remaining (250 nM starting concentration) verus time when incubated at 37°C for the indicated number of hours with 0.12 units/μL DNase II as described in Example 14. Digestion products were separated from full-length aptamer by polyacrylamide gel electrophoresis and bands visualized with SYBR Gold. Percent full-length aptamer, as indicated by aptamer identification no. (Aptamer ID No.), was determined by desitometry. The plot for aptamer ID no. 4149-8_379 is the same plot as shown in Figure 30.

DETAILED DESCRIPTION

[00133] Reference will now be made in detail to representative embodiments of the invention. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that the invention is not intended to be limited to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents that may be included within the scope of the present invention as defined by the claims.

[00134] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in and are within the scope of the practice of the present invention. The present invention is in no way limited to the methods and materials described.

[00135] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art(s) to which this invention belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

[00136]

[00137] As used in this disclosure, including the appended claims, the singular forms "a," "an," and "the" include plural references, unless the content clearly dictates otherwise, and are used interchangeably with "at least one" and "one or more." Thus, reference to "an aptamer" includes mixtures of aptamers, and the like.

Date Reçue/Date Received 2020-06-26

[00138] As used herein, the term "about" represents an insignificant modification or variation of the numerical value such that the basic function of the item to which the numerical value relates is unchanged.

[00139] 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 does not include only those elements but may include other elements not expressly listed or inherent to such process, method, product-by-process, or composition of matter.

[00140] As used herein, the term "nucleotide" refers to a ribonucleotide or a deoxyribonucleotide, or a modified form thereof, as well as an analog thereof. Nucleotides include species that include purines (*e.g.*, adenine, hypoxanthine, guanine, and their derivatives and analogs) as well as pyrimidines (*e.g.*, cytosine, uracil, thymine, and their derivatives and analogs).

[00141] 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.

[00142] 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 some embodiments, 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.

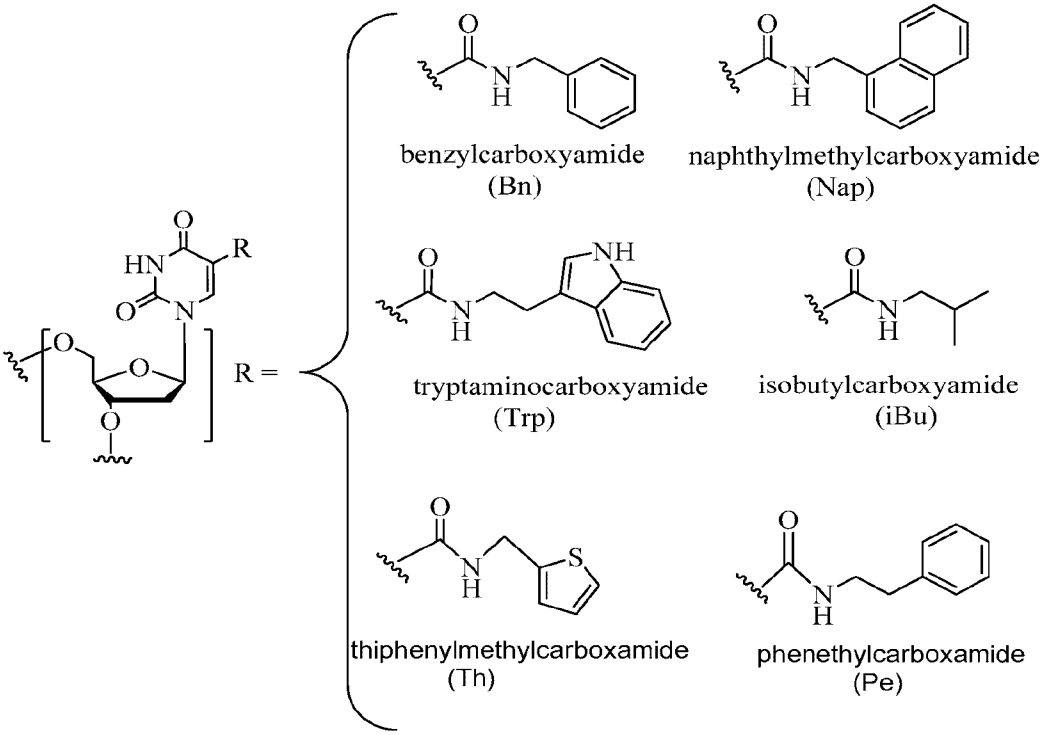
[00143] Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose 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)NR_2 ("amidate"), P(O)R, P(O)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 (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or aralkyl. 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.

[00144] 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.

[00145] 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.

[00146] 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 in FIG. 12. Examples of a C-5 modified pyrimidine include those described in U.S. Patent 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.



[00147] 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.

[00148] 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).

[00149] 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.

[00150] 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.

[00151] As used herein, A, C, G, U and T denote dA, dC, dG, dU and dT respectively, unless otherwise specified.

[00152] As used herein, "nucleic acid ligand," "aptamer," and "clone" are used interchangeably to refer to a non-naturally occurring nucleic acid that has a desirable action on a target molecule. A desirable action includes, but is not limited to, binding of the target, catalytically changing the target, reacting with the target in a way that modifies or alters the target or the functional activity of the target, covalently attaching to the target (as in a suicide inhibitor), and facilitating the reaction between the target and another molecule. In some embodiments, the action is specific binding affinity for a target molecule, such target molecule being a three dimensional chemical structure other than a polynucleotide that binds to the nucleic acid ligand through a mechanism which is independent of Watson/Crick base pairing or triple helix formation, wherein the aptamer is not a nucleic acid having the known

physiological function of being bound by the target molecule. Aptamers to a given target include nucleic acids that are identified from a candidate mixture of nucleic acids, where the aptamer is a ligand of the target, by a method comprising: (a) contacting the candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to other nucleic acids in the candidate mixture can be partitioned from the remainder of the candidate mixture; (b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and (c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids, whereby aptamers of the target molecule are identified. 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, non-target, components in a mixture or sample. An "aptamer" or "nucleic acid ligand" 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. "Aptamers" refer to more than one such set of molecules. Different aptamers can have either the same or different numbers of nucleotides. Aptamers may be DNA or RNA and may be single stranded, double stranded, or contain double stranded or triple stranded regions.

[00153] As used herein, a "SOMAmer" or Slow Off-Rate Modified Aptamer refers to an aptamer (including an aptamers comprising at least one nucleotide with a hydrophobic modification) with an off-rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes. In some embodiments, SOMAmers are generated using the improved SELEX methods described in U.S. Patent No. 7,947,447, entitled "Method for Generating Aptamers with Improved Off-Rates".

[00154] As used herein, "protein" is used synonymously with "peptide," "polypeptide," or "peptide fragment." A "purified" polypeptide, protein, peptide, or peptide fragment is substantially free of cellular material or other contaminating proteins from the cell, tissue, or cell-free source from which the amino acid sequence is obtained, or substantially free from chemical precursors or other chemicals when chemically synthesized.

[00155] As used herein, "co-crystal structure" or "co-crystal complex" is a crystal structure comprising two or more interacting molecules.

[00156] As used herein, "cardiovascular condition or disease" means a condition or disease related to heart and its vascular system. Some examples of such conditions or diseases are aneurysm, angina, arrhythmia, atherosclerosis, atrial fibrillation, congestive heart failure,

cardiomyopathy, coronary heart disease, restenosis, ischemia, left ventricular hypertrophy, peripheral vascular disease, myocardial infarction, hypertension, valvular heart disease and restrictive heart disease.

[00157] As used herein, "fibrosis" means a disease or condition caused by the formation of an excessive and abnormal amount of fibrous connective tissue in an organ resulting in thickening and scarring of connective tissue, leading to malfunction of the organ. Examples of such diseases and conditions are pulmonary fibrosis, renal fibrosis, liver fibrosis and cystic fibrosis.

[00158] As used herein, "AMD" or "age related macular degeneration" or "macular degeneration" means a condition of the eye that is caused by damage to the retina and results in a loss of vision in the center of the visual field, called the macula. AMD occurs in "wet" and "dry" forms. In "wet" AMD, blood vessels grow from the choroid behind the retina. In "dry" AMD, cellular debris (drusen) accumulates between the retina and choroid. In either form, the retina can become detached.

[00159] As used herein, "ophthalmic disease" or "ophthalmic condition" or "ocular disease" or "ocular condition" refers to any disease or condition that affects or involves occular neovascularization disorders, such as macular degeneration ("wet" and "dry"), retinopathy of prematurity, diabetic retinopathy, neovascular glaucoma, corneal neovascularization, proliferative diabetic retinopathy (the most severe stage of diabetic retinopathy), uveitis (an inflammatory condition of the eye that often leads to macular edema), cystoid macular edema following cataract surgery, myopic degeneration (a condition in which a patient with a high degree of nearsightedness develops choroidal neovascularization), inflammatory macular degeneration (a condition in which a patient with inflammation in the macular area due to infections or other causes, develops choroidal neovascularization), and iris neovascularization (a serious complication of diabetic retinopathy or retinal vein occlusion involving new blood vessel growth on the surface of the iris).

[00160] As used herein, "renal disease" or "renal condition" refers to any disease or condition that affects or involves proliferative kidney disorders such as glomerulonephritis, masangial proliferative renal diseases, polycystic kidney disease, kidney cancers, acute kidney failure, nephropathy, amyloidosis, edema, fibrosis, glomerular diseases, renal infarction and nephritis.

[00161] As used herein "cancer" means a disease or condition involving unregulated and abnormal cell growth. Some examples of common cancers are bladder cancer, lung cancer,

breast cancer, melanoma, colon and rectal cancer, lymphoma, endometrial cancer, pancreatic cancer, liver cancer, renal cancer, prostate cancer, leukemia and thyroid cancer.

[00162] As used herein, "modulate" means to alter, either by increasing or decreasing, the level of a peptide or polypeptide, or to alter, either by increasing or decreasing, the stability or activity of a peptide or a polypeptide. The term "inhibit" means to decrease the level of a peptide or a polypeptide or to decrease the stability or activity of a peptide or a polypeptide. In one embodiment, the protein which is modulated or inhibited is PDGF.

[00163] As used herein, the term "bioactivity" indicates an effect on one or more cellular or extracellular process (*e.g.*, via binding, signaling, etc.) which can impact physiological or pathophysiological processes.

[00164] As used herein, the terms "platelet-derived growth factor" and "PDGF" refer to PDGF A, B, C, and D isoforms and their homo or heterodimers AA, BB, AB, CC and DD. In some instances, context will determine which isoform and/or heterodimer of PDGF is meant. For example, in some embodiments, the PDGF aptamers described herein bind to the PDGF-B isoform and homo- and heterodimers comprising that isoform, although the aptamers may be described as binding to PDGF. Specifically included in the definition are naturally-occurring human PDGF AA, AB, and BB isoforms and variants. As used herein, PDGF includes all mammalian species of PDGF, including human, canine, feline, murine, primate, equine, and bovine. A nonlimiting exemplary human PDGF-B isoform precursor has the sequence shown in Swiss-Prot Accession No. P01127.1. Nonlimiting exemplary human PDGF-B isoform mature proteins may have the sequence of amino acids 82 to 241 or 82 to 190 of Swiss-Prot Accession No. P01127.1 (referred to herein as amino acids 1 to 160 or 1 to 109 of PDGF-B).

[00165] As used herein, "PDGF receptor" refers to a receptor that is bound by and activated by PDGF, such as PDGF receptor α and PDGF receptor β. PDGF receptors include the receptors of any mammalian species, including, but not limited to, human, canine, feline, murine, equine, primate, and bovine. A nonlimiting exemplary human PDGFRβ precursor has the sequence shown in Swiss-Prot Accession No. P09619.1. A nonlimiting exemplary human PDGFRβ mature protein has the sequence of amino acids 33 to 1106 of Swiss-Prot Accession No. P09619.1. A nonlimiting exemplary human PDGFRα precursor has the sequence shown in Swiss-Prot Accession No. P16234.1. A nonlimiting exemplary human PDGFRα mature protein has the sequence of amino acids 24 to 1089 of Swiss-Prot Accession No. P16234.1.

[00166] A "PDGF aptamer" is an aptamer that is capable of binding to and modifying the activity of PDGF. In some embodiments, a PDGF aptamer inhibits the activity of PDGF *in vitro*. In some embodiments, a PDGF aptamer inhibits the activity of PDGF *in vivo*. A nonlimiting exemplary activity of PDGF is PDGF-mediated phosphorylation of the PDGF receptor, such as PDGF receptor α (PDGF Rα) or PDGF receptor β (PDGF Rβ).

[00167] In some embodiments, the "VEGF aptamer" as defined herein is a monomer, dimer or a multimer construct, optionally connected by a linker.

[00168] As used herein, the terms "vascular endothelial growth factor", and "VEGF" refer to naturally-occurring VEGF, including isoforms and variants, such as VEGF-121, VEGF-145, VEGF-165, VEGF-183, VEGF-189, and VEGF-206. As used herein, VEGF includes all mammalian species of VEGF, including human, canine, feline, murine, primate, equine, and bovine. A nonlimiting exemplary human VEGF percursor has the sequence shown in Swiss-Prot Accession No. P15692.2. VEGF-121 is described, *e.g.,* in Tee *et al.* (2001) Biochem. J. 359:219; Bornes *et al.* (2004) J. Biol. Chem. 279:18717.

[00169] As used herein, "VEGF receptor" refers to a receptor that is bound by and activated by VEGF, such as VEGFR-1 and VEGFR-2. VEGF receptors include the receptors of any mammalian species, including, but not limited to, human, canine, feline, murine, equine, primate, and bovine. A nonlimiting exemplary human VEGFR-1 precursor has the sequence shown in Swiss-Prot Accession No. P17948.2. A nonlimiting exemplary human VEGFR-1 mature protein has the sequence of amino acids 27 to 1338 of Swiss-Prot Accession No. P17948.2. A nonlimiting exemplary human VEGFR-2 precursor has the sequence shown in Swiss-Prot Accession No. P35968.2. A nonlimiting exemplary human VEGFR-2 mature protein has the sequence of amino acids 20 to 1356 of Swiss-Prot Accession No. P35968.2.

[00170] A "VEGF aptamer" is an aptamer that is capable of binding to and modifying the activity of VEGF. In some embodiments, a VEGF aptamer inhibits the activity of VEGF *in vitro*. In some embodiments, a VEGF aptamer inhibits the activity of VEGF *in vivo*. Nonlimiting exemplary activities of VEGF include VEGF-mediated phosphorylation of the VEGF receptor, such as VEGFR-1 or VEGFR-2. In some embodiments, a VEGF aptamer is provided that competes for binding to VEGF-121 with aptamer 4867-31_183.

[00171] In some embodiments, the "VEGF aptamer" as defined herein is a monomer, dimer or a multimer construct, optionally connected by a linker.

[00172] The terms "PDGF/VEGF aptamer construct" and "VEGF/PDGF aptamer construct" are used interchangeably to refer to a construct comprising a PDGF aptamer and a VEGF aptamer. The order of the words "PDGF" and "VEGF" in "PDGF/VEGF aptamer construct"

and "VEGF/PDGF aptamer construct" is not indicative of how the aptamers are linked, *e.g.*, the order does not indicate which aptamer is located at the 5'-most position of an aptamer construct and which aptamer is located at the 3'-most position in the aptamer construct. In some embodiments, a PDGF/VEGF aptamer construct is capable of binding PDGF and VEGF simultaneously. In some embodiments, a PDGF/VEGF aptamer construct is capable of binding each of PDGF and VEGF separately. In a PDGF/VEGF aptamer construct, the PDGF aptamer and the VEGF aptamer may be linked covalently or non-covalently, *e.g.,* through a binding pair such as streptavidin and biotin. A PDGF/VEGF aptamer construct may comprise a linker between the PDGF aptamer and the VEGF aptamer.

[00173] As used herein, "disease or condition mediated by PDGF" refers to diseases or conditions in which PDGF activity may directly or indirectly lead to the disease or condition. Nonlimiting exemplary diseases or conditions mediated by PDGF include cardiovascular diseases such as atherosclerosis, restenosis, cardiac hypertrophy related conditions, and vascular disorders; ophthalmic diseases such as macular degeneration; fibrosis; and cancers.

[00174] As used herein, "disease or condition mediated by VEGF" refers to diseases or conditions in which VEGF activity may directly or indirectly lead to the disease or condition. Nonlimiting exemplary diseases or conditions mediated by VEGF include cardiovascular diseases, autoimmune diseases, inflammatory rheumatic diseases, ophthalmic diseases, and cancers at various stages in the disease process. Nonlimiting examples of cardiovascular diseases are atherosclerosis, restenosis, cardiac hypertrophy related conditions, and vascular disorders. Non-limiting examples of ophthalmic diseases are retinitis, macular degeneration, choroiditis, retinopathy, edema, glaucoma, and cataract.

[00175] As used herein, the term "pharmaceutically acceptable" 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. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered and includes, but is not limited to, such sterile liquids as water and oils.

[00176] As used herein, the term "pharmaceutically acceptable salt" or "salt" of a PDGF aptamer, VEGF aptamer or a PDGF/VEGF aptamer construct is a product of the disclosed compound that contains an ionic bond and is typically produced by reacting the disclosed 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.

[00177] As used herein, the term "pharmaceutical composition" is a formulation comprising a PDGF aptamer, a VEGF aptamer, or a PDGF/VEGF aptamer construct 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.

[00178] As used herein, the term "therapeutically effective amount" generally means the amount necessary to ameliorate at least one symptom of a disorder or condition to be prevented, reduced, or treated as described herein. The phrase "therapeutically effective amount" as it relates to the PDGF aptamers, VEGF aptamers, or PDGF/VEGF aptamer constructs of the present disclosure means the aptamer dosage that provides the specific pharmacological response for which the aptamer is administered in a significant number of individuals in need of such treatment. It is emphasized that a therapeutically effective amount of an aptamer that is administered to a particular individual in a particular instance will not always be effective in treating the conditions/diseases described herein, even though such dosage is deemed to be a therapeutically effective amount by those of skill in the art.

[00179] The terms "SELEX" and "SELEX process" are used interchangeably herein to refer generally to a combination of (1) the selection of nucleic acids that interact with a target molecule in a desirable manner, for example by 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 molecule.

[00180] 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. Patent 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. Patent No.

5,705,337 entitled "Systematic Evolution of Nucleic Acid Ligands by Exponential Enrichment: Chemi-SELEX."

[00181] 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. Patent No. 5,660,985, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," which describes oligonucleotides containing nucleotide derivatives chemically modified at the C5 and/or 2'-positions of pyrimidines. U.S. Patent 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 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.

[00182] SELEX can also be used to identify aptamers that have desirable off-rate characteristics. See U.S. Patent No.7,947,447, entitled "Method for Generating Aptamers with Improved Off-Rates," which describes improved SELEX methods for generating aptamers that can bind to target molecules. Methods for producing aptamers and photoaptamers 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 dissociate and do not reform, while complexes with slow dissociation rates remain intact. Additionally, the methods include the use of modified nucleotides in the production of candidate nucleic acid mixtures to generate aptamers with improved off-rate performance (see U.S. Patent Publication No. 2009/0098549, entitled "SELEX and PhotoSELEX"). (See also U.S. Patent No. 7,855,054 and U.S. Patent Publication No. 2007/0166740).

[00183] In some embodiments, methods of selecting aptamers that bind to a target molecule are provided, comprising: (a) preparing a candidate mixture of nucleic acids, wherein the candidate mixture comprises modified nucleic acids in which at least one pyrimidine in at least one, or in each, nucleic acid of the candidate mixture is chemically modified at the C5-position; (b) contacting the candidate mixture with a target molecule, wherein nucleic acids

Date Reçue/Date Received 2020-06-26

having an increased affinity to the target molecule relative to other nucleic acids in the candidate mixture bind the target molecule, forming nucleic acid-target molecule complexes; (c) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and (d) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched in nucleic acid sequences that are capable of binding to the target molecule with increased affinity, whereby an aptamer to the target molecule is identified. In certain embodiments, the method further includes performing a slow off-rate enrichment process.

[00184] "Target" or "target molecule" or "target" refers herein to any compound upon which a nucleic acid can act in a desirable manner. A target molecule can be a protein, peptide, nucleic acid, carbohydrate, lipid, polysaccharide, glycoprotein, hormone, receptor, antigen, antibody, virus, pathogen, toxic substance, substrate, metabolite, transition state analog, cofactor, inhibitor, drug, dye, nutrient, growth factor, cell, tissue, any portion or fragment of any of the foregoing, *etc.*, without limitation. Virtually any chemical or biological effector may be a suitable target. Molecules of any size can serve as targets. A target can also be modified in certain ways to enhance the likelihood or strength of an interaction between the target and the nucleic acid. A target can also include any minor variation of a particular compound or 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" or "target" is a set of copies of one type or species of molecule or multimolecular structure that is capable of binding to an aptamer. "Target molecules" or "targets" refer to more than one such set of molecules. Embodiments of the SELEX process in which the target is a peptide are described in U.S. Patent No. 6,376,190, entitled "Modified SELEX Processes Without Purified Protein."

EXEMPLARY PDGF APTAMERS

[00185] The PDGF aptamers of the instant disclosure were identified using the improved SELEX method for identifying aptamers having slow off-rates as described in Example 1, which describes a representative method for the selection and production of an aptamer that binds PDGF with a slow dissociation rate. A random DNA library composed of benzyl-dU (Bn-dU), dA, dC and dG was used for the selection. Using this method, the DNA aptamer to PDGF-BB designated as aptamer 4149-8_1 (SEQ ID NO: 1) was identified.

[00186] Using aptamer 4149-8_1 (SEQ ID NO: 1), studies were conducted to identify the minimum sequence length required to maintain strong affinity for PDGF. Systematic truncation from the 5' and 3' ends led to identification of a core motif consisting of 29 nucleotides (4149-8_38; SEQ ID NO. 38). Aptamer 4149-8_38 exhibited high affinity binding to PDGF-BB (K_d value of 20 pM; FIG. 6).

[00187] Additional sequencing studies were conducted on the sequence pool from which aptamer 4149-8_1 (SEQ ID NO: 1) was selected. 454 sequencing, which is a large-scale, high throughput method that uses parallel pyrosequencing, provides unbiased sample preparation and very accurate sequence analysis. The sequencing data was used to identify a consensus sequence for a PDGF aptamer as shown in FIG. 3. Furthermore, nucleotide substitution studies illustrated in FIG. 6 led to the discovery that six of eight BndU positions in the consensus sequence were desirable for PDGF binding, but four BndU positions could be replaced with dT with little or no loss of binding activity. A consensus sequence is shown in FIG 3A, along with a graphic representation of the nucleotide frequency at each position relative to the apatmer 4149-8_1 (SEQ ID NO: 1).

[00188] In some embodiments, a PDGF aptamer comprises the sequence:
5'-NZVSL$_n$S'V'ZACNN$_m$GCGZZZAZAGCG-3' (SEQ ID NO: 500),
wherein
V is selected from an A, C or G;
V' is selected from a C, G or Z, wherein V' is complementary to V;
S and S' are independently selected from a C or G, wherein S and S' are complementary to each other;
each N is independently selected from any naturally occurring or modified nucleotide;
each Z is independently selected from a modified pyrimidine;
L is selected from any naturally occurring or modified nucleotide, a hydrocarbon linker, a polyethylene glycol linker or a combination thereof;
n is 0 to 20; and m is 0 to 20; and wherein one or more nucleotide insertions are optionally included.

[00189] In some embodiments, a PDGF aptamer comprises the sequence:
5'-ZZVSL$_n$S'V'ZACNN$_m$GCGZZZAZAGCG-3' (SEQ ID NO: 501), wherein V, V', N, S, S', Z, L, n, and m are as defined above.

[00190] In some embodiments, a PDGF aptamer comprises the sequence:
5'-ZZVCL$_n$GV'ZACNMGCGZZZAZAGCG-3' (SEQ ID NO: 502),

wherein Z, V, V', N, Z, L, and n are are as defined above and M is selected from C and A.

[00191] In some embodiments, a PDGF aptamer comprises the sequence: 5'-ZZACL$_n$GZZACACGCGZZZAZAGCG-3' (SEQ ID NO: 503), wherein Z, L, and n are as defined above.

[00192] In some embodiments, a PDGF aptamer comprises the sequence: 5'-ZZACGACZACGZZACACGCGZZZAZAGCG-3' (SEQ ID NO: 504), wherein Z is as defined above.

[00193] In some embodiments, a PDGF aptamer comprises the sequence: 5'-ZVSL$_n$S'V'ZACNN$_m$GCGZZZAZAG-3' (SEQ ID NO: 507),

wherein

V is selected from an A, C or G;

V' is selected from a C, G or Z, wherein V' is complementary to V;

S and S' are independently selected from a C or G, wherein S and S' are complementary to other;

each N is independently selected from modified or unmodified nucleotide;

each Z is independently selected from a modified pyrimidine;

L is selected from a substituted or unsubstituted C_2-C_{20} linker and a modified or unmodified nucleotide;

n is 1 to 50; and m is 0 to 50; and

wherein one or more nucleotide insertions are optionally included.

[00194] In some embodiments, a PDGF aptamer comprises the sequence: 5'-Z'ZVSL$_n$S'V'ZACNN$_m$GCGZZZAZAGC-3' (SEQ ID NO: 508), wherein Z' is a modified pyrimidine or dT; and V, V', N, S, S', Z, L, n, and m are as defined above.

[00195] In some embodiments, a PDGF aptamer comprises the sequence: 5'-Z'ZVCL$_n$GV'ZACNMGCGZZZAZAGC-3' (SEQ ID NO: 509),

wherein Z, Z', V, V', N, Z, L, and n are as defined above and M is selected from C and A.

[00196] In some embodiments, a PDGF aptamer comprises the sequence: 5'-Z'ZACL$_n$GZZACACGCGZZZAZAGC-3' (SEQ ID NO: 510), wherein Z, Z', L, and n are as defined above.

[00197] In some embodiments, a PDGF aptamer comprises the sequence: 5'-Z'ZACGACZACGZZACACGCGZZZAZAGC-3' (SEQ ID NO: 511), wherein Z and Z' are as defined above.

[00198] In some embodiments, a PDGF aptamer comprises the sequence:

5'-ZABL$_p$GYZABK$_q$GCGZZYDYAG-3' (SEQ ID NO: 505)

wherein each Z is, independently, a modified pyrimidine;

each B is independently selected from C and a substituted or unsubstituted C_2-C_{10} linker;

each L is independently selected from a substituted or unsubstituted C_2-C_{10} linker, a hexaethylene glycol linker, and a modified or unmodified nucleotide, wherein p is 1 to 10;

each Y is independently selected from a modified or unmodified pyrimidine;

each K is independently selected from a substituted or unsubstituted C_2-C_{10} linker, a hexaethylene glycol linker, and a modified or unmodified nucleotide, wherein q is 1 to 5; and

D is selected from A and a substituted or unsubstituted C_2-C_{10} linker.

[00199] In some embodiments, a PDGF aptamer comprises the sequence:

5'-XZABL$_n$GYZABL$_n$GCGZZYDYAGBE-3' (SEQ ID NO: 506),

wherein X is selected from a modified or unmodified pyrimidine and a substituted or unsubstituted C_2-C_{10} linker, or is absent; and E is selected from G and a substituted or unsubstituted C_2-C_{10} linker, or is absent.

[00200] An aptamer construct comprising the sequences NZVS (SEQ ID NO 761) and S'V'ZACNN$_m$GCGZZZAZAGCG (SEQ ID NO: 762),

wherein

V is selected from an A, C or G; V' is selected from a C, G or Z, wherein V' is selected from a C, G or Z, wherein V' is complementary to V;

S and S' are independently selected from a C or G, wherein S and S' are complementary to each other;

N is independently selected from any naturally occurring or modified nucleotide;

Z is independently selected from a modified pyrimidine;

m is 1 to 20; and

wherein one or more nucleotide insertions are optionally included.

[00201] In some embodiments, Z is a modified uridine. In some embodiments, each Z is independently selected from the C-5 modified pyrimidines as defined herein. In some embodiments, each Z is independently 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.

[00202] In certain embodiments, portions of the PDGF and/or VEGF aptamer (Y) may not be necessary to maintain binding and certain portions of the contiguous PDGF and/or VEGF aptamer can be modified, including, but not limited to, replacement with a spacer or linker

moiety. In these embodiments, for example, Y can be represented as Y'-Q-Y"-Q'-Y"', wherein Y', Y" and Y"' are parts of a PDGF and/or VEGF aptamer or segments of different PDGF and/or VEGF aptamers and Q and/or Q' are spacers or linker molecules that modify certain nucleic acid features of the original PDGF and/or VEGF aptamer. When Q and Q' are not present, Y', Y", and Y"' represent one contiguous PDGF and/or VEGF aptamer (Y).

[00203] As used herein a "linker" is a molecular entity that connects two or more molecular entities through covalent bond or non-covalent interactions and can allow spatial separation of the molecular entities in a manner that preserves the functional properties of one or more of the molecular entities. A linker can also be known as a spacer. Appropriate linker sequences will be readily ascertained by those of skill in the art based upon the present disclosure.

[00204] As used herein, a linker can comprise one or more molecules or sub-components, selected from the group including, but not limited to, a polynucleotide, a polypeptide, a peptide nucleic acid, a locked nucleic acid, an oligosaccharide, a polysaccharide, an antibody, an affibody, an antibody mimic, an aliphatic, aromatic or heteroaromatic carbon molecule, a polyethylene glycol (PEG) molecule, a cell receptor, a ligand, a lipid, any fragment or derivative of these structures, any combination of the foregoing, or any other chemical structure or component.

[00205] In some embodiments, at least one L is a polyethylene glycol linker. In some embodiments, at least one L is a hexaethylene glycol linker. In some embodiments, L is a substituted or unsubstituted C_2-C_{10} linker. In some embodiments, p is 1, 2, 3, 4, 5, 6, 7, or 8. In some embodiments, p is 1, 2, or 3. In some embodiments, at least one K is a polyethylene glycol linker. In some embodiments, at least one K is a hexaethylene glycol linker. In some embodiments, K is a substituted or unsubstituted C_2-C_{10} linker. In some embodiments, q is 1 or 2. In some embodiments, q is 1.

[00206] In various embodiments, m may be 0 to 20, 0 to 19, 0 to 18, 0 to 17, 0 to 16, 0 to 15, 0 to 15, 0 to 14, 0 to 13, 0 to 12, 0 to 11, 0 to 10, 0 to 9, 0 to 8, 0 to 7, 0 to 6, 0 to 5, 0 to 4, or 0 to 3.

[00207] In some embodiments, L may be a linker such as an 18-atom hexaethylene glycol linker. In some embodiments, the L may be a combination of nucleotides and a linker. As a nonlimiting example, the following aptamers (SEQ ID NOs 67 and 69) include a hexaethylene glycol (Heg) linker:

(SEQ ID NO. 67) 5'-Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-3'

(SEQ ID NO. 69) 5'-Bn-Bn-A-C-G-Heg-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-3'

wherein Bn is benzyl-dU and Heg is a hexaethylene glycol linker.

[00208] In some embodiments, an N may be replaced by a linker, such as in the following aptamers:

(SEQ ID NO. 329) 5'-Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-C3-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-3'

(SEQ ID NO. 408) 5'-Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-C3-C-G-Bn-Bn-Bn-A-Bn-A-G-3'

wherein Bn is benzyl-dU, Heg is a hexaethylene glycol linker and C3 is a three carbon linker.

[00209] Further PDGF aptamers were identified using the improved SELEX method for identifying aptamers having slow off-rates as described in Example 5, which describes a representative method for the selection and production of an aptamer that binds PDGF with a slow dissociation rate. A random DNA library composed of napthyl-dU (Nap-dU), dA, dC and dG was used for the selection. PDGF aptamer 5169-4_26 was identified in the screen.

[00210] In some embodiments, an aptamer that specifically binds PDGF is provided, wherein the aptamer competes for binding to PDGF with PDGF aptamer 5169-4_26. In some such embodiments, the aptamer comprises at least one modified nucleoside comprising a hydrophobic nucleobase modification. Further, in some such embodiments, the hydrophobic nucleobase modification is a modified pyrimidine. In some embodiments, each modified pyrimidine may be independently 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-isobutylcarboxyamide)-2'-O-methyluridine, 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, and 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine).

[00211] In some embodiments, an aptamer that specifically bind PDGF is provided, wherein the aptamer comprises the sequence:

5'-ACAL$_n$ZGZAZGL$_m$ZLZ-3' (SEQ ID NO. 512);

wherein each Z is, independently, a modified pyrimidine; each L is independently selected from a substituted or unsubstituted C_2-C_{50} linker, a polyethylene glycol linker, and a modified or unmodified nucleotide; n is 1 to 5; and m is 1 to 10.

[00212] In some embodiments, each is independently 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-isobutylcarboxyamide)-2'-O-methyluridine, 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, and 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine). In some embodiments, at least one, at least two, at least three, at least four, or each Z is 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU). In some embodiments, n is 1, 2, 3, 4, or 5. In some embodiments, m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, n is 3. In some embodiments, m is 4. In some such embodiments, each L is independently selected from a modified nucleotide, an unmodified nucleotide, and a C_3 linker.

[00213] A C_2-C_{50} linker or spacer may be a backbone comprising a chain of 2 to 50 carbon atoms (C_2-C_{50}) (saturated, unsaturated, straight chain, branched or cyclic), 0 to 10 aryl groups, 0 to 10 heteroaryl groups, and 0 to 10 heterocyclic groups, optionally comprising an ether (-O-) linkage, (*e.g.*, one or more alkylene glycol units, including but not limited to one or more ethylene glycol units -O-(CH_2CH_2O)-; one or more 1,3-propane diol units -O-($CH_2CH_2CH_2O$)-, *etc.*); an amine (-NH-) linkage; an amide (-NC(O)-) linkage; and a thioether (-S-) linkage; *etc.*; wherein each backbone carbon atom may be independently unsubstituted (*i.e.*, comprising –H substituents) or may be substituted with one or more groups selected from a C_1 to C_3 alkyl, -OH, -NH_2, -SH, -O-(C_1 to C_6 alkyl), -S-(C_1 to C_6 alkyl), halogen, -OC(O)(C_1 to C_6 alkyl), -NH-(C_1 to C_6 alkyl), and the like. In some embodiments, a C_2-C_{50} linker is a C_2-C_{20} linker, a C_2-C_{10} linker, a C_2-C_8 linker, a C_2-C_6 linker, a C_2-C_5 linker, a C_2-C_4 linker, or a C_3 linker, wherein each carbon may be independently substituted as described above.

[00214] In some embodiments, one or more nucleosides of a PDGF aptamer comprise a modification selected from a 2'-position sugar modification (such as a 2'-amino (2'-NH_2), a 2'-fluoro (2'-F), or a 2'-O-methyl (2'-OMe)), a modification at a cytosine exocyclic amine, an internucleoside linkage modification, and a 5-methyl-cytosine. In some embodiments, a PDGF aptamer comprises a 3' cap, a 5' cap, and/or an inverted deoxythymidine at the 3' terminus.

[00215] In some embodiments, a PDGF aptamer comprises at least one modified internucleoside linkage. In some embodiments, at least one, at least two, at least three, at least four, or at least five internucleoside linkages are phosphorothioate linkages.

[00216] In some embodiments, a PDGF aptamer has a sequence selected from the sequences shown in Tables 1, 2 and 6 to 9 (SEQ ID NOS: 1 to 499 and 517 to 545). In some embodiments, a PDGF aptamer has a sequence selected from the sequences shown in Table 1 and the sequences shown in Tables 6 to 9 that bind PDGF with an affinity (K_d) of less than 10 nM. In some embodiments, a PDGF aptamer has a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequences shown in Tables 1, 2, and 6 to 9 (SEQ ID NOS: 1 to 1 to 499 and 517 to 545). In some embodiments, a PDGF aptamer has has a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequences shown in Table 1 and the sequences shown in Tables 6 to 9 that bind PDGF with an affinity (K_d) of less than 10 nM.

[00217] The terms "sequence identity", "percent sequence identity", "percent identity", "% identical", "% identity", and variations thereof, when used in the context of two nucleic acid sequences, are used interchangeably to refer to the number of nucleotide bases that are the same in a query nucleic acid or a portion of a query nucleic acid, when it is compared and aligned for maximum correspondence to a reference nucleic acid, divided by either (1) the number of nucleotide bases in the query sequence between and including the most 5' corresponding (*i.e.*, aligned) nucleotide base and the most 3' corresponding (*i.e.*, aligned) nucleotide base, or (2) the total length of the reference sequence, whichever is greater. Exemplary alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, 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.* (1987), Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience).

[00218] One example of an algorithm that is suitable for determining percent sequence identity is the algorithm used in the basic local alignment search tool (hereinafter "BLAST"), see, *e.g.* Altschul *et al.* (1990) J. Mol. Biol. 215:403 and Altschul *et al.* (1997) Nucleic Acids Res. 15:3389. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (hereinafter "NCBI"). The default parameters used in determining sequence identity using the software available from NCBI, *e.g.*, BLASTN (for nucleotide sequences) are described in McGinnis *et al.* (2004) Nucleic Acids Res. 32:W20.

[00219] As used herein, when describing the percent identity of a nucleic acid, such as a PDGF 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. Further, it is intended that a nucleotide base is considered "identical" for the purposes of determining percent identity, when the nucleotide base (1) is the same as the nucleotide base in the reference sequence, or (2) is derived from the nucleotide base in the reference sequence, or (3) is derived from the same nucleotide base from which the nucleotide base in the reference sequence is derived. For example, 5-methyl cytosine is considered to be "identical" to cytosine for the purposes of calculating percent identity. Similarly, the modified uridines shown in FIG. 12 are considered to be identical to one another for the purpose of determining percent identity. The reference sequence may be any one of the nucleotide sequences shown in SEQ ID NOS: 1 to 437.

[00220] In some embodiments, the present disclosure provides a PDGF aptamer that, upon binding PDGF, modulates a PDGF function. In some embodiments, a PDGF aptamer described herein inhibits PDGF-mediated phosphorylation of a PDGF receptor, such as PDGF Rα or PDGF Rβ. In some embodiments, a PDGF aptamer described herein inhibits PDGF-mediated phosphorylation of PDGF Rβ. In various embodiments, the PDGF aptamer modulates a PDGF function *in vivo*, such as inhibiting PDGF-mediated receptor phosphorylation *in vivo*. In various embodiments, the PDGF aptamer has a sequence selected from the sequences of SEQ ID NOS: 1 to 437. In various embodiments, the PDGF aptamer is selected from the aptamers shown in Tables 1 and 2. In various embodiments, the PDGF aptamer is selected from the aptamers shown in Table 1. In some embodiments, the PDGF aptamer comprises at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of a sequence selected from SEQ ID NOS: 1 to 1 to 499 and 517 to 545. In some embodiments, a PDGF aptamer consists of at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides that are identical in nucleobase sequence to a sequence selected from SEQ ID NOS: 1 to 1 to 499 and 517 to 545. In some embodiments, the PDGF aptamer comprises at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of an aptamer shown in Table 1, 2, 6, 7, 8 or 9. In some embodiments, the PDGF aptamer comprises at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of an aptamer shown in Table 1 or an aptamer shown in one of Tables 6 to 9 that binds PDGF with an affinity (K_d) of less than 10 nM. In some embodiments, a PDGF aptamer consists of at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of an aptamer shown in Table 1, 2, 6, 7, 8 or 9. In some embodiments, a PDGF aptamer consists of at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30

contiguous nucleotides of an aptamer shown in Table 1 or an aptamer shown in one of Tables 6 to 9 that binds PDGF with an affinity (K_d) of less than 10 nM.

[00221] In some embodiments, a PDGF aptamer has a nucleobase sequence selected from the sequences of SEQ ID NOS. 500 to 512; 761 and 762. In some embodiments, a PDGF aptamer has the sequence of any one of SEQ ID NOS: 1 to 1 to 499 and 517 to 545. In some embodiments, a PDGF aptamer is at least 95% identical, at least 90% identical, at least 85% identical, at least 80% identical, or at least 75% identical to any one of SEQ ID NOS: 1 to 499 and 517 to 545. In any of the embodiments herein, a PDGF aptamer may comprise additional nucleotides or other chemical moieties on the 5' end, the 3' end, or both the 5' and the 3' end of the aptamer.

[00222] The PDGF aptamer can contain any number of nucleotides in addition to the PDGF binding region. In various embodiments, the PDGF aptamer can 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, or up to about 20 nucleotides.

[00223] In some embodiments, the PDGF aptamer is selected from an aptamer that has similar binding characteristics and ability to treat PDGF associated atherosclerosis, macular degeneration, fibrosis, or cancer conditions as an aptamer selected from SEQ ID NOS: 1 to 499 and 517 to 545. In some embodiments, a PDGF aptamer binds to the same region of a PDGF-B monomer (in the context of a PDGF-BB or PDGF-AB dimer) as an aptamer selected from the aptamers shown in Tables 1, 2, and 6 to 9. In some embodiments, a PDGF aptamer binds to the same region of a PDGF-B monomer (in the context of a PDGF-BB or PDGF-AB dimer) as an aptamer selected from the aptamers shown in Table 1. In some embodiments, a PDGF aptamer binds to the same region of a PDGF-B monomer (in the context of a PDGF-BB or PDGF-AB dimer) as an aptamer selected from the aptamers shown in Table 6. In some embodiments, a PDGF aptamer binds to the same region of a PDGF-B monomer (in the context of a PDGF-BB or PDGF-AB dimer) as PDGF aptamer 4149-8_260. In some embodiments, a PDGF aptamer binds to a region of PDGF-B comprising amino acids 24 to 86 of PDGF-B. In some such embodiments, the PDGF aptamer competes for binding to PDGF with PDGF aptamer 4149-8_260. In some embodiments, a PDGF aptamer binds to PDGF-B with less than 15%, less than 14%, less than 13%, less than 12%, less than

11%, less than 10%, less than 9%, less than 8%, less than 7%, or less than 6% polar contacts to protein contact atoms. Polar contacts are defined as the sum of hydrogen bonds and charge-charge interactions. In some embodiments, a PDGF aptamer binds to PDGF-B with a ratio of polar contacts to interface area of less than 0.01, less than 0.009, less than 0.008, less than 0.007, or less than 0.006. In some embodiments, a PDGF aptamer binds to the same region of a PDGF-B monomer (in the context of a PDGF-BB or PDGF-AB dimer) as PDGF aptamer 5169-4_26.

[00224] In some embodiments, a PDGF aptamer has any combination of the following characteristics:

(a) binds to a region of PDGF-B comprising amino acids 24 to 86 of PDGF-B;

(b) competes for binding to PDGF with PDGF aptamer 4149-8_260;

(c) competes for binding to PDGF with PDGF aptamer 5169-4_26;

(d) binds to PDGF-B with a ratio of polar contacts to interface area of less than 0.01, less than 0.009, less than 0.008, less than 0.007, or less than 0.006; and/or

(e) binds to PDGF-B with less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, or less than 6% polar contacts to protein contact atoms.

[00225] The PDGF aptamer can be selected to have any suitable dissociation constant (K_d) for PDGF. In some embodiments, a PDGF aptamer has a dissociation constant (K_d) for PDGF of less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, less than 10 nM, less than 9 nM, less than 8 nM, less than 7 nM, less than 6 nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, or less than 1 nM. Dissociation constants may 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 in Example 3, below. In some embodiments, the PDGF aptamer is an aptamer with a K_d that is less than or equal to the K_d of an aptamer shown in any one of Tables 1, 2 or 6 to 9. In some embodiments, the PDGF aptamer is an aptamer with a K_d that is less than or equal to the K_d of an aptamer shown in Table 1 or Table 6.

[00226] Aptamer 4149-8_1 binds in a 1:1 stoichiometry with a PDGF monomer. Since PDGF forms a tight homodimer that is required for reaction with its target receptors, a more efficient inhibition of PDGF activity might be achieved by using a dimeric or other multimeric form of aptamer 4149-8_1. Thus, in some embodiments, the PDGF aptamer is a multimerization of any combination of the sequences of aptamer 4149-8_1, 4149-8_379, and SEQ ID NOS 500 to 512. In some embodiments, an aptamer construct comprises a first

aptamer selected from any of the PDGF aptamers described herein, and a second aptamer comprising any of the PDGF aptamers described herein, wherein the first aptamer and the second aptamer may be the same or different. The first aptamer and the second aptamer of the PDGF aptamer construct may be covalently or noncovalently linked. Nonlimiting exemplary linkages are known in the art and/or are described herein. In some embodiments, a PDGF aptamer construct may be capable of binding two PDGF monomers simultaneously. In some embodiments, a PDGF aptamer construct binds PDGF with an affinity (K_d) of less than 10 nM.

EXEMPLARY VEGF APTAMERS

[00227] The VEGF aptamers of the instant disclosure were identified using the improved SELEX method for identifying aptamers having slow off-rates as described in Example 7, which describes a representative method for the selection and production of an aptamer that binds VEGF with a slow dissociation rate.

[00228] We truncated a clone from a Nap-dU VEGF-121 SELEX experiment to a minimal sequence of 29 nucleotides. This SOMAmer binds to both VEGF-121 and VEGF-165 with high affinity (K_d values of 90 pM and 20 pM, respectively). The SOMAmer also potently inhibits the ability of both VEGF isoforms to induce VEGFR2 phosphorylation in human umbilical vein endothelial cells *in vitro* (see Example 9), supporting the notion that it binds to and blocks the receptor-binding domain on VEGF.

[00229] The present disclosure provides the first identification of an inhibitory aptamer to VEGF-121. Thus, the present VEGF aptamers represent broad inhibitors of VEGF, similar to protein-based drugs like bevacizumab (Avastin®), ranibizumab (Lucentis®) and aflibercept (Eylea®) (Papadopoulos *et al.* (2012) Angiogenesis 15:171; Yu *et al.* (2011) Biochem. Biophys. Res. Commun. 408:276. Thus, the present VEGF aptamers may more effectively inhibit VEGF signaling than Macugen®, which is a selective inhibitor of VEGF-165.

[00230] A truncated clone from the successful Nap-dU VEGF-121 SELEX experiment provided a sequence of 29 nucleotides. This aptamer (or SOMAmer) (4867-31 binds to both VEGF-121 and VEGF-165 with high affinity (K_d values of 90 pM and 20 pM, respectively). This SOMAmer also potently inhibits the ability of both VEGF isoforms to induce VEGFR2 phosphorylation in human umbilical vein endothelial cells *in vitro*, supporting the concept that it binds to and blocks the receptor-binding domain on VEGF.

[00231] Aptamer 4867-31_192 binds in a 1:1 stoichiometry with a VEGF monomer. Since VEGF forms a tight homodimer that is required for reaction with its target receptors, a more

efficient inhibition of VEGF activity might be achieved by using a dimeric or other multimeric form of aptamer 4867-31_192. Thus, in some embodiments, the VEGF aptamer is a multimerization of any combination of the sequences of aptamer 4867-31_192, SEQ ID NOS 513 to 516. In some embodiments, an aptamer construct comprises a first aptamer selected from any of the VEGF aptamers described herein, and a second aptamer comprising any of the VEGF aptamers described herein, wherein the first aptamer and the second aptamer may be the same or different. The first aptamer and the second aptamer of the VEGF aptamer construct may be covalently or noncovalently linked. Nonlimiting exemplary linkages are known in the art and/or are described herein. In some embodiments, a VEGF aptamer construct may be capable of binding two VEGF monomers simultaneously. In some embodiments, a VEGF aptamer construct binds VEGF with an affinity (K_d) of less than 10 nM.

[00232] In some embodiments, a VEGF aptamer binds VEGF-121 with a K_d of less than 10 nM. In some embodiments, the VEGF aptamer comprises one or more modified nucleotides. In some embodiments, the VEGF aptamer comprises one or more modified nucleotides comprising hydrophobic modification. In some embodiments, the VEGF aptamer comprises one or more modified pyrimidines. In some embodiments, the VEGF aptamer comprises one or more modified pyrimidines shown in FIG. 12. In some embodiments, the VEGF aptamer comprises one or more modified pyrimidines shown in FIG. 12, groups II to V. In some embodiments, the VEGF aptamer comprises one or more modified pyrimidines shown in FIG. 12, groups III to V. In some embodiments, the VEGF aptamer comprises one or more modified pyrimidines shown in FIG. 12, groups III and IV. In some embodiments, the VEGF aptamer comprises one or more (N-naphthylmethylcarboxyamide)-2'-deoxyuridines (NapdUs).

[00233] In some embodiments, a VEGF aptamer comprises the sequence:
5'-GZZQAAEZECZZEZDRGAZZZAAAZGG-3' (SEQ ID NO. 513)
wherein each Z is a modified pyrimidine;
Q is selected from any modified or unmodified nucleotide and a substituted or unsubstituted C_2-C_{50} linker, or is absent;
each E is independently selected from a G and a substituted or unsubstituted C_2-C_{50} linker;
D is selected from A and a substituted or unsubstituted C_2-C_{50} linker; and
R is selected from any modified or unmodified nucleotide and a substituted or unsubstituted C_2-C_{50} linker.

[00234] In some embodiments, a VEGF aptamer comprises a sequence selected from:

5'-CGZZQAAEZECZZEZDRGAZZZAAAZG-3' (SEQ ID NO. 514);
5'-GZZQAAEZECZZEZDRGAZZZAAAZGG-3' (SEQ ID NO. 513);
5'-CGZZQAAEZECZZEZDRGAZZZAAAZGG-3' (SEQ ID NO. 515); and
5'-CCGZZQAAEZECZZEZDRGAZZZAAAZGG-3' (SEQ ID NO. 516);
wherein Z, Q, E, D, and R are as defined above.

[00235] In some embodiments, Z is a modified uridine. In some embodiments, each Z is independently selected from the C-5 modified pyrimidines as defined herein. In some embodiments, each Z is independently 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.

[00236] In some embodiments, each Z is independently selected from the modified pyrimidines shown in FIG. 12, groups II to V. In some embodiments, each Z is independently selected from the modified pyrimidines shown in FIG. 12, groups III to V. In some embodiments, at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight Zs are 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU). In some embodiments, each Z is independently selected from the modified pyrimidines shown in FIG. 12, groups III to IV. In some embodiments, each Z is 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU).

[00237] A C_2-C_{50} linker or spacer may be a backbone comprising a chain of 2 to 50 carbon atoms (C_2-C_{50}) (saturated, unsaturated, straight chain, branched or cyclic), 0 to 10 aryl groups, 0 to 10 heteroaryl groups, and 0 to 10 heterocyclic groups, optionally comprising an ether (-O-) linkage, (*e.g.*, one or more alkylene glycol units, including but not limited to one or more ethylene glycol units -O-(CH_2CH_2O)-; one or more 1,3-propane diol units -O-($CH_2CH_2CH_2O$)-, *etc.*); an amine (-NH-) linkage; an amide (-NC(O)-) linkage; and a thioether (-S-) linkage; *etc.*; wherein each backbone carbon atom may be independently unsubstituted (*i.e.*, comprising –H substituents) or may be substituted with one or more groups selected from a C_1 to C_3 alkyl, -OH, -NH_2, -SH, -O-(C_1 to C_6 alkyl), -S-(C_1 to C_6 alkyl), halogen, -OC(O)(C_1 to C_6 alkyl), -NH-(C_1 to C_6 alkyl), and the like. In some embodiments, a C_2-C_{50} linker is a C_2-C_{20} linker, a C_2-C_{10} linker, a C_2-C_8 linker, a C_2-C_6 linker, a C_2-C_5 linker, a C_2-C_4 linker, or a C_3 linker, wherein each carbon may be independently substituted as described above.

[00238] In some embodiments, each substituted or unsubstituted C_2-C_{50} linker is independently selected from a substituted or unsubstituted C_2-C_{20} linker, a substituted or

unsubstituted C_2-C_{10} linker, a substituted or unsubstituted C_2-C_8 linker, a substituted or unsubstituted C_2-C_6 linker, a substituted or unsubstituted C_2-C_5 linker, a substituted or unsubstituted C_2-C_4 linker, and a substituted or unsubstituted C_3 linker. In some embodiments, each substituted or unsubstituted C_2-C_{50} linker is a substituted or unsubstituted C_2-C_{10} linker. In some such embodiments, each substituted or unsubstituted C_2-C_{10} linker is a substituted or unsubstituted C_2-C_8 linker, a substituted or unsubstituted C_2-C_6 linker, a substituted or unsubstituted C_2-C_5 linker, a substituted or unsubstituted C_2-C_4 linker, or a substituted or unsubstituted C_3 linker.

[00239] In some embodiments, one or more nucleosides of a VEGF aptamer comprise a modification selected from a 2'-position sugar modification (such as a 2'-amino (2'-NH_2), a 2'-fluoro (2'-F), or a 2'-O-methyl (2'-OMe)), a modification at a cytosine exocyclic amine, an internucleoside linkage modification, and a 5-methyl-cytosine. In some embodiments, a VEGF aptamer comprises a 3' cap, a 5' cap, and/or an inverted deoxythymidine at the 3' terminus.

[00240] In some embodiments, a VEGF aptamer comprises at least one modified internucleoside linkage. In some embodiments, at least one, at least two, at least three, at least four, or at least five internucleoside linkages are phosphorothioate linkages.

[00241] In some embodiments, a VEGF aptamer has a sequence selected from the sequences shown in Tables 10 to 14. In some embodiments, a VEGF aptamer has a sequence selected from the sequences shown in Tables 10 to 14that have a K_d of less than 10 nM. In some embodiments, a VEGF aptamer has a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequences shown in Tables 10 to 14. In some embodiments, a VEGF aptamer has a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the sequences shown in Tables 10 to 14 that have a K_d of less than 10 nM. Percent identity is determined as described above for PDGF aptamers, except that the reference sequences are the VEGF aptamer sequences shown in Tables 10 to 14, such as the sequences that have a K_d of less than 10 nM.

[00242] In some embodiments, the present disclosure provides a VEGF aptamer that, upon binding VEGF, modulates a VEGF function. In some embodiments, a VEGF aptamer inhibits VEGF-mediated phosphorylation of a VEGF receptor, such as VEGFR1 or VEGFR2. In some embodiments, a VEGF aptamer inhibits VEGF-mediated phosphorylation of VEGF receptor. In various embodiments, the VEGF aptamer modulates a VEGF function *in vivo*,

such as inhibiting VEGF-mediated receptor phosphorylation *in vivo*. In various embodiments, the VEGF aptamer has a sequence selected from the sequences shown in Tables 10 to 14. In various embodiments, the VEGF aptamer is selected from the aptamers shown in Tables 10 to 14 that have a K_d of less than 10 nM. In various embodiments, the VEGF aptamer is selected from the aptamers shown in Tables 10 to 14. In some embodiments, the VEGF aptamer comprises at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of a sequence shown in Tables 10 to 14 that have a K_d of less than 10 nM. In some embodiments, a VEGF aptamer consists of at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides that are identical in nucleobase sequence to a sequence shown in Tables 10 to 14 that have a K_d of less than 10 nM. In some embodiments, the VEGF aptamer comprises at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of an aptamer shown in Tables 10 to 14. In some embodiments, the VEGF aptamer comprises at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of an aptamer shown in Tables 10 to 14 that have a K_d of less than 10 nM. In some embodiments, a VEGF aptamer consists of at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of an aptamer shown in Tables 10 to 14. In some embodiments, a VEGF aptamer consists of at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 contiguous nucleotides of an aptamer shown in Tables 10 to 14 that have a K_d of less than 10 nM.

[00243] In any of the embodiments herein, a VEGF aptamer may comprise additional nucleotides or other chemical moieties on the 5' end, the 3' end, or both the 5' and the 3' end of the aptamer.

[00244] The VEGF aptamer can contain any number of nucleotides in addition to the VEGF binding region. In various embodiments, the VEGF aptamer can 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, or up to about 20 nucleotides.

[00245] In some embodiments, the VEGF aptamer is selected from an aptamer that has similar binding characteristics and ability to treat VEGF associated atherosclerosis, macular degeneration, fibrosis, and cancer conditions as an aptamer shown in Tables 10 to 14 have a K_d of less than 10 nM. In some embodiments, a VEGF aptamer binds to the same region of VEGF-121 as an aptamer selected from the aptamers shown in Tables 10 to 14 that have a K_d of less than 10 nM. In some embodiments, a VEGF aptamer binds to the same region of a VEGF-121 as a VEGF aptamer shown in Table 10, 11, 12, 13 or 14 that have a K_d of less than 10 nM. In some embodiments, a VEGF aptamer binds to the same region of VEGF-121 as VEGF aptamer 4867-31_183.

[00246] The VEGF aptamer can be selected to have any suitable dissociation constant (K_d) for VEGF. In some embodiments, a VEGF aptamer has a dissociation constant (K_d) for VEGF-121 of less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, less than 10 nM, less than 9 nM, less than 8 nM, less than 7 nM, less than 6 nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, or less than 1 nM. Dissociation constants may 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 in Example 3, below.

[00247] In some embodiments, an aptamer construct comprises a first aptamer selected from any of the VEGF aptamers described herein, and a second aptamer comprising any of the VEGF aptamers described herein, wherein the first aptamer and the second aptamer may be the same or different. The first aptamer and the second aptamer of the VEGF aptamer construct may be covalently or noncovalently linked. Nonlimiting exemplary linkages are known in the art and/or are described herein. In some embodiments, a VEGF aptamer construct may be capable of binding two VEGF monomers simultaneously. In some embodiments, a VEGF aptamer construct binds VEGF with an affinity (K_d) of less than 10 nM.

EXEMPLARY PDGF/VEGF APTAMER CONSTRUCTS

[00248] There is considerable evidence that more efficient blocking of tumor-associated and ocular angiogenesis, coupled with new blood vessel regression, is possible with combined inhibition of VEGF and PDGF-B signaling pathways (Bergers, G., *et al.* (2003) J. Clin. Invest. 111:1287; Jo, N., *et al.* (2006) Am. J. Pathol. 168:2036). This effect is mediated by the disruption of tight cell-cell association between endothelial cells, which form initial capillary sprouts, and periendothelial cells (or pericytes), which encircle the new blood vessels as they mature, rendering the blood vessels less susceptible to VEGF inhibitors (Benjamin, L. E., *et al.* (1998) Development 125:1591; Benjamin, L. E., *et al.* (1999) J. Clin. Invest. 103:159). The aptamers described herein can form the basis of such a dual inhibitor.

[00249] In some embodiments, a PDGF/VEGF aptamer construct comprises any of the PDGF aptamers described herein linked to any of the VEGF aptamers described herein. In some embodiments, a PDGF/VEGF aptamer construct comprises any of the PDGF aptamers shown in Table 1 linked to any of the VEGF aptamers shown in Table 10 to 14 that have a K_d less than 10 nM. The linkage may be covalent or noncovalent.

[00250] The PDGF/VEGF aptamer construct may comprise a PDGF aptamer and a VEGF aptamer in any orientation, such as a PDGF aptamer linked at or near its 3' end to a point at or near the 5' end of a VEGF aptamer, or a VEGF aptamer linked at or near its 3' end to a point at or near the 5' end of a PDGF aptamer, or any other orientation that preserves the binding properties of each aptamer of the construct.

[00251] In some embodiments in which the linkage is covalent, the PDGF/VEGF aptamer construct may be linked through a phosphate or phosphorothioate linkage. Many other covalent linkages are also contemplated, such as linkages through various linker moieties, including, but not limited to, hexaethylene glycol linkers, polyethylene glycol linkers, substituted or unsubstituted hyrdrocarbon linkers, etc. One skilled in the art can select a suitable covalent linkage for linking a PDGF aptamer to a VEGF aptamer.

[00252] In some embodiments, the PDGF aptamer and the VEGF aptamer are linked via a noncovalent linkage. Noncovalent linkages include, but are not limited to, biotin / streptavidin; metal-binding peptides / metals; hybridizable modified and/or unmodified oligonucleotides; etc. One of ordinary skill in the art can select a suitable noncovalent linkage for linking a PDGF aptamer to a VEGF aptamer.

Pharmaceutical Compositions Comprising Aptamers and Aptamer Constructs

[00253] In some embodiments, pharmaceutical compositions comprising at least one aptamer or aptamer construct described herein and at least one pharmaceutically acceptable carrier are provided. Suitable carriers are described in "Remington: The Science and Practice of Pharmacy, Twenty-first Edition," published by Lippincott Williams & Wilkins. Pharmaceutical compositions that include at least one aptamer or aptamer construct described herein and at least one pharmaceutically acceptable carrier may also include one or more active agents that is not a PDGF or VEGF inhibitor.

[00254] The aptamers described herein can be utilized in any pharmaceutically acceptable dosage form, including, but not limited to, injectable dosage forms, liquid dispersions, gels, aerosols, ointments, creams, lyophilized formulations, dry powders, tablets, capsules, controlled release formulations, fast melt formulations, delayed release formulations, extended release formulations, pulsatile release formulations, mixed immediate release and controlled release formulations, *etc.* Specifically, the aptamers described herein can be formulated: (a) for administration selected from any of oral, pulmonary, intravenous, intra-arterial, intrathecal, intra-articular, rectal, ophthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration; (b) into a dosage form selected from any of liquid dispersions, gels, aerosols, ointments, creams, tablets, sachets and capsules; (c) into a dosage form selected from any of lyophilized formulations, dry powders, fast melt formulations, controlled release formulations, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations; or (d) any combination thereof.

[00255] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can comprise one or more of the following components: (1) a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; (2) antibacterial agents such as benzyl alcohol or methyl parabens; (3) antioxidants such as ascorbic acid or sodium bisulfite; (4) chelating agents such as ethylenediaminetetraacetic acid; (5) buffers such as acetates, citrates or phosphates; and (5) agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. A parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

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[00256] Pharmaceutical compositions suitable for injectable use may include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition should be sterile and should be fluid to the extent that easy syringability exists. The pharmaceutical composition should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The term "stable", as used herein, means remaining in a state or condition that is suitable for administration to a subject.

[00257] The carrier can be a solvent or dispersion medium, including, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and inorganic salts such as sodium chloride, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00258] Sterile injectable solutions can be prepared by incorporating the active reagent (*e.g.*, an aptamer, and/or an aptamer construct) in an appropriate amount in an appropriate solvent with one or a combination of ingredients enumerated above, as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating at least one aptamer, and/or aptamer construct into a sterile vehicle that contains a basic dispersion medium and any other desired ingredient. In the case of sterile powders for the preparation of sterile injectable solutions, exemplary methods of preparation include vacuum drying and freeze-drying, both of which will yield a powder of an aptamer, and/or an aptamer construct plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00259] In some embodiments, an aptamer, and/or an aptamer construct is formulated for intravitreal injection. Suitable formulations for intravitreal administration are described, *e.g.*, in. Ocular drug delivery is discussed, *e.g.*, in Rawas-Qalaji *et al.* (2012) Curr. Eye Res. 37:

345; Bochot *et al.* (2012) J. Control Release 161:628; Yasukawa *et al.* (2011) Recent Pat. Drug Deliv. Formul. 5:1; and Doshi *et al.* (2011) Semin. Ophthalmol. 26:104. In some embodiments, a pharmaceutical composition comprising an aptamer, and/or an aptamer construct is administered by intravitreal injection once per week, once per two weeks, once per three weeks, once per four weeks, once per five weeks, once per six weeks, once per seven weeks, once per eight weeks, once per nine weeks, once per 10 weeks, once per 11 weeks, once per 12 weeks, or less often than once per 12 weeks.

[00260] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed, for example, in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the aptamer, and/or aptamer construct can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

[00261] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressured container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, a nebulized liquid, or a dry powder from a suitable device. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active reagents are formulated into ointments, salves, gels, or creams, as generally known in the art. The reagents can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00262] In some embodiments, an aptamer, and/or an aptamer construct is prepared with a carrier that will protect against rapid elimination from the body. For example, a controlled release formulation can be used, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

[00263] Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00264] Additionally, suspensions of an aptamer, and/or an aptamer construct may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also include suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

[00265] In some cases, it may be especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of an aptamer and/or aptamer construct calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of aptamers and/or constructs described herein are dictated by and directly dependent on the characteristics of the particular aptamer and/or aptamer construct and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active agent for the treatment of individuals.

[00266] Pharmaceutical compositions comprising at least one aptamer, and/or aptamer construct can include one or more pharmaceutical excipients. Examples of such excipients include, but are not limited to, binding agents, filling agents, lubricating agents, suspending agents, sweeteners, flavoring agents, preservatives, buffers, wetting agents, disintegrants, effervescent agents, and other excipients. Such excipients are known in the art. Exemplary excipients include: (1) binding agents which include various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102, silicified microcrystalline cellulose (ProSolv SMCC™), gum tragacanth and gelatin; (2) filling agents such as various starches, lactose, lactose monohydrate, and lactose anhydrous; (3) disintegrating agents such as alginic acid, Primogel, corn starch, lightly crosslinked polyvinyl pyrrolidone, potato starch, maize starch, and modified starches, croscarmellose sodium, cross-povidone, sodium starch glycolate, and mixtures thereof; (4) lubricants,

including agents that act on the flowability of a powder to be compressed, and including magnesium stearate, colloidal silicon dioxide, such as Aerosil® 200, talc, stearic acid, calcium stearate, and silica gel; (5) glidants such as colloidal silicon dioxide; (6) preservatives, such as potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic compounds such as phenol, or quaternary compounds such as benzalkonium chloride; (7) diluents such as pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing; examples of diluents include microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102; lactose such as lactose monohydrate, lactose anhydrous, and Pharmatose® DCL21; dibasic calcium phosphate such as Emcompress®; mannitol; starch; sorbitol; sucrose; and glucose; (8) sweetening agents, including any natural or artificial sweetener, such as sucrose, saccharin sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and acesulfame; (9) flavoring agents, such as peppermint, methyl salicylate, orange flavoring, Magnasweet® (trademark of MAFCO), bubble gum flavor, fruit flavors, and the like; and (10) effervescent agents, including effervescent couples such as an organic acid and a carbonate or bicarbonate. Suitable organic acids include, for example, citric, tartaric, malic, fumaric, adipic, succinic, and alginic acids and anhydrides and acid salts. Suitable carbonates and bicarbonates include, for example, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, magnesium carbonate, sodium glycine carbonate, L-lysine carbonate, and arginine carbonate. Alternatively, only the sodium bicarbonate component of the effervescent couple may be present.

[00267] In various embodiments, the formulations described herein are substantially pure. As used herein, "substantially pure" means the active ingredient (*e.g.*, an aptamer, and/or an aptamer construct) is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition). In some embodiments, a substantially purified fraction is a composition wherein the active ingredient comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will include more than about 80% of all macromolecular species present in the composition. In various embodiments, a substantially pure composition will include at least about 85%, at least about 90%, at least about 95%, or at least about 99% of all macromolecular species present in the composition. In various embodiments, the active ingredient is purified to homogeneity (contaminant species cannot be detected in the

composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

Kits Comprising Aptamers and Aptamer Constructs

[00268] The present disclosure provides kits comprising any of the aptamers, and/or aptamer constructs described herein. Such kits can comprise, for example, (1) at least one aptamer, and/or aptamer constructs; 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.

Methods of Treatment

[00269] The present disclosure provides methods of preventing or treating (*e.g.*, alleviating one or more symptoms of) medical conditions through the use of a PDGF aptamer or aptamer construct, a VEGF aptamer or aptamer construct, and/or a VEGF/PDGF aptamer construct. The methods comprise administering a therapeutically effective amount of such aptamers and/or aptamer constructs to a subject in need thereof. The described aptamers can also be used for prophylactic therapy. In some embodiments, the aptamer and/or aptamer construct is administered orally or intravenously.

[00270] The aptamer and/or aptamer construct used in methods of treatment can be: a PDGF aptamer or aptamer construct a VEGF aptamer or aptamer construct, and/or a VEGF/PDGF aptamer construct described herein, or a pharmaceutically acceptable salt thereof, or a prodrug thereof.

[00271] The individual or subject can be any animal (domestic, livestock or wild), including, but not limited to, cats, dogs, horses, pigs and cattle, and preferably humans. As used herein, the terms patient, individual, and subject may be used interchangeably.

[00272] As used herein, "treating" describes the management and care of a patient for the purpose of treating a disease, condition, or disorder and includes the administration of an aptamer, and/or an aptamer construct to prevent the onset of the symptoms or complications of a disease, condition or disorder; to alleviate symptoms or complications of the disease, condition, or disorder; or to eliminate the presence of the disease, condition or disorder in the patient. More specifically, "treating" includes reversing, attenuating, alleviating, minimizing, suppressing or halting at least one deleterious symptom or effect of a disease (disorder) state,

disease progression, disease causative agent or other abnormal condition. Treatment is generally continued as long as symptoms and/or pathology ameliorate.

[00273] As used herein, "preventing" means preventing in whole or in part; ameliorating or controlling; reducing, lessening, or decreasing; or retarding or halting.

[00274] In various embodiments, the disclosed compositions and methods are used to treat cardiovascular diseases, cancers, fibrosis, renal diseases or ophthalmic diseases.

[00275] In some embodiments, the disclosed compounds or pharmaceutically acceptable salts thereof, or prodrugs, can be administered in combination with other treatments that improve or eradicate the disease conditions as described above. Compositions including the disclosed aptamers and/or aptamer constructs may contain, for example, more than one aptamer. In some examples, a composition containing one or more aptamers is administered in combination with another useful cardiovascular agent or anticancer agent or antifibrotic agent etc. In general, the currently available dosage forms of the known therapeutic agents for use in such combinations will be suitable.

[00276] "Combination therapy" (or "co-therapy") includes the administration of an aptamer and/or aptamer construct composition and at least one second agent as part of a specific treatment regimen intended to provide the beneficial effect from the co-action of these therapeutic agents. The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days or weeks depending upon the combination selected).

[00277] "Combination therapy" may, but generally is not, intended to encompass the administration of two or more of these therapeutic agents as part of separate monotherapy regimens that incidentally and arbitrarily result in the combinations of the present disclosure. "Combination therapy" is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Substantially simultaneous administration can be accomplished, for example, by administering to the subject a single dose having a fixed ratio of each therapeutic agent or in multiple, single doses for each of the therapeutic agents.

[00278] The dosage regimen utilizing the aptamers and/or aptamer constructs is selected in accordance with a variety of factors, including, for example, type, species, age, weight, gender and medical condition of the subject; the severity of the condition to be treated; the

route of administration; the renal and hepatic function of the subject; and the particular aptamer and/or aptamer constructs or salts thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition.

[00279] In general, the dosage, *i.e.*, the therapeutically effective amount, ranges from about 1 μg to about 100 mg/kg body weight of the subject being treated, per day.

EXAMPLES

[00280] The following examples are provided for illustrative purposes only and are not intended to limit the scope of the invention as defined by the appended claims. All examples described herein were carried out using standard techniques, which are well known and routine to those of skill in the art. Routine molecular biology techniques described in the following examples can be carried out as described in standard laboratory manuals, such as Sambrook *et al.* (2001) Molecular Cloning: A Laboratory Manual, 3rd. ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Example 1. PDGF Aptamer Selection and Sequences

[00281] Preparation of Candidate Mixtures: A candidate mixture of partially randomized ssDNA oligonucleotides was prepared by polymerase extension of a DNA primer annealed to a biotinylated ssDNA template.

[00282] SELEX Conditions: Aptamers to the PDGF-BB protein (R&D Systems) were selected by SomaLogic Inc, as described (Gold *et al.* (2010) PLoS One 5:e15004), from a library containing a 40-nucleotide random region in which Bn-dU was substituted for dT. The forward primer was 5'-CGCCCTCGTCCCATCTC (SEQ ID NO: 837), and the reverse primer was 5'-CGTTCTCGGTTGGTGTTC (SEQ ID NO: 838). The PDGF-BB protein was biotinylated and partitioned on streptavidin MyOne-SA (Dynal) beads. Preferential selection of aptamers with slow dissociation rates was achieved using a kinetic challenge wherein protein-DNA complexes were incubated in the presence of 10 mM dextran sulfate at 37°C with increased incubation times and decreased protein concentrations in successive rounds. Kinetic challenge was initiated in round 4 of the selection and was continued through the final 8th round with incubation times as follows: 5 minutes round 4, 15 minutes rounds 5-7, 30 minutes round 8.

[00283] Pool Sequencing: Oligonucleotide sequences from the 8th round pool were cloned and several clones were sequenced. This led to the identification of a family of related sequences, as exemplified by the 4149-8_1.

[00284] Deep Sequencing of PDGF SELEX Pool: To evaluate more completely the sequences within the 4149-8_1 aptamer family, the 8th round pool was sequenced using 454 pyrosequencing technology. The pool DNA was amplified with 454 primers and the PCR product was purified and normalized using a Sequal normalization plate (Invitrogen, Cat# A10510-01). The eluate was run on a gel to confirm the size and purity of each amplicon. The purified PCR product was sequenced at the 454 pyrosequencing facility at the University of Colorado Health Science Center in Aurora CO.

[00285] The 454 sequences were aligned with 4149-8_1 by CLUSTAL analysis. The sequence data set from the pool contained 10,803 full length sequences (*i.e.*, those sequences containing both primer sequences) of which 3,839 were unique. These 3,839 unique sequences were searched for the motif "5'-ZACNCGCGZZZAZAGCG" (identity = 0.65) (SEQ ID NO: 839) and then "ZZ" (identity = 1.0) upstream from this. There were 436 sequences found that contained both of these motifs. In addition, 58 other sequences contained only the first pattern but with a generally low identity and with no evident hairpin structure upstream. The 436 sequences were then aligned as follows, (1) with respect to "ZZ", (2) with respect to the center of the loop, and (3) with respect to "ZACNCGCGZZZAZAGCG" (SEQ ID NO: 839). For all the sequences, the percentage identity at each position with 4149-8_1 was calculated as listed in FIG. 3A. Tables 1 and 2 lists a number of sequences representative of the 4149-8_1 aptamer family of sequences.

[00286] Aptamer Synthesis: The modified deoxyuridine-5-carboxamide amidite reagents used for solid-phase synthesis were prepared by: condensation of 5'-*O*-(4,4'-dimethoxytrityl)-5-trifluoroethoxycarbonyl-2'-deoxyuridine (Nomura *et al.* (1997) Nucl. Acids Res. 25:2784) with the appropriate primary amine (RNH_2,1.2 eq; Et_3N, 3 eq.; acetonitrile; 60 °C; 4h); 3'-*O*-phophitidylation with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (1.2 eq.; iPr_2EtN, 3 eq.; CH_2Cl_2; -10 to 0 °C; 4 h); and purification by flash chromatography on neutral silica gel (Still, *et al.* (1978) J. Org. Chem. 43:2923). Aptamers were prepared by solid phase synthesis using the phosphoramidite method (Beaucage and Caruthers (1981) Tetrahedron Lett. 22:1859) with some adjustments to the protocol to account for the unique base modifications described herein. Detritylation was accomplished with 10% dichloroacetic acid in toluene for 45 seconds; coupling was achieved with 0.1 M phosphoramidites in 1:1 acetonitrile:dichloromethane activated by 5-benzylmercaptotetrazole and allowed to react 3

times for 5 minutes; capping and oxidation were performed according to instrument vendor recommendations. Deprotection was effected with 1:1:2, t-butylamine:methanol:water (Mullah 1998), reacted for 24 hours at 37 degrees centigrade. Aptamers were synthesized at 200 nmol scale and purified from a polyacrylamide gel using UV shadowing as described (Fitzwater and Polisky (1996) Methods Enzymol. 267:275) with Costar Spin-X (not including siliconized glass wool or spun polypropylene prefilter) and Amicon YM3 concentration per manufacturer's recommendations.

[00287] Modified nucleotide structure activity relationship and affinity maturation: To examine the contribution of each of the eight benzyl side chains to binding, we performed another series of systematic point substitutions by chemically synthesizing 5-position variants with a custom-made library of modified dU phosphoramidites. For this purpose, we designed a library to allow us to probe the microenvironment of each of the positions by varying the size, polarity, disposition of H-bond donors and acceptors, linker length, and orientation of the 5-position substituents. In choosing the functional groups for this analysis, we aimed to include variations on a theme of the original modification (in this case, the benzyl group), amino acid side chains overrepresented in complementarity determining regions (CDRs) of antibodies (like tryptophan and tyrosine) (Mian, IS, *et al.* (1991) J. Mol. Biol. 217:133; Ramaraj T. *et al.* (2012) Biochim. Biophys. Acta. 1824:520), and "privileged" fragments of small-molecule drugs (Welsch *et al.* (2010) Curr. Opin. Chem. Biol. 14:347). In a sense, we endeavored to combine elements of affinity maturation in antibodies and structure-activity relationship (SAR) optimization in medicinal chemistry. Although we utilized a single modified nucleotide during SELEX, post-SELEX optimization is constrained only by the synthetic accessibility of the modified monomers and compatibility with solid-phase synthesis.

[00288] The effect of individual substitutions of the benzyl group with fourteen alternative moieties at the 5-position is summarized in FIGS. 1C and D, and FIG. 6B, with relative affinities expressed as dissociation constant ratios and relative PDGF Rβ phosphorylation expressed as percent phospho-PDGF Rβ ratios. Substitution with dT, which only has a methyl group at the 5-position, represents the most drastic change, and in that sense is comparable to alanine scanning mutagenesis in proteins (Cunningham, B.C. *et al.* (1989) Science 243:1330). Not surprisingly, this was the least tolerated substitution at six of the eight modified nucleotide positions. The exceptions were nucleotides 1 and 7, where this substitution was well-tolerated. These two positions also tolerated many other substitutions, with some replacements yielding up to 5-fold improvement in binding affinity (FIG. 6B). In

contrast, nucleotides 8, 17 and 18 exhibited the highest sensitivity to changes. The best single substitutions were then combined, yielding additional variants including 4149-8_255 and 4149-8_260 (FIG. 6B). Aptamer 4149-8_260, which combined phenethyl-dU (Pe-dU) at nucleotide 17 and thiophene-dU (Th-dU) at nucleotide 18, showed excellent binding to both PDGF-BB and PDGF-AB (FIG. 6B). It is worth noting that the affinity of the originally selected SOMAmer was already so high (K_d=20 pM) that it approached the detection limit of the binding assay, so it is possible that the degree of affinity improvement is underestimated. We have applied similar post-SELEX optimization strategies to other SOMAmers with weaker initial binding (*e.g.*, K_d values ranging from 100 pM to >10 nM), and have observed affinity improvements of up to 100-fold.

[00289] Homodimers of PDGF aptamer 4149-8_260 (SL5): Since PDGF forms a covalently linked homodimer, and two SOMAmers bind to each PDGF homodimer, we determined the effect on binding of homodimerized SOMAmers. The affinity of the PDGF aptamer homodimers could be substantially improved compared to the affinity of the corresponding monomers, due to avidity effects. The crystal structure showed that the 5' ends of the SOMAmer were 38 Å apart, while the 3' ends were 74 Å apart. Connecting the 5' to 3' end would require at least 63 Å since the shortest path between the two points bisected the protein. Two types of homodimers were ordered, based on readily available chemistry. These were 1) head-to-tail homodimers connected by two to six Heg linkers, which provide ~20 Å distance per Heg, and 2) 3'-3' homodimers connected via a synthetic doubler support, combined with one to three Hegs. The homodimers of 4149-8_260 were tested in the PDGF-BB Zorbax binding assay. The binding assay was performed with limiting amount of SOMAmer, and would not distinguish binding of one SOMAmer per protein dimer versus binding of two SOMAmers per protein dimer. The structure of the homodimers is shown in Table 1 (sequences 4149-8_334 through 4149-8_342). The K_d values obtained in the Zorbax assay suggested that in the 5' to 3' configuration, a longer linker was desirable, and gave up to 10-fold improvement in binding affinity, as shown in Table 1a. In the 3'-3' linked homodimers, the shorter linker actually appeared to perform better than the longer linker. This was corroborated by cellular phosphorylation results, see Table 1a.

[00290] Based on these sequences, an exemplary consensus sequence is:

5'-ZZVCL$_n$GV'ZACNMGCGZZZAZAGCG-3' (SEQ ID NO: 502),

wherein

V is selected from an A, C or G;

V' is selected from a C, G or Z, wherein V' is complementary to V;

N is independently selected from any naturally occurring or modified nucleotide;
M is selected from a C or A;
Z is independently selected from a modified pyrimidine; L is a spacer selected from any naturally occurring or modified nucleotide, a hydrocarbon linker, a polyethylene glycol linker or a combination thereof; and
n is 0 to 20;
wherein one or more nucleotide insertions are optionally included.

[00291] Sequence Truncation Studies: Systematic truncation from the 5' and 3' ends of 4149-8_1 was performed to define a minimum length required to retain full binding activity of the aptamer to human PDGF-BB, as shown in Table 3. K_d values for a subset of the truncations are shown. Z = Benzyl-deoxyuridine (Bn-dU); A, C, G and T are deoxyribonucleotides.

Protein Expression and Purification, and Aptamer Complex Formation

[00292] For crystallography studies, recombinant human PDGF-BB protein was purchased from Creative BioMart (Shirley, NY). The recombinant protein was expressed in *E. coli* cells. Aptamer solutions were thawed and then annealed by heating to ~95 °C for 5 minutes, then incubating at 40°C for 5 minutes, then cooling to room temperature. The annealed aptamer solution was mixed with protein at a 1.1:1 ratio of DNA to protein. The complex was diluted 5-fold in buffer containing 20 mM Na/K phosphate (pH 7) and 100 mM NaCl. The resulting mixture was concentrated to ~4 mg/mL in protein in a 1.5 mL Amicon centrifugal filter. Final concentration was estimated from the final volume of retentate.

Example 2. Crystallization and Structure of PDGF-Aptamer Complex

[00293] Crystals were grown using the sitting drop vapor diffusion method in Compact, Jr. plates (Emerald BioSystems, WA) set up at 16 °C. Crystals for data collection were obtained from a primary screen (ProPlex, Molecular Dimensions). The crystal for the PDGF-BB:4149-8_255 complex was grown from 100 mM magnesium acetate, 100 mM sodium acetate (pH 4.5) and 8% (w/v) PEG 8000. The crystal for the PDGF-BB:4149-8_260 complex was grown from 100 mM magnesium acetate, 100 mM sodium cacodylate (pH 6.5) and 15% (w/v) PEG 6000. Crystals were harvested with Litho Loops and cryoprotected by quick transfer to reservoir solution containing 33% (v/v) ethylene glycol before flash-cooling by plunging directly into liquid nitrogen.

[00294] Data collection and structure determination: Data for both structures were collected at beamline 19-ID of the Advanced Photon Source (Argonne, IL). The data sets were processed using XDS (Kabsch 2010). The structure of the PDGF-BB:4149-8_260 complex was initially phased by molecular replacement using Phaser from the CCP4 software suite (CCP4, 1994) with the protein model of PDGF from the structure of the PDGF-BB:Beta-type PDGF receptor complex (PDB entry 3MJG) as the search model. Molecular replacement located a single protein monomer per asymmetric unit. Inspection of the electron density maps following an initial round of restrained refinement in REFMAC showed features consistent with nucleic acid adjacent to the protein model. The model of the aptamer was subsequently built through a process of "bootstrapping", i.e., partial models were subjected to iterative rounds of refinement; resulting in marginally improved maps which allowed further model building. First, phosphate ions were built in nucleic acid backbone density. Secondly, phosphates were replaced by dT residues. Following refinement, modified residues could be discerned by protrusions of positive difference electron density. Identification of the modified residues facilitated determination of the sequence register of the aptamer, and in the final steps dT residues were replaced with the correct nucleobases. All manual building was performed using the Crystallographic Object-Oriented Toolkit (Coot) (Emsley & Cowtan, 2004). The structure of the PDGF-BB:4149-8_255 structure was solved by molecular replacement using the finished model of the 4149-8_260 complex.

[00295] In each structure, a protuberance of electron density was observed contiguous with the electron density of the Oγ atom of residues Thr88 and Thr90. Although O-mannosylation has been reported at these sites for recombinant PDGF-B expressed in yeast (Settineri, *et al.*, (1990)) there is little reason to expect similar post-translational modifications in the PDGF-B expressed in *E. coli*. As the observed electron density suggested less than full occupancy, the threonine residues were modeled without any post-translational modification.

[00296] Table 4 discloses data-collection statistics and refinement and model statistics of two aptamer ligands with 4149-8_260 (SEQ ID. NO. 211) and 4149-8_255 (SEQ ID. NO. 207) respectively.

[00297] Table 5 illustrates base-pair parameters for the PDGF BB aptamer compared to B-form DNA. The PDGF BB aptamer adopts deviated B-form conformations in the 5' stem loop domain and in both stems of the miniknot. Where appropriate, mean values and standard deviations (in parenthesis) are given. Aptamer values are based on analysis using web3DNA (Zheng *et al.* (2009) Nucleic. Acids Res. 37:W240) and B-DNA values (as found in high

resolution crystal structures) were determined using 3DNA as described and reported in Olson, *et al.* (2001) J. Mol. Biol. 313(1): 229.

[00298] The monomeric subunits of PDGF-BB form twisted β-sheets that dimerize in an anti-parallel orientation characteristic of the cystine knot family of proteins (Oefner *et al.* (1992) EMBO J. 11:3921). SL5 (4149-8_260) binds two homologous sites at either end of the long axis, crossing the homodimer interface and contacting each of the three PDGF loops (FIG. 7A). The SOMAmer is composed of two domains connected by a network of hydrophobic aromatic interactions (FIG. 7B). At the 5' end, a short stem is capped with a Heg loop (disordered in the crystal structure), while the remainder of the molecule folds into an extraordinarily small H-type pseudoknot (Aalberts, D.P. *et al.* (2005) Nucleic Acids Res. 33:2210), with modified nucleotides clustering at the stem loop/pseudoknot junction. Remarkably, all eight modified nucleotides are in contact with PDGF. Seven modified nucleotides cluster together along a hydrophobic groove on the protein, while Bn-dU1 adopts an extended conformation, following a channel at the PDGF homodimer interface. Two natural nucleotides also contact PDGF, with the remaining natural nucleotides contributing to internal structure (FIG. 2 and FIG. 7). The secondary structure elements of SL5, a stem-loop and a pseudoknot, are well-known nucleic acid structural motifs. However, replacement of certain conventional bases with modified nucleotides offers novel functional groups for alternative interactions. This distinguishing feature of SL5 results in an extensive hydrophobic surface for protein binding as well as unique intramolecular contacts between canonical and modified nucleotides.

[00299] Although the 3' end of SL5 exhibits hallmark characteristics of an H-type pseudoknot (Staple, D.W. *et al.* (2005) PLoS Biol. 3:e213), this categorization understates the unconventional nature of this signature "miniknot" motif. Compared to the smallest structurally reported H-type pseudoknot which requires 21 nucleotides (Nonin-Lecomte S. *et al.* (2006) Nucleic Acids Res. 34:1847), the SL5 miniknot consists of a mere 16 nucleotides (FIG. 7B). Furthermore, deletion of the terminal mG24:dC12 base pair of stem 2 (S2) results in undiminished binding affinity (FIG. 3), demonstrating the functional integrity of a 14-nucleotide miniknot. With unprecedented backbone twists and stacking interactions, the miniknot represents a novel pseudoknot variant in which unusually small size is attained via stabilization contributed by packing of the hydrophobic moieties of the modified nucleotides.

[00300] Miniknot stem 1 (S1) formally consists of just two Watson-Crick base pairs (FIG. 8A), while loop 2 (L2) is composed nominally of 5 bases, Pe-dU17, Th-dU18, dA19, Bn-dU20 and mA21. While interactions between L2 and S1 are a defining feature of

pseudoknots, they are typically limited to H-bonding. In contrast, the SOMAmer miniknot makes atypical loop-to-stem stacking interactions, supported by unconventional base pairing. In particular, S1 is stabilized by stacking with a non-canonical Bn-dU17:Bn-dU20 base pair derived from L2 (FIG. 8C and FIG. 8B), effectively creating a three base-pair S1 with a novel backbone discontinuity. In contrast to previously described U:U imino carbonyl base pairs, the Pe-dU17:Bn-dU20 base pair utilizes a single H-bond between N3 of Bn-dU17 and the carbonyl oxygen in the amide linker of Bn-dU20 (FIG. 8D). The *syn* conformation about the glycosyl bond of Bn-dU20 impedes H-bonding with Bn-dU17, but allows Bn20 to stack with Bn-dU8 base without steric clashing with the sugar of Bn-dU8. The unconventional Pe-dU17:Bn-dU20 base pair is made possible by a 280° turn in the backbone between nucleotides 18 and 20 (FIG. 8C). This dramatic strand reversal allows Bn-dU20 base to stack with the sugar of dA9 and form a hydrogen bond with Pe-dU17. Importantly, the Pe-dU17:Bn-dU20 base pair derives additional stabilization through hydrophobic interactions conferred by the modified nucleotides; the ethylene (linker) portion of the Pe-dU17 side chain is directed toward Bn16 (CH::π) while its benzyl group is stacked in π-π edge-to-face interactions with Bn2 and Th18 (FIG. 8E). One additional interaction between L2 and S1 is a base triple (mA21:dG15:dC10; FIG. 8F), a recurrent motif in pseudoknots (Chen, G. *et al.* (2009) Proc. Natl. Acad. Sci. USA 106:12706). This is the only long-range tertiary interaction in SL5 that does not involve the modified nucleotides.

[00301] Loop 1 (L1) consists of a single extruded nucleotide, mA11 that allows the backbone to make a tight 94° turn, with the intrastrand phosphate distance between mA11 and dC12 compressing to just 5.9 Å (FIG. 8G). H-type pseudoknots often have one or two nucleotides in L1, which typically form hydrogen bonds with S2 and stack into the helical junction (Nonin-Lecomte, S. *et al.* (2006) Nucleic Acids Res. 34:1847; Michiels, P.J. *et al.* (2001) J. Mol. Biol. 310:1109). The extruded L1 nucleotide is necessary to keep the structure condensed so that the 5' stem domain can interface with the miniknot through the hydrophobic moieties of the modified nucleotides. As expected, the extruded base is not conserved (FIG. 3) and can be replaced with a single C3 spacer (FIG. 6A), however, its deletion abrogates binding, presumably due to interference with the miniknot formation.

[00302] The Watson-Crick base pairs of miniknot S1 (dA9:Bn-dU16, dC10:dG15) assemble by the favored H-type pseudoknot arrangement in which strand one of S2 leads directly into strand two of S1, providing efficient stacking of the stems (Klein, D.J. *et al.* (2009) Nat. Struct. Mol. Biol. 16:343). The three base pairs of S2 are composed entirely of Watson-Crick interactions and form a slightly undertwisted B-form helix (FIG. 8H). This understwisting

results in helical parameters that more closely resemble A-form helices, as expected for pseudoknot topology; however, the relevance of these calculations is equivocal, given the short length of the helices in this structure. S2 does not form a conventional coaxial stack with S1 due to severe helical overwinding at the junction (twist angle of 70°) formed by dC10:dG15 of S1 and dC14:dG22 of S2. Continuous stacking of the stems is nevertheless maintained as dC14 stacks with dG15, and dG22 stacks with mA21 from the base triple (FIG. 8H). The extensive helical twist at this junction is necessary to allow mA21 to bridge the major groove of S2 while broadening the minor groove for base triple formation. This configuration is typical in pseudoknots with one or two nucleotides in L1 (Nonin-Lecomte, S. *et al.* (2006) Nucleic Acids Res. 34:1847; Michiels, P.J. *et al.* (2001) J. Mol. Biol. 310:1109).

[00303] The SL5 5' stem is composed of two Watson-Crick base pairs (mA3:Bn-dU7 and dC4:dG6) and a non-canonical Bn-dU2:Bn-dU8 base pair at the base of the stem (FIG. 8I). The Bn-dU2:Bn-dU8 base pair contains two hydrogen bonds, a typical 4-carbonyl-N3 and a unique 4-carbonyl from Bn-dU2 base to amide linker of Bn-dU8 bond (FIG. 8J). Analysis of related sequences in the affinity-enriched pool shows that the length and base composition of the 5' stem can change, with the notable exception of the invariant Bn-dU:Bn-dU brace at the base of the stem (FIG. 3A and B), highlighting the importance of this non-canonical base pair in the overall structure and function of SL5. Stability of the 5' stem helix is further bolstered by stacking of dU8, Bn20 and Pro82 of PDGF (FIG. 9H). The 5' stem-loop and miniknot domains of SL5 converge where the backbone makes a sharp 111° bend. Significant twist angles and radial displacement of the base pairs in the 5' stem results in bases 2-4 and 6-7 having greater stacking overlap (because of helix undertwisting) than in conventional B-form helices, while Bn-dU8 base is shifted out and Bn-dU7 base stacks with the amide linker of Bn-dU8 (FIG. 8I). This atypical helix facilitates critical interactions with the rest of SL5 and with PDGF; Bn-dU8 base stacks with Bn20 while Bn8 lies perpendicularly between the rings of Bn16 and Bn20 in consecutive π-π edge-to-face interactions. These long-range tertiary interaction define a precise hinge between the miniknot and the stem-loop domains (FIG. 8L and 8K). The lack of curvature between the first two nucleotides prevents clashing of Bn-dU1 base with Bn2, augmenting stacking of the rings (FIG. 8I). Bn2 sits in the middle of a hydrophobic cluster created by Bn7 and Bn8 (from the 5' stem) and Bn16, Pe17 and Bn20 (from the miniknot) (FIG. 7B, Fig. 8I and FIG. 8K). This hydrophobic cluster contributes to stabilization of the SOMAmer, supported by the observation that SL5 exhibits a T_m of 64°C, which is >30°C higher than its analog that lacks the modified nucleotides.

[00304] In addition to SL5, we also solved the structure of SL4 (4149-8_255), which is identical to SL5 except for the replacement of Bn-dU8 with isobutyl-dU (iB-dU). When iB-dU8 was combined with Pe-dU17 and Th-dU18 in variant SL4, the SOMAmer showed substantially weaker binding (~20-50-fold *vs.* SL5) and a 75-fold lower *in vitro* inhibitory activity (FIG. 1A, FIG. 1B and FIG. 6B). The smaller non-aromatic isobutyl side chain cannot form the energetically favorable π-π edge-to-face stacking seen with the benzyl side chains of Bn-dU20, Bn-dU8 and Bn-dU16 in SL5 (FIG. 8M and FIG. 8N). This creates a hole in the center of the hydrophobic cluster at the protein interface, effectively unlocking the hinge between the 5' stem and the miniknot domains. The structural effect of this substitution is directly analogous to a Phe to Leu mutation in the hydrophobic core of a protein. Such protein mutations are well-described (Kadonosono, T. *et al.* (2003) Biochemistry 42:10651; Lin, H.J. *et al.* (2003) Biochim. Biophys. Acta. 1649:16; Baase, W.A. *et al.* (2010) Protein Sci. 19:631) and usually have a significant destabilizing effect. Junctions between secondary structure motifs are known to play a critical role in determining nucleic acid tertiary structure (Pyle, A.M. *et al.* (2011) Curr. Opin. Struct. Biol. 21:293).

[00305] Despite markedly weaker target-binding affinity, SL4 exhibits similar thermal melting profile to SL5 in the absence of ligand (T_m values of 62°C and 64°C, respectively). This is consistent with the notion that the cavity and altered junction topology created by iB-dU8 substitution in SL4 destabilizes the protein-binding interface, while leaving the intradomain structures of the SOMAmer intact. The conformations of free SOMAmers in solution may well be very different from those in the complex with the protein, which could also diminish the relationship between T_m and binding affinity. In fact, since the energetic cost of solvating a large hydrophobic surface of the SOMAmer is likely to be substantial, we expect the uncomplexed SOMAmer to collapse around the hydrophobic side chains and adopt a conformation in which the hydrophobic side chains are partially protected from the solvent.

[00306] In contrast to previously described protein:aptamer complexes, hydrophobic interactions dominate the interface between SL5 and PDGF (FIG. 2, FIG. 4 and FIG. 9). Binding to PDGF-BB creates a buried surface area of ~1225 $Å^2$ per SOMAmer. The eight modified nucleotides of SL5 create an extensive hydrophobic interface that interacts with 13 non-polar amino acids of PDGF (Ala35, Phe37, Leu38, Val39, Trp40, Pro42, Cys52, Cys53, Ile75, Ile77, Pro82, Ile83, and Phe84), which account for approximately half of the total non-polar contacts, with the remainder comprising aliphatic regions of polar or charged amino acids such as Glu24, Arg27, Asn36, Asn54, Asn55, Arg56, Arg73, Lys74, Lys80, Lys85, and Lys86 (FIG. 9). Similar interactions between completely non-polar residues and non-polar

moeities of charged amino acids are often observed in proteins. Thus, the structural diversity afforded by the modified nucleotides in SOMAmers enables them to mimic the rich repertoire of interactions accessible to proteins. The striking difference in the extent of hydrophobic contacts made by the SOMAmer compared to traditional aptamers is evident when the interface atoms are displayed on the surfaces of the target proteins. SL5 exhibits remarkably few polar interactions, having just six H-bonds and one charge-charge interaction with PDGF (FIG. 4), despite close proximity to basic amino acids. Relative to the contact surface area, this is significantly lower than what is typical for aptamers. The total number of H-bonds and charge-charge interactions (that is, polar contacts) for six traditional aptamers increases approximately linearly in direct proportion to the interface area (FIG. 10A, FIG. 22A) with a correlation coefficient of 0.91 and an average of 1.9±0.4 polar contacts per 100 $Å^2$ interface area. SL5, as well as two additional SOMAmers in other co-crystal structures, clearly fall outside of the 99% confidence intervals of this trend, with less than half the number of polar contacts per interface area (average of 0.7±0.2 per 100 $Å^2$ interface area), while exhibiting a trend toward higher binding affinities for their targets (FIG. 22C). In terms of ligand efficiency (free energy of binding per non-hydrogen contact atom) (Kuntz, I.D. *et al.* (1999) Proc. Natl. Acad. Sci. USA 96:9997), aptamers and SOMAmers do not appear to be different (FIG. 22C), encompassing a range of values observed with protein-based and small molecule-based ligands (Wells, J.A. *et al.* (2007) Nature 450:1001). Free energies of binding per interface area are also similar (FIG. 22C). What is different, however, is the value of free energy of binding per polar contact, which is about twice as large for SOMAmers than for aptamers (FIG. 22 B and C), consistent with the notion that SOMAmers derive a larger contribution to binding from hydrophobic interactions.

[00307] Charge-charge interactions often contribute less than 0.2 kcal/mol to the stability of a folded protein (Sali, D. *et al.* (1991) J. Mol. Biol. 220:779. In contrast, burying just a single methylene group is estimated to contribute ~1-1.5 kcal/mol to globular protein stability and/or binding interactions (Kellis, J.T., Jr. *et al.* (1988) Nature 333:784; Pace, C.N. *et al.* (2011) Mol. Biol. 408:514). SOMAmer structures reveal a strong reliance on hydrophobic interactions and in this sense, their binding to proteins more closely resemble typical protein-protein interactions. Consistent with this observation, the affinity of SL5 for PDGF shows virtually no decrease across a broad range of salt concentrations (0.1 to 1.0 M NaCl) or pH values (5.0 to 8.8), in contrast to the effects seen with traditional aptamers (Ahmad, K.M. *et al.* (2011) PLoS ONE 6:e27051; Tang, Q. *et al.* (2007) J. Colloid. Interface. Sci. 315:99).

[00308] Post-SELEX optimization facilitates fine tuning of shape complementary and hydrophobic packing interactions. For example, the exceptional shape complementary of Pe-dU17 and Th-dU18 at the protein interface (FIG. 10B) is corroborated with the structure-activity relationships (FIG. 6B). Bn-dU1 also forms a unique interaction with PDGF-BB, with the benzyl ring sitting in a tunnel formed by the Cys43-Cys52 disulfide bond and a salt bridge between Glu24 of PDGF chain1 and Arg56 of chain 2 (FIG. 10C). The crystal structure suggests that the binding pocket can accommodate a variety of side chains, including larger bicyclic substituents, thereby enhancing this point of contact with the protein. Indeed, we have identified several modified nucleotide substitutions at this position that confer 5- to 10-fold enhancement in binding affinity (FIG. 6B).

[00309] A notable feature of the PDGF-BB:SL5 structure is the degree to which the SOMAmer mimics PDGFRβ. The receptor binds to PDGF primarily through hydrophobic interactions, including seven hydrophobic amino acids at the PDGF interface (Shim, A.H. *et al.* (2010) Proc. Natl. Acad. Sci. USA 107:11307). SL5 binding site largely overlaps that of the receptor with the Bn-dU aromatic rings occupying the same hydrophobic groove on the protein (FIG. 11). PDGF contacts both the receptor and SL5 with 24 residues, of which 10 are shared. These shared or "promiscuous" residues likely represent a hot spot of binding energy on the surface of PDGF (Wells, J.A. *et al.* (2007) Nature 450:1001; Clackson, T. *et al.* (1994) Science 267:383. However, compared with PDGF Rβ, SL5 exhibits a 10-fold higher affinity for PDGF-BB (Lokker, N.A. *et al.* (1997) J. Biol. Chem. 272:33037). Consistent with these observations, SL5 is a potent inhibitor of PDGF-BB (FIG. 1B and FIG. 6B).

Example 3. Binding affinity assays

[00310] For determination of target binding affinity, SOMAmers were 5′ end-labeled using T4 polynucleotide kinase (New England Biolabs) and γ-^{32}P-ATP (Perkin Elmer). Binding assays were performed by incubating radiolabeled SOMAmer (~20,000 c.p.m) at a concentration of ~0.03-0.05 nM and target protein at concentrations ranging from 10^{-7} to 10^{-12} M in 1XSB18T buffer (40 mM HEPES,pH 7.5; 120 mM NaCl; 5 mM KCl; 5 mM $MgCl_2$ and 0.01% TWEEN-20) at 37 °C for 30 minutes. Bound complexes were mixed with Zorbax resin and captured on Durapore filter plates. The fraction of SOMAmer bound was quantified with a PhosphorImager (FUJI FLA-3000). Raw binding data were corrected for nonspecific background binding of radiolabeled SOMAmer to Zorbax resin. Equilibrium dissociation constants (K_d) was determined as previously described (Jellinek *et al.* (1993)

Proc. Natl. Acad. Sci. 91:11227). Competitor tRNA at a concentration of 200 nM was included in isoform specificity studies as indicated in FIG. 5. To determine the salt dependence on the PDGF-BB/SOMAmer and E10030 interactions, binding affinity assays were performed and analyzed as described above in the presence of 40 mM Hepes pH 7.5, 0.01% TWEEN-20 and either 100 mM, 250 mM, 500 mM, 750 mM or 1.0 M NaCl). The log-log plots of the salt concentrations versus the dissociation constants were fit using simple linear regression. The slope of the plots represents the number of counter-ions released from the DNA upon protein binding, as described by the counter-ion condensation theory of (Manning, G.S. (1969) J. Chem. Phys. 51:924). The affinity of aptamer 4149-8_260 (SEQ ID NO. 211) for PDGF showed little change across a broad range of salt concentrations (0.1 to 1.0 M NaCl) or pH values (5.0 to 8.8), in contrast to the effects seen with traditional aptamers (Ahmad, K.M. *et al.* (2011) PLoS One 6:e27051; Tang, Q. *et al.* (2007) J. Colloid. Interface Sci. 315: 99).

Example 4. PDGF-BB Cellular Phosphorylation Assay

[00311] PDGF-BB Activity. For testing the ability of PDGF-BB SOMAmers to inhibit PDGF Rβ activation, Hs27 human foreskin fibroblast cells (American Type Culture Collection) were seeded at 5000 cells/well into a 96-well plate, and serum-starved for 24 hours. SOMAmers (varying concentrations as indicated in the figures) were incubated with PDGF-BB (20 ng/mL) (Creative BioMart) in serum-free media for 30 minutes at 37°C, then the complex was added to serum-starved Hs27 cells. At five minutes post stimulation, the supernatant was discarded and the cells were lysed in Lysis Buffer #9 (R&D Systems: 1% NP-40 Alternative, 20 mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate, 10 μg/mL Aprotinin, and 10 μg/mL Leupeptin) on ice for 5 minutes. Elisa detection of phospho-PDGF Rβ was performed using the DuoSet Phospho-PDGF Rβ kit (R&D Systems) according to the manufacturer's instructions. The percent phospho-PDGF Rβ was measured at OD_{450}, corrected for plate absorbance and background signal with a no stimulant control. Experiments were generally performed in duplicate or triplicate. Data were plotted in GraphPad Prism 3.0 and fit to a one site competition curve using nonlinear regression. Representative plot of the IC_{50} determination is shown in FIG. 13 for SOMAmers 4149-8_379 and 5' amino-linker modified SOMAmer 4149-8_379, with IC_{50} values of 1.6 nM and 1.7 nM, respectively.

[00312] For activity screening of variants of clone 4149-8, percent inhibition of PDGF-BB-induced PDGF Rβ phosphorylation in Hs27 fibroblasts, under the same conditions as described above but at a single concentration of SOMAmer variants (generally 20 nM), was evaluated.

Example 5. Additional PDGF Ligands Based on Nap-dU Modification

[00313] To identify additional aptamers that bind to PDGF-BB with high affinity, we have performed another SELEX experiment with a library comprising Nap-dU modified nucleotides. The selections were performed in a manner substantionally analogous to the one described in Example 1 above and resulted in the identification of the Nap-dU aptamer clone 5169-4.

[00314] Deep Sequencing of PDGF Nap-dU SELEX Pool: To evaluate more completely the sequences within the 5169-4_1 aptamer family, the 7th round pool was sequenced using 454 pyrosequencing technology. The pool DNA was amplified with 454 primers and the PCR product was purified and normalized using a Sequal normalization plate (Invitrogen, Cat# A10510-01). The eluate was run on a gel to confirm the size and purity of each amplicon. The purified PCR product was sequenced at the 454 pyrosequencing facility at the University of Colorado Health Sciences Center in Aurora, CO.

[00315] The sequence data set from the pool contained 8,273 full length sequences (*i.e.*, those sequences containing both primer sequences) of which 1,629 were unique. These 1,629 unique sequences were used to find statistically significant n-mer patterns by counting all possible n-mers in the sequence set, from 4-mers to 30-mers. By comparing the counts for each identified n-mer to counts expected at random for n-mers from a pool of the same composition, statistically significant patterns were found. Two major patterns were identified in the sequence set and 5169-4_1 was found aligned within the second pattern defined by the conserved sequence motif "APGPAPGCACAPCP" found in 11 sequences. A search through all unique sequences for this motif (identity = 0.75) found 51 sequences that were then aligned by the motif. For all the sequences, the fractional identity at each position in the alignment was calculated as listed in FIG. 14, with the consensus sequence indicated.

[00316] Sequence Truncation Studies: Systematic truncation from the 5' and 3' ends of the 50-nucleotide 5169-4 clone was performed to define a minimum length required to retain full binding activity of the aptamer to human PDGF-BB, as shown in Table 6. K_d values for these truncations are shown (P = Napthyl-deoxyuridine (Nap-dU); A, C, G and T are deoxyribonucleotides). The 5169-4 clone proved highly amenable to truncation and a 21-

nucleotide sequence was identified (5169-4_26) that bound to PDGF-BB with improved binding affinity compared to the 50-mer (17 pM and 29 pM, respectively). The 5169-4_26 21-mer contained 5 Nap-dU modified nucleotides versus 9 Nap-dU modified nucleotides in the 50-mer.

[00317] C3 Spacer Single Substitutions in 5169-4_26 (21-mer): The first round of post-SELEX modifications of the Nap-dU PDGF-BB aptamer included a C3 spacer walk at all positions in the 21-mer 5169-4_26. The C3 spacer walk is meant to identify bases not required for high affinity binding that could potentially be removed altogether, replaced with the C3 spacer or other linkers such as hexaethylene glycol (Heg) or polyethylene glycol (PEG) linkers. The results for the C3 spacer substitutions are shown in Table 7. In this table, "P" denotes Nap-dU, "C3" denotes C3 spacer; A, C and G denote deoxyribonucleotides, and "NB" denotes no binding up to 100 nM PDGF-BB. Three sites tolerated C3 substitution with a modest decrease in binding affinity: C1, G6 and C7 (numbering refers to the 21-mer, as shown below). One position, C15, tolerated a C3 spacer substitution with no affect on the binding affinity, compared to 5169-4_26.

[00318] 2'-O-methyl Single Substitutions in 5169-4_26 (21-mer): 2'O-methyl substitutions were made at all natural bases in order to identify positions that could tolerate this nuclease-resistant substitution. With the 2'O-methyl Nap phosphoramidite synthesized in our laboratories (Nap-mU), we also evaluated the Nap-dU positions that would tolerate Nap-mU single substitutions. In addition, deoxythymidine (T) was substituted for Nap-dU to assess the importance of each Nap-dU. The binding affinity results are shown in Table 8, and demonstrate that all positions tolerated 2'O-methyl substitutions to varying degrees. The affect of 2'O-methyl substitutions at each deoxycytidine position (C) resulted in no change in the binding affinity, compared to 5169-4_26, up to a 2- fold decrease in binding affinity. The four deoxyguanosine positions (G) showed varying results when substituted with 2'O-methyl from 2.5-fold increased binding affinity at G14, to 5.5-fold decreased binding affinity at G10, compared to 5169-4_26. The 2'O-methyl substitutions at the six deoxyadenosine positions (A) had from zero to greater than 50-fold adverse affect on the binding affinity. The deoxyadenosines towards the 5' end of the aptamer (A3, A5 and A8) were the three most sensitive to 2'O-methyl substitution. Only the Nap-dU at position 21 completely tolerated Nap-mU substitution with no affect on the binding affinity while the Nap-mU substitution at position 11 showed a 2.5-fold decrease in binding affinity, compared to 5169-4_26. The remaing Nap-mU substitutions resulted in a 15- to 30-fold decrease in binding affinity. The only substitutions that completed eliminated binding (at PDGF-BB concentrations up to 100

nM) were the deoxythymidine substitutions at positions 12 and 21, with the remaining deoxythymidine substitutions having a significant negative affect (> 400 fold) on the binding affinity. In Table 8, P=5-naphthalene modified dU, a superscript 1 indicates a 2'-O-methyl modified nucleoside. A, C, G, and T represent the naturally occurring deoxyribonucleotides and "NB" denotes no binding up to 100 nM PDGF-BB.

[00319] Multiple 2'-O-methyl Substitutions in 5169-4_26 (21-mer). The combined effects of 2'-O-methyl substitutions in 5169-4_26 lead to the identification of several variants with improved binding affinity, including variant 5169-4_146. This 21-mer has 11 positions that are nuclease-protected by 2'O-methyl and its binding affinity is at least 20-fold greater than the parent truncate, 5169-4_26 (0.60 pM vs 17 pM, respectively). Many other variants also had significant improvements (approximately 3-fold) in binding affinity with combinations of 3 to 10 2'O-methyls. In Table 9, P=5-naphthalene modified dU, a superscript 1 indicates a 2'-O-methyl modified nucleoside, A, C and G represent the naturally occurring deoxyribonucleotides and "NB" denotes no binding up to 3.2 nM PDGF-BB.

Example 6. PDGF Nap-dU Aptamer Activity Assay

[00320] To analyze the inhibitory impact of PDGF Nap-dU aptamers on PDGF Rβ activation cellular phosphorylation inhibition assays were performed as described in Example 4. The four aptamer sequences tested inhibited PDGF Rβ activation with IC_{50} values as follows: 5169-4_26, IC_{50} = 1.6 nM; 5169-4_84, IC_{50}=3.3 nM; 5169-4_85, IC_{50}=7.3 nM; 5169-4_112, IC_{50}=1.0 nM.

Example 7. VEGF Aptamer Selection and Sequences

[00321] Preparation of Candidate Mixtures: A candidate mixture of partially randomized ssDNA oligonucleotides was prepared by polymerase extension of a DNA primer annealed to a biotinylated ssDNA template.

[00322] VEGF SELEX Conditions: Aptamers to recombinant human VEGF-121 protein (both from R&D Systems) were selected by SomaLogic Inc, as described (Gold *et al.* (2010) PloS One 5:e15004), from a library containing a 40-nucleotide random region in which Nap-dU was substituted for dT. For VEGF-121, the forward primer was 5'-GCCACACCCTGCCCTC-3' and the reverse primer was 5'-GAGGACACAGACAGACAC-3'. VEGF-121 protein was biotinylated and partitioned on streptavidin MyOne-SA (Dynal) beads. Preferential selection of aptamers with slow dissociation rates was achieved using a kinetic challenge wherein protein-DNA complexes were incubated in the presence of 10 mM

dextran sulfate at 37°C with increased incubation times and decreased protein concentrations in successive rounds. In the VEGF-121 SELEX, rounds 4 and 5 included a 15 minute kinetic challenge while rounds 6 and 7 (final round) included a 30 minute kinetic challenge.

[00323] The smallest alternatively spliced form of vascular endothelial growth factor, VEGF-121, is a difficult protein target for SELEX. With naturally occurring DNA or RNA libraries, or with nucleic acid libraries modified at the 2'-position of ribose, we have previously failed to obtain even a modest degree of affinity improvement. This is notable for two reasons. First, among members of the cystine knot superfamily, VEGF-121 has the highest structural similarity to PDGF-BB, with a root-mean-square deviation of 1.9 Å for 124 Cα atoms (Muller et al., 1997). Second, the larger and the most prevalent VEGF isoform, VEGF-165, has proved to be a good target for SELEX. For example, pegaptanib (Macugen), the only aptamer-based therapeutic to have received regulatory approval to date (for the treatment of macular degeneration), binds only to VEGF-165 through the heparin-binding exon-7-encoded domain, which is lacking in VEGF-121 (Lee et al., 2005; Ruckman et al., 1998). One difference between VEGF-121, VEGF-165 and PDGF-BB is the overall charge, with pI values of 5.8, 8.5 and 10.1, respectively. This points to the importance of polar interactions in aptamer binding. Successful affinity enrichment for VEGF-121 was ultimately achieved with a SELEX Nap-dU library.

[00324] Identification of VEGF-121 Nap-dU Aptamer Sequences: Two highly related high affinity variants that differ at a single position (4867-15 and 4867-31) were identified from a Nap-dU SELEX experiment performed as described above. Clone 4867-31 has been truncated to a 29-mer in a series of deletion experiments (Table 7). It is worth noting that truncation of both high affinity clones (4867-15 and 4867-31) results in the same 29-mer since the single nucleotide difference is outside the 5' boundary of the minimal sequence. Truncated variants that encompass the shorter sequence with high affinity biding, 29-mer 4867-31_143 (5'-CCGPP CAAGP GCPPG PAGGA PPPAA APGG-3'; where "P" is the single-letter designation for Nap-dU) and its close variants, bind to human VEGF-121, human VEGF-165, mouse VEGF-120 and rat VEGF-164 with comparable affinities, ranging from 0.1 – 1 nM (Table 10). In this table, "P" denotes Nap-dU; A, C, and G denote the naturally occurring deoxyribonucleotides and "NB" denotes no binding up to 100 nM VEGF.

[00325] C3 Spacer Single Substitutions in 4867-15_2 (50-mer). The first round of post-SELEX modifications of the VEGF-121 aptamer was a C3 spacer walk at all positions in the 50mer 4867-15_2 (truncated 50-mer). The C3 spacer walk is meant to identify bases not

required for high affinity binding that could potentially be removed altogether, replaced with the C3 spacer or other linkers such as hexaethylene glycol (Heg) or polyethylene glycol (PEG) linkers. The results for the C3 spacer substitutions are shown in Table 11. In this table, "P" denotes Nap-dU, "V" denotes C3 spacer; A, C, and G denote the naturally occurring deoxyribonucleotides and "NB" denotes no binding up to 100 nM VEGF. At least three internal sites tolerated C3 substitution: C17, G26 and G29 (numbering refers to the 50-mer, as shown below).

[00326] 2'-O-methyl Single Substitutions in 4867-31_43 (32-mer). 2'O-methyl substitutions were made at natural bases in order to identify positions that could tolerate this nuclease-resistant substitution. In addition, 2'-OMe-uridine (2'-OMeU) was substituted for Nap-dU to assess the importance of each Nap-dU. In addition, C3 spacers were tested at certain internal positions hypothesized to be extruded bases, now in the context of the 32mer. Internal deletions and alternative bases were tested at each of the three positions as well. The binding affinity and cell culture inhibition data for select SOMAmers (single concentration of 20 nM) are shown below. The results are shown in Table 12, and demonstrate that C8 (C17 in Table 11) did not completely tolerate substitution to C3 in the context of the shorter truncate. The other two putative extruded bases (G17 and G20) retain good binding and functional activity as C3 or alternative base substitutions in this context. Internal deletions at those positions were not tolerated. None of the Nap-dU modifications could be replaced with 2'OMe-U in this experiment. Several internal sites tolerated 2'OMe modifications, however. In Table 12, P=5-naphthalene modified dU, and a superscript 1 indicates a 2'-OMe modified nucleoside, V=3 carbon spacer, and an empty box indicates a nucleoside deletion. A, C, G, and U represent the naturally occurring deoxyribonucleotides.

[00327] 2'-O-methyl Nap-dU Substitutions and Multiple 2'-O-methyl Substitutions in 4867-31_143 (29-mer). With the 2'O-methyl Nap phosphoramidite synthesized in our laboratories (Nap-mU), we evaluated the Nap-dU positions that would tolerate Nap-mU single substitutions. In addition, we tested combinations of 2'OMe and C3 linker substitutions at each of the natural bases. The binding affinity and cell culture inhibition data for select SOMAmers (single concentration of 20 nM) are shown below. As shown in Table 13 below, most of the Nap-dU residues did not tolerate OMe substitution, but substitution of Nap-mU for Nap-dU at position 22 gave a 10-fold increase in affinity, and excellent inhibitory activity. In this table, superscript "1" denotes 2'-O-methyl substitution, "V" denotes C3 spacer. The 2'-OMe combinations were mostly well-tolerated based on affinity, but certain combinations showed a striking loss of inhibitory activity.

[00328] The combined effects of 2'-O-methyl and C3 substitutions led to the identification of several variants with improved binding affinity, including variant 4867-31_188. This 29-mer has 10 positions that are nuclease-protected by either 2'OMe or C3 and its binding affinity is about 3-fold tighter than the parent truncate, 4867-31_143 (38 pM vs 140 pM, respectively). Variant 4867-31_188 retains comparable cellular inhibition activity relative to the parent SOMAmer. *See* Table 13.

[00329] The only position that tolerated Nap-mU was nucleotide 22 (using 4867-31_143 truncate as the parent sequence). This Nap-mU substitution was next placed into the background of the best combined 2'OMe SOMAmer, 4867-31_188. In addition, the substitution of the original dG at position 15 with a C3 spacer was compared with 2'-OMe substitution at that position to examine the possibility that nuclease-protected base might add rigidity to the molecule and hence increase binding. The best aggregate result was obtained with variant 4867-31_192, which now has 9 protected positions compared to the parent 29-mer truncated variant 4867-31_143 (see Table 14 below; superscript "1" denotes 2'-O-methyl substitution and "V" denotes C3 spacer).

[00330] Modified nucleotide structure activity relationship and affinity maturation: To examine the contribution of each of the ten napthyl side chains to binding, we performed another series of systematic point substitutions by chemically synthesizing 5-position variants with a custom-made library of modified dU phosphoramidites. For this purpose, we designed a library to allow us to probe the microenvironment of each of the positions by varying the size, polarity, disposition of H-bond donors and acceptors, linker length, and orientation of the 5-position substituents. In choosing the functional groups for this analysis, we aimed to include variations on a theme of the original modification (in this case, the napthyl group), amino acid side chains overrepresented in complementarity determining regions (CDRs) of antibodies (like tryptophan and tyrosine) (Mian, I.S. *et al.* (1991) J. Mol. Biol. 217:133; Ramaraj, T. *et al.* (2012) Biochim. Biophys. Acta. 1824:520), and "privileged" fragments of small-molecule drugs (17). FIG. 15 shows the results of these substitutions, represented as the ratio of K_d values (substituted/unsubstituted). Of the 17 different modification substitutions tested each of the ten Nap-dU positions, only four substitutions (Trp-dU 27, NE-dU 16, MBn-dU 10 and BT-dU 16) had little to no effect of the binding affinity. All other substitutions resulted in weaker binding affinity, to varying degrees.

[00331] Deep Sequencing of VEGF SELEX Pool: To evaluate more completely the sequences within the 4149-8_1 aptamer family, the enriched pool was sequenced using 454 pyrosequencing technology. The pool DNA was amplified with 454 primers and the PCR

product was purified and normalized using a Sequal normalization plate (Invitrogen, Cat# A10510-01). The eluate was run on a gel to confirm the size and purity of each amplicon. The purified PCR product was sequenced at the 454 pyrosequencing facility at the University of Colorado Health Sciences Center in Aurora, CO.

[00332] The 454 sequences were aligned with 4867-31 by CLUSTAL analysis. The sequence data set from the pool contained 13,139 full-length sequences (*i.e.*, those sequences containing both primer sequences) of which 2,235 were unique. These 2,235 unique sequences were searched for the motif 5'-CCGPP CAAGP GCPPG PAGGA PPPAA APGG-3'. There were 86 sequences found that contained this motifs. For all the sequences, the percentage identity at each position with 4867-31 was calculated as listed in FIG. 16.

Example 8. VEGF Binding affinity assays

[00333] For determination of target binding affinity, SOMAmers were 5′ end-labeled using T4 polynucleotide kinase (New England Biolabs) and γ-^{32}P-ATP (Perkin Elmer). Binding assays were performed by incubating radiolabeled SOMAmer (~20,000 c.p.m) at a concentration of ~0.03-0.05 nM and target protein at concentrations ranging from 10^{-7} to 10^{-12} M in 1XSB18T buffer (40 mM HEPES,pH 7.5; 120 mM NaCl; 5 mM KCl; 5 mM $MgCl_2$ and 0.01% TWEEN-20) at 37 °C for 30 minutes. Bound complexes were mixed with Zorbax resin and captured on Durapore filter plates. The fraction of SOMAmer bound was quantified with a PhosphorImager (FUJI FLA-3000). Raw binding data were corrected for nonspecific background binding of radiolabeled SOMAmer to Zorbax resin. Equilibrium dissociation constants (K_d) was determined as previously described (Jellinek *et al.* (1993) Proc. Natl. Acad. Sci. 90:11227).

Example 9. VEGF Activity Assay

[00334] To analyze the inhibitory impact of VEGF121 SOMAmers on the cellular kinase activity of VEGF-R2 (Vascular Endothelial Growth factor Receptor 2), we used human umbilical vein endothelial cells (HUVECs) (Lonza, #CC-2519) which expresses endogenously high level of VEGF-R2. HUVEC cells were plated in EGM-2 (Endothelial Cell Growth Medium) supplemented with EGM-2 BulletKit (#CC-3162) containing 2% FBS, growth factors (hEGF, Hydrocortisone, VEGF, HFGF-B, R3-IGF-1), heparin, ascorbic acid and GA-1000 (Gentamicin, Amphotericin-B). When HUVEC cells reached 70 to 80% confluence, they were plated in 24-well plate (10^5 cells/ well) and starved overnight with serum-free medium.

[00335] SOMAmers (a single concentration at 20 nM or a range of concentrations) were added to the cultures with 20 ng/mL (1 nM) of VEGF-121 (R&D System, #4464-VS) containing 1% BSA at 37°C for 30 minutes. The cells were washed in PBS two times and stimulated with the pre-incubated VEGF-121/SOMAmers complex for 5 minutes. The treated cells were washed again with PBS two times and added ice-cold lysis buffer (1% NP-40 Alternative, 20 mM Tris (pH8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate, 10 μg/ mL Aprotinin and 10 μg/mL leupeptin supplemented with a Halt phosphatase inhibitor (Thermo Scientific, #78428). The cell lysates were measured for phosphorylation of VEGF-R2 by using Human Phospho-VEGF R2/KDR Kit (R&D, DYC 1766-2).

[00336] In functional activity experiments *in vitro*, various truncated variants of clone 4867-31 at a screening concentration of 20 nM are capable of essentially completely inhibiting VEGFR2 phosphorylation induced by VEGF-121 or VEGF-165 (1-4 nM) in immortalized or primary human umbilical vein endothelial cells (HUVECs). A representative plot of the IC_{50} determination is shown in FIG. 17 for VEGF aptamers 4867-31_43 and 4867-31_192, with IC_{50} values of 2.2 nM and 2.1 nM, respectively.

[00337] For activity screening of variants of clone 4867-31, we have evaluated percent inhibition of VEGF-induced VEGF R2 phosphorylation in HUVECs, under the same conditions as described above but at a single concentration of SOMAmer variants (generally 20 nM).

Example 10. Homodimer Constructs of PDGF and VEGF Aptamers

[00338] Both PDGF-BB and VEGF are disulfide linked homodimers that exert their biological effects by dimerizing their tyrosine kinase receptors leading to receptor autophosphorylation and signal transduction. If more than one aptamer can bind to its protein target, as is the case with PDGF-BB aptamer 4149-8_260 (based on the crystal structures), such aptamers can be covalently linked in a multimeric construct in a manner that permits simultaneous binding of individual aptamer subunits to the protein. This can lead to improvement in affinity through avidity effect. Two types of homodimers were synthesized, based on readily available chemistry. These were 1) head-to-tail homodimers connected by zero to six Heg linkers, which provide ~20 Å distance per Heg, and 2) 3'-3' homodimers connected via a synthetic doubler support, combined with one to three Hegs on each side (that is, two, four or six Hegs total in the dimer). The homodimers of 4149-8_379, 5169-4_26 and 4867-31_192 were tested in a competition binding assay. For determination of

competitor binding affinities, aptamer ligands were 5′ end-labeled using T4 polynucleotide kinase (New England Biolabs) and γ-^{32}P-ATP (Perkin Elmer). Competition assays were performed by pre-mixing a fixed concentration of radiolabeled ligand (1.0 nM) with varying concentrations of competitor aptamer (10^{-11} to 10^{-6} M). The ligand and competitor dilutions were incubated with the target protein (100 pM) in 1XSB18T buffer (40 mM HEPES, pH 7.5; 120 mM NaCl; 5 mM KCl; 5 mM $MgCl_2$ and 0.01% TWEEN-20) at 37°C for 60 minutes. Bound complexes were mixed with Zorbax resin and captured on Durapore filter plates. The fraction of ligand bound was quantified with a PhosphorImager (FUJI FLA-3000). Raw binding data were normalized to binding without addition of competitor. Data were plotted in GraphPad Prism 3.0 and fit to a one site competition curve using nonlinear regression to determine the equilibrium dissociation constants for the competitor aptamers (K_i). **PDGF Homodimers:** The structure of the PDGF homodimers of sequences 4149-8_379 (sequences 4149-8_438 through 4149-8_447) and 5169-4_26 (sequences 5169-4_134 through 5169-4_143) are shown in Table 15. For the 4149-8_379 based homodimers, the K_i values obtained in the competition assay suggested that in the 5' to 3' configuration, a longer linker was desirable, since a greater than 10-fold improvement in binding affinity was measured with five Heg linkers compared to no Heg linker (0.25 pM vs 4.2 pM, respectively). In the 3' to 3' linked 4149-8_379 homodimers, the longer four and six Heg linkers also performed at least 10-fold better than no linker and approximately 2-fold better than the two Heg linkers. For the 5169-4_26 based homodimers, the K_i values indicated a longer Heg linker was advantageous in the 5' to 3' configuration, since the K_i imporved from 28 pM for no Heg linker to 3.6 pM for six Heg linkers. There was no difference in the K_i values for five and six Heg linkers in the 5' to 3' configuration. In the 3' to 3' linked 5169-4_26 based homodimers the same pattern was observed, with the K_i improving as the Heg linker length increased. The six Heg linker showed a 5-fold improvement in the $_{Ki}$ compared to no Heg linker (2.0 pM vs. 11 pM, respectively). In Tables 15 and 16 , Z = Benzyl-deoxyuridine (Bn-dU), P=5-naphthalene modified dU (Nap-dU), M=methylenedioxybenzyl-dU (MBn-dU), a superscript 1 indicates a 2'-O-methyl modified nucleoside, no superscript indicates deoxyribonucleotides, “C3” indicates a three carbon linker and “H” indicates a hexaethylene glycol linker.

Example 11. PDGF/VEGF Heterodimer Aptamer Constructs

[00339] Heterodimers based on PDGF aptamer 4149-8 and VEGF aptamer 4867-31. With the aim of developing constructs with specificity for PDGF and VEGF, we designed and tested a variety of aptamer constructs comprising a VEGF aptamer linked to a PDGF

aptamer. The first aptamer constructs tested combined PDGF variant 4149-8_273 and VEGF 4867-31_183. Aptamer constructs were synthesized head-to-tail, connected by zero to three hexaethylene glycol (Heg) linkers, in both orientations (either with the PDGF aptamer at the 5' end or VEGF aptamer at the 5' end). The results are shown in Table 17 and 18 below. In Table 17, "Z" denotes Bn-dU, "P" denotes Nap-dU, superscript "1" denotes 2'-O-methyl substitution, no superscript indicates deoxyribonucleotides, "V" denotes C3 spacer and "H" denotes hexaethylene glycol (Heg) linker. In Table 18, percent activity remaining denotes fractional PDGF βR phosphorylation levels in Hs27 fibroblasts in the presence of 20 nM aptamer relative to control (no aptamer).

[00340] Based on binding affinity for PDGF-BB, -AB, VEGF-121, and VEGF-165, aptamer construct 4149-8_320 appeared to give the best results in this experiment. We also tested the aptamer constructs in the PDGF cellular phosphorylation assay, as shown in Table 18. Based on the functional assay data, all aptamer constructs tested inhibited PDGF-BB-induced PDGF βR phosphorylation in Hs27 fibroblasts. Aptamer constructs 4149-8_313, 4149-8_314, 4149-8_315, 4149-8_316, 4149-8_319 and 4149-8_320 inhibited PDGF-BB-induced PDGF βR phosphorylation with IC_{50} values of <20 nM. Aptamer constructs 4149-8_317 and 4149-8_318 had IC_{50} values of ~20 nM.

[00341] We synthesized 4149-8_401, based on the configuration of 4149-8_320 (5'PDGF-3Heg-VEGF3'), comprising PDGF aptamer 4149-8_379 and VEGF aptamer 4867-31_192. *See* Table 19. In this table, "Z" denotes Bn-dU, "P" denotes Nap-dU, M denotes MBn-dU, superscript "1" denotes 2'-O-methyl substitution, no superscript denotes deoxyribonucleotides, "C3" denotes C3 spacer and "H" denotes hexaethylene glycol (Heg) linker. Aptamer construct 4149-8_401 showed binding affinity for PDGF-BB and VEGF121 that was equivalent or better than the binding affinity of its precursor aptamer construct, 4149-8_320. *See* Table 20.

[00342] Aptamer constructs 4149-8_320 and 4149-8_401 inhibited PDGF-BB-induced were PDGF-Rβ phosphorylation in Hs27 fibroblasts with IC_{50} values of about 1 nM and 5 nM, respectively. Further, aptamer construct 4149-8_401 comprising a 5' amino linker conjugated to either 20 kDa or 40 kDa PEG maintained the ability to inhibit PDGF-BB-induced PDGF-Rβ phosphorylation in Hs27 fibroblasts with IC_{50} values of about 1 nM. Those results are consistent with stoichiometric titration/inhibition of all of the PDGF in the assay (1 nM monomer).

[00343] We tested another set of aptamer constructs comprising aptamers 4149-8_379 and 4867-31_192 to determine the effect of overall linker length and the orientation of the PDGF

and VEGF aptamers. *See* Table 21, below. With VEGF at the 5' end, we tested one to six Heg linkers. With PDGF at the 5' end, we tested two to six Heg linkers, including 4149-8_401, which has three Heg linkers. One Heg linker variant was not tested in this orientation because it exhibited somewhat reduced binding in a related variant 4149-8_318. Binding data are shown in Table 16. Most of the aptamer constructs performed well, with the exception of 4149-8_408 and 4149-8_409, which showed somewhat weaker affinity for PDGF-BB. The binding affinity of the Ophthotech aptamer E10030 (Fovista) is included for comparison.

[00344] The ability of the aptamer construct 4149-8_401 to inhibit the activity of both PDGF and VEGF *in vitro* was tested in the receptor phosphorylation experiments as described above. Aptamer construct 4149-8_401 inhibited PDGF-induced PDGFRβ phosphorylation in Hs27 fibroblasts with comparable potency to that of the PDGF monomer 4149-8_379 (IC_{50} values of 2.4 nM and 1.7 nM, respectively). Similarly, aptamer construct 4149-8_401 inhibited VEGF-induced VEGFR2 phosphorylation in HUVEC cells with comparable potency to that of the VEGF monomer 4867-31_192 (IC_{50} values of 0.7 nM and 2.1 nM, respectively). FIG. 18 shows the results of that experiment. FIG. 18A shows (A) inhibition of PDGF-induced PDGF Rβ phosphorylation in Hs27 fibroblasts with PDGF aptamer 4149-8_379 (open circles) and PDGF/VEGF aptamer construct 4149-8_401 (closed circles), and (B) inhibition of VEGF-induced VEGF R2 phosphorylation in HUVECs with VEGF aptamer 4867-31_192 (open circles) and PDGF/VEGF aptamer construct 4149-8_401 (closed circles).

[00345] Heterodimers based on PDGF aptamer 5169-4 and VEGF aptamer 4867-31. We have designed and tested additional heterodimer constructs based on the variants of PDGF aptamer 5169-4_26 and VEGF aptamer 4867-31_192. Aptamer constructs were synthesized head-to-tail, connected by one to six hexaethylene glycol (Heg) linkers, in both orientations (either with the PDGF aptamer at the 5' end or VEGF aptamer at the 5' end). The results are shown in Table 22. With VEGF at the 5' end Heg linkers between three and six resulted in the highest affinities. The affinites in general were slightly lower when the VEGF-121 aptamer sequence was on the 3' end, with most K_d values falling in the 100-300 pM range, except for the five Heg linker sequence which had a K_d of 56 pM. With PDGF at the 5' end, the K_d values ranged from 11 pM for three Heg linkers to 0.54 pM for four Heg linkers, with the remaing K_d values falling in between for all other Heg linker lengths. When PDGF was at the 3' end there was a trend towards higher binding affinity as the linker length increased, with one Heg linker having a K_d of 5.3 pM and six Heg linkers having a K_d of 0.20

pM. In Table 22, "P" denotes Nap-dU, superscript "1" denotes 2'-O-methyl substitution, no superscript denotes deoxyribonucleotides and "H" denotes hexaethylene glycol (Heg) linker.

Example 12. Simultaneous Binding of PDGF/VEGF Aptamer Constructs to VEGF and PDGF

[00346] To demonstrate the ability of the PDGF/VEGF aptamer constructs to bind VEGF and PDGF simultaneously, a sandwich assay was developed. Briefly, Nunc Maxisorp® plates were coated with either human PDGF-BB or human VEGF-121 (20 ng/mL). After blocking the wells with a 1% BSA solution, PDGF/VEGF aptamer construct was added (10 nM) and allowed to bind to the adsorbed protein target. After washing, the biotinylated complementary protein (2 nM PDGF-BB for VEGF-121 coated plates and 2 nM VEGF-121 or VEGF-165 for PDGF-BB coated plates) was allowed to bind to form a ternary complex. Following another wash, horseradish peroxidase conjugated streptavidin (HRP-SA) was added and allowed to form a quaternary complex. After a final wash, a color forming horse radish peroxidase substrate was added according to the manufacturer's directions (Thermo Scientific TMB substrate kit 34021) and the reaction stopped when appropriate by addition of 1.6 M H_2SO_4. The absorbance per well at 450 nm was determined with the Spectramax M5 plate reader with auto check on. In parallel to the method described above, a set of four control experiments were executed in which one of the 4 components that make up the quaternary complex was excluded.

[00347] As shown in FIG. 19, PDGF/VEGF aptamer construct SL1012 (20 kDa PEG-N-4149-8_401) was able to bind simultaneously to human VEGF-121 and PDGF-BB. A strong signal was observed when all components of the quaternary complex were added (complete) while the absence of any one of the 4 components resulted in background or near background signal. Similar results were obtained with PDGF and VEGF coated plates, indicating that the order of protein addition to the aptamer construct did not matter. As shown in FIG. 19, SL1012 was able to bind simultaneously to human VEGF-165 and PDGF-BB. FIG. 19A shows microtiter plates coated with VEGF with addition of biotinylated PDGF. FIG.19B shows microtiter plates coated with PDGF with addition of biotinylated VEGF. Data are presented as the mean + 95% confidence interval (n=3). A strong signal was observed when all components of the quaternary complex were added (complete) while the exclusion of any of the four components resulted in background or near background signal. The data also demonstrate that the addition of a PEG moiety to the 5'-terminus of the aptamer construct does not preclude simultaneous binding activity.

[00348] Simultaneous binding of human VEGF-165 and human PDGF-BB to (A) SL1012 or and (B) SL1013 (40 kDA PEG-N-4149-8-401) is shown in FIG.20. Microtiter plates were coated with PDGF with addition of biotinylated VEGF. Complete means addition of all components of the quaternary complex, while each condition without one of the four components is shown in the graphs. Data are presented as the mean + 95% confidence interval (n=3)

[00349] A similar experiment was performed with various aptamer constructs that did not contain the PEG moiety. In this experiment, only a no aptamer control was included because the previous results demonstrated the requirement for the other components of the complex to generate signal. As shown in FIG. 21, aptamer constructs 4149-8_317, 4149-8_318, 4149-8_320, 4149-8_401 and 4149-8_414 simultaneously bound to PDGF and VEGF regardless of the order of protein addition. FIG. 21(A) shows microtiter plates coated with VEGF with addition of biotinylated PDGF and FIG. 21(B) shows microtiter plates coated with PDGF with addition of biotinylated PDGF.

Add data on simultaneous binding of heterodimer constructs based on the variants of PDGF aptamer 5169-4 and VEGF aptamer 4867-31.

Example 13 . Intravitreal Pharmacokinetic Studies

[00350] Initial ocular pharmacokinetic testing was performed to understand how the aptamers and aptamer constructs behave in the eye. Four aptamers and aptamer constructs were tested as shown in Table 23.

[00351] For each aptamer or aptamer construct, a single intravitreal injection was performed into both eyes of five New Zealand White rabbits (10 eyes). Animals received either a 0.5 mg/eye dose (SL1010 and SL1011) or a 1.0 mg/eye dose (SL1012 and SL1013). These doses represent the weight of the aptamer or aptamer construct only (PEG weight was excluded from the calculations). All test articles were formulated in phosphate buffered saline. For each aptamer or aptamer construct test article, vitreous humor samples were collected from both eyes from one animal at 2, 24, 48, 96 or 192 hours post dose. Vitreous humor samples were stored frozen until they were assayed.

[00352] The vitreous humor concentrations of the aptamers or aptamer constructs were determined by ultra performance liquid chromatography (UPLC) assay methods with detection by absorbance at 260 nanometers (nm). Briefly, the vitreous hydrogel was sheered by passing it several times through a 20 gauge needle. Vitreous proteins were precipitated by the addition of 2 volumes of 2-ethoxyethanol. Following centrifugation, the supernatant was

recovered and injected onto an Acquity® C18 column (0.2 x 100 mm). The column temperature was 80°C and the flow rate was maintained at 0.2 mL/min. Buffer A consisted of TEAA pH 7.0 and 5% acetonitrile. Buffer B consisted of 100% acetonitrile. The program held 50% buffer B for 1 minute following the injection of sample and then buffer B was increased linearly to 70% over 4 minutes. Detection was accomplished by absorbance at 260 nm. Concentrations (free acid equivalent) of aptamer or aptamer construct in the vitreous humor were determined by interpolation of the peak absorbance units of the unknown samples to those obtained by a standard curve prepared with known concentrations of aptamer or aptamer construct.

[00353] The results of that experiment are shown in Table 24.

[00354] An ordinary linear regression fit of the natural logarithm of the vitreous concentration versus time resulted in estimates for the vitreous half-lives of 105, 47, 69 and 92 hours for SL1010, SL1011, SL1012, and SL1013, respectively. Table 25 shows the results, along with the 95% condfidence interval.

[00355] These vitreous half-lives compare favorably to the half-lives in NZW rabbits of similar sized therapeutic VEGF inhibitors, such as Macugen (83 hours, Eyetech Study Group (2002) Retina 22:143) and Lucentis (70 hours, Gaudreault *et al*. (2007) Retina 27:1260). Therefore, these aptamers and aptamer constructs may be useful for the treatment of ocular diseases such as AMD and diabetic retinopathy.

Example 14 . Materials and Methods

[00356] This Example provides a summary of the general methods and materials used in the proceeding examples.

[00357] Determination of nuclease sensitive sites by liquid chromatography coupled to mass spectrometry (LCMS): For digestion by DNase I, polyacrylamide-gel-purified aptamer (500 nM final concentration) was incubated with recombinant human DNase I (Cell Sciences, Cat No. CSI10719) (0.3 units for 4149-8_379 and 3 units for 5169-4_146) in nuclease buffer (510 mM Tris HCl pH 7.6, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$) at 37°C in a total reaction volume of 150 μL. Aliquots (50 μL) were collected at 0, 30 and 60 minutes and the reaction terminated by incubating at 95°C for 5 minutes. For digestion with DNase II 500 nM of gel purified aptamer was incubated with porcine DNase II (Worthington Biochemical Corporation) (18 units for 4867-31_192, 1 unit for 4149-8_379) in nuclease buffer (100 mM sodium acetate pH 4.6, 2 mM $MgCl_2$, 15 mM NaCl) at 37°C in a total reaction volume of 150 μL. Aliquots were collected and the reactions stopped as described above. The 5'-amine

containing versions of 4867-31_192 and 4149-8_379 and the 5'-OH version of 5169-4_146 were utilized for these experiments.

[00358] Following nuclease digestion, the full-length and aptamer fragments were separated by liquid chromatography and detected by ultraviolet absorbance and mass spectrometry (LCMS). Briefly, samples were analyzed on an LCMS system consisting of an Agilent 1100 HPLC equipped with a vacuum degasser (Model # G1322A), an autosampler (Model # G1313A), a binary pump module (Model # G1312A), a column manager with temperature control (Model #G1316A) and a variable wavelength detector (Model # G1314A)) coupled to a Bruker Esquire 3000 ion trap mass spectrometer. LCMS runs were performed with Agilent Chemstation® software (version B.01.03). The chromatography was performed using a Hamilton PRP-3, 2.1 x 150 mm column at 80°C. Mobile phase A contained 10 mM piperidine and 10 mM imidazole, pH approximately 10 (not adjusted); mobile phase B contained 10 mM piperidine and 10 mM imidazole in 75/25 acetonitrile/water. The flow rate was 0.25 mL/min and the gradient elution was 0-1 minutes: 0%B; 1-20 minutes:0-53% B. Samples (20 μL) were injected and absorbance determined at 254 nm. Typical source conditions for mass spectrometric detection were as follows: negative ion electrospray ionization mode, nebulizer pressure at 50 psi, drying gas at 7L/min, source temperature was set at 325°C, and the capillary voltage was set at 3000 V. Trap conditions were scan range 200 to 2200 m/z, accumulation time 50 msec, trap drive 92, averaging 25 spectra.

[00359] Data were processed using Daltonics DataAnalysis® 2.0. Mass spectra were obtained by summing across the observed peak in the TIC, and smoothing using a Svitsky-Golay algorithm (smoothing width=0.8 m/z, cycles=2). Spectra were deconvoluted using the algorithm within the Daltonics software. Parameters included mass range between 2000-30000 Da, abundance cutoff of 10%, minimum peaks in component of 3, and molecular weight agreement of 0.05%.

[00360] DNase I aptamer stability assay: Polyacrylamide-gel-purified aptamers at a final concentration of 250 nM were incubated with 2 or 10 units/mL of recombinant human DNase I (Cell Sciences, Cat No. CSI10719) in nuclease buffer (10 mM Tris HCl pH 7.6, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$) at 37°C in a total reaction volume of 100 μL. At various times, a 20 μL aliquot was collected and the reaction stopped by adding an equal volume of 2x gel loading buffer (93.85% formamide, 0.03% SDS, 20 mM Na_2EDTA, 0.01% xylene cylanol and 0.01% Orange G) and heating at 95°C for 2 minutes. Samples were loaded onto a 15% TBE polyacrylamide denaturing gel (8 M urea) and electrophoresis performed at 200 V for 20 minutes. Gels were stained with approximately 2 μM SYBR® Gold (Molecular Probes,

Cat No. S11494) for 10 minutes to visualize the bands. The amount of full-length aptamer remaining at each time point was quantified using FlourChem®Q analysis software (Alpha Innotech). If necessary, the intensity of each band was determined following a background subtraction and data are presented as a percentage remaining of full-length input DNA at the zero time point. Aptamers with a hydroxyl at the 5'-terminus were utilized for these experiments except for aptamer 4149-8_379 which harbored a 5'-terminal amine.

[00361] DNase II aptamer stability assay: Polyacrylamide-gel-purified aptamers at a final concentration of 250 nM were incubated with 120 units/mL or 240 units/mL of porcine DNase II (Worthington Biochemical Corporation) in nuclease buffer (0.1M NaOAc pH 4.6, 2.0 mM $MgCl_2$, 15 mM $NaCl_2$) at 37°C in a total reaction volume of 100 μL. At various times, a 20 μL aliquot was collected and the reaction stopped by adding an equal volume of 2x gel loading buffer (93.85% formamide, 0.03% SDS, 20 mM Na_2EDTA, 0.01% xylene cylanol and 0.01% Orange G) and heating at 95°C for 2 minutes. Samples were loaded onto a 15% TBE polyacrylamide denaturing gel (8 M urea) and electrophoresis performed at 200 V for 20 minutes. Gels were stained with approximately 2 μM SYBR® Gold (Molecular Probes, Cat No. S11494) for 10 minutes to visualize the bands. The amount of full-length SOMAmer remaining at each time point was quantified using FlourChem® Q analysis software (Alpha Innotech). If necessary, the intensity of each band was determined following a background subtraction and data are presented as a percentage remaining of full-length input DNA at the zero time point. Aptamers with a hydroxyl at the 5'-terminus were utilized for these experiments except for aptamer 4149-8_379 which harbored a 5'-terminal amine.

[00362] SOMAmer stability assay in rabbit vitreous humor: Polyacrylamide-gel-purified aptamers at a final concentration of 500 nM were incubated in 90% pooled vitreous humor from New Zealand White rabbits (Bioreclamation IIC, Cat No. RAB-VITHUM. Samples were incubated at 37°C in a total reaction volume of 200 μL (samples also contained a final concentration of 0.067% phosphate buffered saline). At various time points, a 20 μL aliquot was collected and the reaction stopped by adding an equal volume of 2x gel loading buffer (93.85% formamide, 0.03% SDS, 20 mM Na_2EDTA, 0.01% xylene cylanol and 0.01% Orange G). Samples were stored at -20°C until processed. To each sample 100 μL of water was added followed by 150 μL of 25:24:1 Phenol/Chloroform/Isoamyl alcohol (AMRESCO). Samples were mixed and centrifuged at 16,100 x *g* for 15 minutes. The aqueous phase was collected and frozen at -20°C until gel analysis. Samples (15 μL) were loaded onto a 15% TBE polyacrylamide denaturing gel (8 M urea) and electrophoresis performed for approximately 20 minutes at 200 V. Gels were stained with approximately 2 μM SYBR®

Gold (Molecular Probes, Cat No. S11494) for 10 minutes to visualize the bands. The amount of full-length SOMAmer remaining at each time point was quantified using FlourChem® Q analysis software (Alpha Innotech). If necessary, the intensity of each band was determined following a background subtraction and data presented as a percentage remaining of full-length input DNA at the zero time point.

[00363] Competition Binding Assay Methods: Aptamers to be used as ligands in the competition binding assay were 5'-end-labeled using T4 polynucleotide kinase (New England Biolabs) and γ-[^{32}P]ATP (Perkin Elmer). The competition assay was performed by combining equal volumes of 2 nM ligand with unlabeled competitor (concentrations ranging from 10^{-6} to 10^{-11} M) in 1 x SB18T buffer [40 mM Hepes (pH 7.5), 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, and 0.01% TWEEN-20]. Human PDGF-BB (Creative Biomart) at a concentration of 200 pM in 1 X SB18T buffer was added to the ligand/competitor mixtures and the reactions were incubated at 37°C for 40 minutes. Bound complexes were mixed with Zorbax® resin (Agilent Technologies) and captured on Durapore® filter plates. The signal in each well was quantified with a PhosphorImager (FUJI FLA-3000). Raw binding data were corrected for background signal and normalized to fraction bound for no competitor. To determine the inhibitor constants (K_i), the data were plotted in GraphPad Prism® 6 and fit to a one-site competition curve.

Example 15. VEGF Aptamers with Improved Nuclease Stability

[00364] This Example provides the chemical modifications applied to VEGF aptamers that improve the nuclease stability of the aptamers. In this Example, the following abbreviations are used in context with the aptamer: P is NapdU and 2'-OMe is a 2'-O-methyl nucleotide. To enhance the nuclease resistance of the NapdU-containing Slow Off Rate Modified Aptamers (SOMAmers) selected to bind to human vascular endothelial growth factor (VEGF), 2'-O-methyl (2'-OMe) groups were added at nucleotide positions in which binding to VEGF protein was not significantly affected. These efforts led to the discovery of aptamer 4867-31_192 which contains nine 2'-OMe groups within the 29 nucleotide sequence. The use of Nap-dU nucleotides, the 2'-OMe substitutions and the addition of an inverted deoxythymidine at the 3'-terminus in 4867-31_192 together impart a substantial degree of nuclease protection compared with unmodified DNA Nevertheless, 4867-31_192 is still not fully stable in the presence of some nucleases. For example, while stable against human recombinant DNase I (Figure 23A), 4867-31_192 is digested by porcine DNase II (Figure 23B). As shown in Figure 23B, a decrease in the amount of full-length aptamer over time indicates sensitivity to digestion by DNase II.

[00365] To determine the feasibility of further stabilizing the aptamer by using phosphorothioate linkages, a systematic substitution of phosphorothioates in place of individual phosphodiester bonds (the "phosphorothioate walk" experiment) was performed in order to determine which positions of aptamer 4867-31_192 could accept a phosphorothioate linkage without significantly impacting binding to VEGF121. For all sequences in this example and the proceeding examples, a nucleotide having a superscript "1" indicates that the nucleotide has a 2'-O-methyl modification, and a nucleotide having a superscript "2" indicates that the nucleotide has a 3'-phosphorothioate group and links that nucleotide to the nucleotide immediately 3' to it). A nucleotide having both superscript "1" and "2" indicates that the nucleotide has both a 2'-O-methyl modification and a 3'-phosphorothioate group and links that nucleotide to the nucleotide immediately 3' to it. All aptamers were tested for binding with the inclusion of an inverted deoxythymidine at the 3'-terminus. The results of this walk are shown in Table 26. Most, but not all, positions tested could accept a phosphorothioate linkage without severely impacting binding. An important exception was the guanine at position 3 (4867-31_410) where substitution of a phosphorothioate linkage for the natural phosphordiester linkage reduced affinity for VEGF121 by 29,259-fold.

[00366] Since nuclease sensitive sites cannot be predicted, the position(s) of the DNase II sensitive cleavage sites were identified by mass spectrometry following incubation with porcine DNase II as described in Example 14 (data not shown). For aptamer 4867-31_192 (with the addition of an inverted deoxythymidine at the 3'-terminus) two major fragments were identified. Fragment one contained nucleotides 1 through 24 while fragment two contained nucleotides 1 through 25. Therefore, a series of aptamers were created in which phosphorothioate linkages were substituted for phosphodiester linkages in various combinations between nucleotide positions 24 and 25, 25 and 26, and 26 and 27 as shown in Table 27. Substitutions at all 3 of these positions were allowed since none significantly affected binding (Table 26). Phosphorothioate substitutions were tested with or without the addition of a 2'-OMe cytosine at position 1 (Table 27). For nuclease stability experiments all subsequent aptamers were studied with the inclusion of an inverted deoxythymidine at the 3'-terminus.

[00367] The susceptibility to DNase II was examined as described in Example 14 and the data presented in Figure 24. All aptamers tested that had phosphorothioate linkages between positions 24 and 25 and 25 and 26 were more stable in this assay than aptamers that were not modified at these two positions. This trend was observed for aptamers with or without a 2'-OMe at position 1.

[00368] The aptamer 4867-31_192 and a subset of aptamers with phosphorothioate linkages at both key positions were next tested for stability in 90% rabbit vitreous humor (Figure 25). Because we are considering developing aptamers-based antagonists to VEGF and PDGF for the treatment of ocular disorder, stability in the vitreous humor has relevance for their therapeutic effectiveness. As illustrated in the plot of percent full-length aptamers remaining versus incubation time (Figure 25), all of the aptamers modified with phosphorothioate linkages were more stable (at 192 hours) in this biological matrix than 4867-31_192 (without phosphorothioate linkages).

[00369] To ensure these substitutions did not adversely affect binding, an experiment was performed to test each aptamer for binding to human VEGF121 protein. All sequences tested retained high affinity binding to target. The dissociation constants (K_d values) obtained in this experiment are shown in Table 27.

[00370] Figure 24 shows the percentage of full-length aptamer remaining versus incubation time with DNase II as described in Example 14. Aptamers (all at 250 nM), as indicated by aptamer identification no. (Aptamer ID No.), were incubated at 37°C for the indicated number of hours with 0.24 units/μL DNase II and the digestion products separated by polyacrylamide gel electrophoresis. As determined by densitrometry, the percentage of full-length aptamer remaining versus incubation time is plotted. The plot foraptamer ID no. 4867-31_192 in Figure 24 is the same plot as shown in Figure 23B.

[00371] Figure 25 shows the percentage of full-length aptamer remaining versus incubation time in vitreous humor as described in Example 14. Aptamers (all at 500 nM) as indicated by aptamer identification no. (Aptamer ID No.) were incubated at 37°C for the indicated number of hours in 90% vitreous humor obtained from New Zealand White rabbits. Aptamers were resolved by polyacrylamide gel electrophoresis. Figure 25 shows the percent remaining of each full-length aptamer band versus time as determined by densitometry.

[00372] In summary, these data, generally, indicate that the 4867-31 aptamer with phosphorothioate linkage(s) is less sensitive to nuclease digestion than the 4867-31 aptamer without phosphorothioate linkage(s), and further, that the 4867-31 aptamer with phosphorothioate linkage(s) retains binding affinity to the VEGF protein target.

Example 16. PDGF Aptamers with Improved Nuclease Stability

[00373] This Example provides the chemical modifications applied to PDGF aptamers (e.g., 5169-4) that improve the nuclease stability of the aptamers. In this Example, the following

abbreviations are used in context with the aptamer: P is NapdU and 2'-OMe is a 2'-O-methyl nucleotide.

[00374] To enhance the nuclease resistance of the NapdU-containing Slow Off Rate Modified Aptamers (SOMAmers) selected to bind to human platelet derived growth factor (PDGFBB), 2'-O-methyl groups (2'-OMe) were added at nucleotide positions in which binding to PDGF protein was not or only minimally affected. These efforts led to the discovery of aptamer 5169-4_146 which contains eleven 2'-OMe groups within the 21 nucleotide sequence. With these eleven 2'-OMe modifications and an inverted deoxythymidine at the 3'-terminus, 5169-4_146 is stable against DNase II, but not against DNAse I (Figure 26). Therefore, in contrast to the VEGF aptamers 4867-31_192 described in Example 15 which was more sensitive to DNase II than DNase I, PDGF-BB aptamers 5169-4_146 exhibits greater sensitivity to DNase I compared with DNase II. In other words, the sensitivity of 5169-4_146 to DNase I, but not DNase II is opposite to the result found in Example 15 and shows the unpredictability of aptamers sensitivity to nucleases. This suggests that the intrinsic sensitivity to degradation by specific nucleases cannot be predicted and has to be determined empirically. Once this sensitivity to a specific nuclease is determined, additional stability might be achieved with additional phophorothioate substitutions at the remaining sensitive positions. As shown in Figure 26A, a decrease in full-length aptamer over time indicates sensitivity to digestion by DNase I.

[00375] As shown in Figure 26, aptamer 5169-4_146 (250 nM) was incubated at 37°C for the indicated number of hours with 0.01 units/μL DNase I (Figure 26A) or 0.12 units/μL DNase II (Figure 26B) as described in Example 14 and the digestion products separated by polyacrylamide gel electrophoresis. As determined by densitrometry, the percentage of full-length aptamer remaining versus incubation time is plotted.

[00376] To determine the feasibility of further stabilizing the aptamer by using phosphorothioate linkages, a "phosphorothioate walk" experiment was performed on 5169-4_146 in order to determine which positions could accept a phosphorothioate without significantly impacting aptamer binding to PDGF. The results of this walk are shown in Table 28. All aptamers tested included an inverted deoxythymidine at the 3' terminus. Phosphorothioate substitution was well-tolerated at all positions of the 5169-4_146 sequence. The maximum impairment of binding to PDGF was a reduction of affinity by 1.8-fold (5169-4_154).

[00377] Similar to Example 15, the position of the sensitive nucleotides of 5169-4_146 (with an inverted deoxythymidine at the 3' terminus) to cleavage by DNase I were identified by

mass spectrometry and these most sensitive "nuclease sites" protected by substitution of a 3'-phosphorothioate linkage in place of the natural 3'-phosphodiester linkage.

[00378] For aptamer 5169-4_146 several fragments were identified following digestion with human recombinant DNase I. These fragments revealed DNase I sensitive sites between nucleotide position 9 and 10; 10 and 11; 11 and 12; and 12 and 13. Therefore an aptamer was created in which phosphorothioate linkages were substituted for phosphodiester at these four positions and designated as aptamer 5169-4_182 (Table 29). These aptamers were tested for nuclease resistance with the inclusion of an inverted deoxythymidine at the 3' terminus. The susceptibility to DNase I was examined as before (Figure 27). The phosphorothioate-containing aptamer was clearly more stable than 5169-4_146.

[00379] As shown in Figure 27, aptamer 5169-4_182 (250 nM) was incubated at 37°C for the indicated number of hours with 0.01 units/μL DNase I as described in example 14 and the digestion products separated by polyacrylamide gel electrophoresis. Bands were visualized with SYBR gold and, as determined by densitrometry, the percentage of full-length aptamer remaining versus incubation time is plotted.

[00380] The same aptamer was also tested for stability in 90% rabbit vitreous humor (Figure 28). As illustrated in the plot of percent full-length aptamers remaining versus incubation time (Figure 28), aptamer 5169-4_182 (with phosphorothioate linkages) was more stable in this biological matrix than 5169-4_146 (without phosphorothioate linkages).

[00381] As shown in Figure 28, aptamers (at 500 nM) as indicated were incubated at 37°C for the indicated number of hours in 90% vitreous humor obtained from New Zealand white rabbits as described in Example 14. Aptamers were visualized by polyacrylamide gel electrophoresis. Figure 28 shows the percent remaining of each full length aptamer band (SOMAmer) versus time as determined by densitometry.

[00382] Although 5169-4_182 was clearly more stable than 5169-4_146, a DNase I digestion product of 5169-4_182 was clearly visible (data not shown). Using the mass spectrometry described in Example 14, the cleavage site for this major digestion product was identified as between nucleotide positions 4 and 5. This was surprising as nucleotide position 4 was already protected by a 2'-OMe moiety (Table 29). This result illustrates the fact that there are nucleases capable of cleaving phosphodiester bonds adjacent to 2'-OMe-substituted nucleotides. Nonetheless, a 3'-phosphorothioate linkage was substituted for the natural phosphodiester linkage between nucleotide positions 4 and 5 of 5169-4_182 to create aptamer 5169-4_188 (Table 29). The susceptibility to DNase I was examined as before (Figure 29). Aptamers 5169-4_182 and 5169-4_188 were more stable than 5169-4_146. The addition of a

phosphorothioate linkage between nucleotides 4 and 5 in 5169-4_188 eliminated the appearance of the stable digestion product observed for 5169-4_182 (data not shown) which led to a better stability against DNase I (Figure 29).

[00383] As shown in figure 29, aptamers, as indicated by aptamer identification no. (Aptamer ID No.),, were incubated at 37°C for the indicated number of hours with 0.01 units/μL DNase I as described in example 14 and the digestion products separated by polyacrylamide gel electrophoresis. Bands were visualized with SYBR gold and, as determined by densitrometry, the percentage of full-length aptamer remaining versus incubation time is plotted.

[00384] To ensure these substitutions did not adversely affect binding, an experiment was performed to determine the affinity of each aptamers for human PDGFBB protein. All sequences tested retained high affinity binding to target. The dissociation constants (K_d values) obtained in this experiment are shown in Table 29.

[00385] In summary, these data, generally, indicate that the 5169-4 aptamer with phosphorothioate linkage(s) are less sensitive to nuclease digestion than the 5169-4 aptamer without phosphorothioate linkage(s), and further, that the 5169-4 aptamer with phosphorothioate linkage(s) retains binding affinity to the PDGF protein target.

Example 17. Additional PDGF Aptamers with Improved Nuclease Stability

[00386] This Example provides the chemical modifications applied to PDGF aptamers (e.g., 4149-8) that improve the nuclease stability of the aptamers. In this Example, the following abbreviations are used in context with the aptamer: P is a NapdU; 2'-OMe is a 2'-O-methyl nucleotide; Bn is a BenzyldU (BndU); M is a methoxybenzyl (MBndU); V is a 3 carbon spacer; and i is iBudU.

[00387] To enhance the nuclease resistance of the BndU-containing Slow Off Rate Modified Aptamers (SOMAmers) selected to bind to human platelet derived growth factor (PDGFBB), 2'-O-methyl (2'-OMe) groups were added at nucleotide positions in which binding to PDGF protein was not significantly affected. These efforts led to the discovery of aptamer 4149-8_379 which contains seven 2'-OMe groups within the 24 nucleotide sequence. Even with these seven 2'-OMe modifications, 4149-8_379 is still not impervious to nuclease digestion. For example, 4149-8_379 can be digested with recombinant human DNase I (data not shown) and porcine DNase II (Figure 30). As shown in Figure 30, a decrease of the full-length aptamer over time indicates sensitivity to digestion by DNase II. This sensitivity to both

DNase I and DNase II for 4149-8_379 is different than found for examples 15 and 16 and again shows the unpredictability of the nuclease stability of aptamers.

[00388] As shown in Figure 30, aptamer 4149-8_379 (250 nM) was incubated at 37°C for the indicated number of hours with 0.12 units/μL DNase II as described in Example 14 and the digestion products separated by polyacrylamide gel electrophoresis. Bands were visualized with SYBR Gold and, as determined by densitrometry, the percentage of full-length aptamer remaining versus incubation time is plotted.

[00389] In order to further enhance the nuclease resistance of this aptamer, the position of the sensitive nucleotides to the action of DNase I and DNase II were identified by mass spectrometry by the method described in Example 14. DNase I sensitive sites were identified between nucleotide positions 15 and 16 and between nucleotide positions 16 and 17. A DNase II sensitive site was identified between nucleotide positions 9 and 10. Therefore phosphorothioate linkages were substituted for the phosphodiester linkages at these three positions. In addition, a phosphorothioate linkage was substituted for the phosphodiester linkage between nucleotides 17 and 18. Finally the 3, 4 methylene dioxy benzyl-dU nucleotide (or MBndU; designated by M in Tables 30 and 31) at position 2 replaced with a BndU and the BndU at position 16 replaced with a NapdU. This aptamer was designated 4149-8_454 (Table 30). Aptamer 4149-8_455 was created which, in addition to the modifications of 4149-8_454, also contained a 2'-OMe G as position 13 and a phosphorothioate linkage in place of the natural phosphodiester linkage between nucleotides 8 and 9 (Table 30). Although a complete phosphorothioate walk was not performed, a number of sequences were created and tested for affinity to PDGFBB (Table 31). Some of these sequences served as a guide for creating the aptamers are shown in Table 31.

[00390] The susceptibility to DNase II was examined as before (Figure 31) when all aptamers contained an inverted deoxythymidine at the 3' terminus. The results show that aptamer 4149-8_455 was more stable than 4149-8_454 which was more stable than 4149-8_379.

[00391] Aptamers 4149-8_379 and 4149-8_454 with the addition of an inverted deoxythymidine at the 3'-terminus were tested for stability in 90% rabbit vitreous humor (data not shown). However, in this biological matrix no additional stability was obtained with 4149-8_454 as compared to 4149-8_379.

[00392] To ensure these substitutions did not adversely affect binding, an experiment was performed to determine the affinity of each aptamer for human PDGFBB protein. All

sequences tested retained their binding affinity to the target. The dissociation constants (K_d values) obtained in this experiment are shown in Table 30.

[00393] As shown in Figure 31, the aptamers as indicated by aptamer ID no. (i.e., 4149-8_379;4149-8_454 and 4149-8_455) were incubated at 37°C for the indicated number of hours with DNase II and the digestion products separated by polyacrylamide gel electrophoresis. As The percent full-length aptamer, as determined by densitrometry from the gel image, versus incubation time is plotted. The plot for aptamer ID no. 4149-8_379 in Figure 31 is the same plot as shown in Figure 30.

[00394] The foregoing embodiments and examples are intended only as examples. No particular embodiment, example, or element of a particular embodiment or example is to be construed as a critical, required, or essential element or feature of any of the claims. Various alterations, modifications, substitutions, and other variations can be made to the disclosed embodiments without departing from the scope of the present invention, which is defined by the appended claims. The specification, including the figures and examples, is to be regarded in an illustrative manner, rather than a restrictive one, and all such modifications and substitutions are intended to be included within the scope of the invention. Accordingly, the scope of the invention should be determined by the appended claims and their legal equivalents, rather than by the examples given above. For example, steps recited in any of the method or process claims may be executed in any feasible order and are not limited to an order presented in any of the embodiments, the examples, or the claims.

Table 1. Sequences Representative of the 4149-8_1 and Truncated Variants with K_d values for PDGF of 10 nM or less

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_1	C-G-C-C-C-T-C-G-T-C-C-C-A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A-C-C-A-A-C-C-G-A-G-A-A-C-G	1
4149-8_2	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	2
4149-8_4	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A	3
4149-8_5	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G	4
4149-8_6	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G	5
4149-8_7	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G	6
4149-8_8	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C	7
4149-8_9	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A	8
4149-8_10	A-T-C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C	9
4149-8_11	C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	10
4149-8_12	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	11
4149-8_13	Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	12
4149-8_20	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C	13
4149-8_21	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A	14
4149-8_22	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A	15
4149-8_23	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G	16
4149-8_24	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-T	17
4149-8_25	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G	18
4149-8_26	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A	19
4149-8_27	C-T-C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C	20
4149-8_28	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C	21
4149-8_29	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-T	22

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_30	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	23
4149-8_31	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C	24
4149-8_32	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G	25
4149-8_36	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C	26
4149-8_37	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-T	27
4149-8_38	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	28
4149-8_39	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C	29
4149-8_40	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G	30
4149-8_44	Bn-Bn-A[1]-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	31
4149-8_45	Bn-Bn-A-C[1]-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	32
4149-8_46	Bn-Bn-A-C-G[1]-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	33
4149-8_47	Bn-Bn-A-C-G-A[1]-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	34
4149-8_48	Bn-Bn-A-C-G-A-C[1]-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	35
4149-8_49	Bn-Bn-A-C-G-A-C-Bn-A[1]-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	36
4149-8_50	Bn-Bn-A-C-G-A-C-Bn-A-C[1]-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	37
4149-8_51	Bn-Bn-A-C-G-A-C-Bn-A-C-G[1]-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	38
4149-8_52	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A[1]-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	39
4149-8_53	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C[1]-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	40
4149-8_54	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A[1]-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	41
4149-8_55	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C[1]-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	42
4149-8_56	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G[1]-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	43
4149-8_57	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C[1]-G-Bn-Bn-Bn-A-Bn-A-G-C-G	44
4149-8_58	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G[1]-Bn-Bn-Bn-A-Bn-A-G-C-G	45
4149-8_59	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A[1]-Bn-A-G-C-G	46

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_60	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G	47
4149-8_61	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G^1-C-G	48
4149-8_62	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C^1-G	49
4149-8_63	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G^1	50
4149-8_64	C3-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	51
4149-8_67	Bn-Bn-A-C3-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	52
4149-8_68	Bn-Bn-A-C-C3-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	53
4149-8_69	Bn-Bn-A-C-G-C3-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	54
4149-8_70	Bn-Bn-A-C-G-A-C3-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	55
4149-8_71	Bn-Bn-A-C-G-A-C-C3-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	56
4149-8_72	Bn-Bn-A-C-G-A-C-Bn-C3-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	57
4149-8_73	Bn-Bn-A-C-G-A-C-Bn-A-C3-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	58
4149-8_78	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C3-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	59
4149-8_79	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-C3-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	60
4149-8_80	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C3-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	61
4149-8_87	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-C3-Bn-A-G-C-G	62
4149-8_91	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C3-G	63
4149-8_92	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-C3	64
4149-8_93	Bn-Bn-A^1-C-G-A^1-C-Bn-A^1-C-G-Bn-Bn-A-C-A^1-C-G^1-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G^1-C^1-G^1	65
4149-8_94	Bn-Bn-A^1-C-G-A-C-Bn-A^1-C-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	66
4149-8_95	Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	67
4149-8_96	Bn-Bn-A-C-Heg-Heg-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	68
4149-8_97	Bn-Bn-A-C-G-Heg-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	69
4149-8_98	Bn-Bn-A-C-C3-C3-C3-C3-C3-C3-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	70

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_99	Bn-Bn-A-C-C3-C3-C3-C3-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	71
4149-8_100	Bn-Bn-A-C-C3-C3-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	72
4149-8_101	Bn-Bn-A^1-C-G-C3-C-C3-C3-C-G-Bn-Bn-A-C-A^1-C-G^1-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G^1-C^1-G^1	73
4149-8_103	U^1-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	74
4149-8_105	Bn-Bn-A-C-G-A-C-U^1-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	75
4149-8_106	Bn-Bn-A-C-G-A-C-Bn-A-C-G-U^1-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	76
4149-8_110	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-U^1-A-Bn-A-G-C-G	77
4149-8_112	Bn-Nap-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	78
4149-8_113	Bn-Bn-A-C-G-A-C-Nap-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	79
4149-8_114	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Nap-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	80
4149-8_115	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Nap-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	81
4149-8_116	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Nap-Bn-Bn-A-Bn-A-G-C-G	82
4149-8_117	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Nap-Bn-A-Bn-A-G-C-G	83
4149-8_118	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Nap-A-Bn-A-G-C-G	84
4149-8_119	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Nap-A-G-C-G	85
4149-8_121	Nap-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	86
4149-8_122	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-U^1-A-G-C-G	87
4149-8_123	Bn-Bn-A^1-C-Heg-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	88
4149-8_124	Bn-Bn-A-C-Heg-Heg-G-Bn-Bn-A-C-A-C-G-C-G-Nap-Bn-Bn-A-Bn-A-G-C-G	89
4149-8_125	Bn-Bn-A-C-Heg-Heg-G-U^1-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	90
4149-8_126	Bn-Bn-A^1-C-Heg-Heg-G-U^1-Bn-A-C-A^1-C-G-C-G-Nap-Bn-Bn-A-Bn-A^1-G-C-G^1	91
4149-8_128	Bn-Nap-A^1-C-Heg-Heg-G-Nap-Nap-A-C-A^1-C-G-C-G-Nap-Nap-Bn-A-Nap-A^1-G-C-G^1	92
4149-8_130	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	93
4149-8_131	Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-A-C-G-C-G-Nap-Bn-Bn-A-Bn-A-G-C-G	94

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_132	Bn-Bn-A-C-Heg-G-U^1-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	95
4149-8_133	Bn-Bn-A^1-C-Heg-G-U^1-Bn-A-C-A^1-C-G-C-G-Nap-Bn-Bn-A-Bn-A^1-G-C-G^1	96
4149-8_135	Pe-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	97
4149-8_136	Bn-Pe-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	98
4149-8_137	Bn-Bn-A^1-C-Heg-G-Pe-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	99
4149-8_138	Bn-Bn-A^1-C-Heg-G-Bn-Pe-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	100
4149-8_139	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Pe-Bn-Bn-A-Bn-A^1-G-C-G^1	101
4149-8_140	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Bn-A-Bn-A^1-G-C-G^1	102
4149-8_141	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Pe-A-Bn-A^1-G-C-G^1	103
4149-8_142	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Pe-A^1-G-C-G^1	104
4149-8_143	BT-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	105
4149-8_144	Bn-BT-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	106
4149-8_145	Bn-Bn-A^1-C-Heg-G-BT-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	107
4149-8_146	Bn-Bn-A^1-C-Heg-G-Bn-BT-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	108
4149-8_147	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-BT-Bn-Bn-A-Bn-A^1-G-C-G^1	109
4149-8_148	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-BT-Bn-A-Bn-A^1-G-C-G^1	110
4149-8_149	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-BT-A-Bn-A^1-G-C-G^1	111
4149-8_150	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-BT-A^1-G-C-G^1	112
4149-8_151	Th-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	113
4149-8_152	Bn-Th-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	114
4149-8_153	Bn-Bn-A^1-C-Heg-G-Th-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	115
4149-8_154	Bn-Bn-A^1-C-Heg-G-Bn-Th-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	116
4149-8_155	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Th-Bn-Bn-A-Bn-A^1-G-C-G^1	117
4149-8_156	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Th-Bn-A-Bn-A^1-G-C-G^1	118

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_157	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Th-A-Bn-A^1-G-C-G^1	119
4149-8_158	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Th-A^1-G-C-G^1	120
4149-8_159	Nap-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	121
4149-8_160	Bn-Nap-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	122
4149-8_161	Bn-Bn-A^1-C-Heg-G-Nap-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	123
4149-8_162	Bn-Bn-A^1-C-Heg-G-Bn-Nap-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	124
4149-8_163	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Nap-Bn-Bn-A-Bn-A^1-G-C-G^1	125
4149-8_164	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Nap-Bn-A-Bn-A^1-G-C-G^1	126
4149-8_165	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Nap-A-Bn-A^1-G-C-G^1	127
4149-8_166	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Nap-A^1-G-C-G^1	128
4149-8_167	Ib-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	129
4149-8_168	Bn-Ib-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	130
4149-8_169	Bn-Bn-A^1-C-Heg-G-Ib-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	131
4149-8_170	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	132
4149-8_171	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Ib-Bn-Bn-A-Bn-A^1-G-C-G^1	133
4149-8_172	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Ib-Bn-A-Bn-A^1-G-C-G^1	134
4149-8_173	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Ib-A-Bn-A^1-G-C-G^1	135
4149-8_174	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Ib-A^1-G-C-G^1	136
4149-8_175	Trp-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	137
4149-8_176	Bn-Trp-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	138
4149-8_177	Bn-Bn-A^1-C-Heg-G-Trp-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	139
4149-8_181	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Trp-A-Bn-A^1-G-C-G^1	140
4149-8_182	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Trp-A^1-G-C-G^1	141
4149-8_183	2Nap-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	142

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_184	Bn-2Nap-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	143
4149-8_185	Bn-Bn-A^1-C-Heg-G-2Nap-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	144
4149-8_187	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-2Nap-Bn-Bn-A-Bn-A^1-G-C-G^1	145
4149-8_188	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-2Nap-Bn-A-Bn-A^1-G-C-G^1	146
4149-8_189	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-2Nap-A-Bn-A^1-G-C-G^1	147
4149-8_190	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-2Nap-A^1-G-C-G^1	148
4149-8_191	2NE-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	149
4149-8_192	Bn-2NE-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	150
4149-8_193	Bn-Bn-A^1-C-Heg-G-2NE-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	151
4149-8_194	Bn-Bn-A^1-C-Heg-G-Bn-2NE-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	152
4149-8_195	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-2NE-Bn-Bn-A-Bn-A^1-G-C-G^1	153
4149-8_197	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-2NE-A-Bn-A^1-G-C-G^1	154
4149-8_198	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-2NE-A^1-G-C-G^1	155
4149-8_199	NE-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	156
4149-8_200	Bn-NE-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	157
4149-8_201	Bn-Bn-A^1-C-Heg-G-NE-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	158
4149-8_202	Bn-Bn-A^1-C-Heg-G-Bn-NE-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	159
4149-8_203	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-NE-Bn-Bn-A-Bn-A^1-G-C-G^1	160
4149-8_204	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-NE-Bn-A-Bn-A^1-G-C-G^1	161
4149-8_205	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-NE-A-Bn-A^1-G-C-G^1	162
4149-8_206	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-NE-A^1-G-C-G^1	163
4149-8_207	MBn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	164
4149-8_208	Bn-MBn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	165
4149-8_209	Bn-Bn-A^1-C-Heg-G-MBn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	166

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_210	Bn-Bn-A^1-C-Heg-G-Bn-MBn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	167
4149-8_211	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-MBn-Bn-Bn-A-Bn-A^1-G-C-G^1	168
4149-8_212	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-MBn-Bn-A-Bn-A^1-G-C-G^1	169
4149-8_213	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-MBn-A-Bn-A^1-G-C-G^1	170
4149-8_214	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-MBn-A^1-G-C-G^1	171
4149-8_215	PP-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	172
4149-8_216	Bn-PP-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	173
4149-8_217	Bn-Bn-A^1-C-Heg-G-PP-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	174
4149-8_218	Bn-Bn-A^1-C-Heg-G-Bn-PP-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	175
4149-8_219	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-PP-Bn-Bn-A-Bn-A^1-G-C-G^1	176
4149-8_220	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-PP-Bn-A-Bn-A^1-G-C-G^1	177
4149-8_221	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-PP-A-Bn-A^1-G-C-G^1	178
4149-8_222	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-PP-A^1-G-C-G^1	179
4149-8_223	Tyr-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	180
4149-8_224	Bn-Tyr-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	181
4149-8_225	Bn-Bn-A^1-C-Heg-G-Tyr-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	182
4149-8_226	Bn-Bn-A^1-C-Heg-G-Bn-Tyr-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	183
4149-8_227	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Tyr-Bn-Bn-A-Bn-A^1-G-C-G^1	184
4149-8_228	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Tyr-Bn-A-Bn-A^1-G-C-G^1	185
4149-8_229	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Tyr-A-Bn-A^1-G-C-G^1	186
4149-8_230	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Tyr-A^1-G-C-G^1	187
4149-8_231	FBn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	188
4149-8_232	Bn-FBn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	189
4149-8_233	Bn-Bn-A^1-C-Heg-G-FBn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	190

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_234	Bn-Bn-A^1-C-Heg-G-Bn-FBn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	191
4149-8_235	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-FBn-Bn-Bn-A-Bn-A^1-G-C-G^1	192
4149-8_236	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-FBn-Bn-A-Bn-A^1-G-C-G^1	193
4149-8_237	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-FBn-A-Bn-A^1-G-C-G^1	194
4149-8_238	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-FBn-A^1-G-C-G^1	195
4149-8_239	Bn-Bn-A^1-Cº-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	196
4149-8_240	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-Cº-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	197
4149-8_241	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-Cº-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	198
4149-8_242	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-Cº-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	199
4149-8_243	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-Cº-G^1	200
4149-8_245	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	201
4149-8_246	Bn-Bn-A^1-C-Heg-G^1-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	202
4149-8_247	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G^1-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	203
4149-8_248	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G^1-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	204
4149-8_249	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G^1-C-G^1	205
4149-8_254	Tyr-Bn-A^1-C-Heg-G-FBn-Bn-A-C-A^1-C-G-C-G-2Nap-Bn-Bn-A-Bn-A^1-G-C-G^1	206
4149-8_255	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	207
4149-8_256	Bn-Bn-A^1-C-Heg-G-2Nap-Ib-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	208
4149-8_257	Bn-Bn-A^1-C-Heg-G-NE-Ib-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	209
4149-8_259	Bn-Bn-A^1-C-Heg-G-Ib-Ib-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	210
4149-8_260	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	211
4149-8_261	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Pe-A-Bn-A^1-G-C-G^1	212
4149-8_262	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Th-Th-A-Bn-A^1-G-C-G^1	213
4149-8_263	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-2Nap-Pe-Th-A-Bn-A^1-G-C-G^1	214

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_264	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Th-Pe-Th-A-Bn-A^1-G-C-G^1	215
4149-8_265	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Ib-Pe-Th-A-Bn-A^1-G-C-G^1	216
4149-8_266	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C-G-C-G-Th-Pe-Th-A-Bn-A^1-G-C-G^1	217
4149-8_267	Bn-Ib-A^1-C-Heg-G-Ib-Ib-A-C-A^1-C-G-C-G-Ib-Bn-Bn-A-Bn-A^1-G-C-G^1	218
4149-8_268	2Nap-2Nap-A^1-C-Heg-G-2Nap-Bn-A-C-A^1-C-G-C-G-2Nap-Bn-Bn-A-Bn-A^1-G-C-G^1	219
4149-8_269	NE-Bn-A^1-C-Heg-G-NE-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-NE-A^1-G-C-G^1	220
4149-8_270	Th-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Th-Bn-Th-A-Bn-A^1-G-C-G^1	221
4149-8_271	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G^1-C-G^1	222
4149-8_272	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G^1-C-G^1	223
4149-8_273	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	224
4149-8_274	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G^1-C-G^1	225
4149-8_275	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C-G-C-G-2Nap-Pe-Th-A-Bn-A^1-G-C-G^1	226
4149-8_279	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C^1-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	227
4149-8_280	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C-G-C-G-Bn-Pe-Th-A^1-Bn-A^1-G-C-G^1	228
4149-8_281	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G^1-C-G^1	229
4149-8_282	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C^1-G-C-G-Bn-Pe-Th-A^1-Bn-A^1-G-C-G^1	230
4149-8_283	Bn-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C^1-G-C-G-Bn-Pe-Th-A^1-Bn-A^1-G^1-C-G^1	231
4149-8_284	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-Cº-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	232
4149-8_285	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C^1-A^1-C^1-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C^1-G^1	233
4149-8_286	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C^1-A^1-C^1-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G^1-C^1-G^1	234
4149-8_287	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-C3-C3-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	235
4149-8_288	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-C3--G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	236
4149-8_289	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-C3-C^1-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	237
4149-8_290	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C3-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	238

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_291	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-Heg-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	239
4149-8_292	Bn-Bn-A-C-G-C-A-C-G-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	240
4149-8_293	Bn-Bn-A-C-C-A-C-G--G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	241
4149-8_294	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-T-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	242
4149-8_295	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-C-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	243
4149-8_296	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-G-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	244
4149-8_297	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-T-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	245
4149-8_298	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-A-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	246
4149-8_299	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-G-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	247
4149-8_300	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-T-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	248
4149-8_301	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	249
4149-8_302	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-G-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	250
4149-8_303	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	251
4149-8_307	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	252
4149-8_308	Bn-Bn-A^1-C-Heg-G-Bn-Bn^1-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	253
4149-8_311	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn^1-A-Bn-A^1-G-C-G^1	254
4149-8_312	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn^1-A^1-G-C-G^1	255
4149-8_321	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Bn-A-MBn-A^1-G-C-G^1	256
4149-8_322	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-MBn-A^1-G-C-G^1	257
4149-8_323	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Th-A^1-Bn-A^1-G-C-G^1	258
4149-8_324	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Th-Pe-Th-A^1-Bn-A^1-G-C-G^1	259
4149-8_325	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn-A^1-Bn-A^1-G-C-G^1	260
4149-8_326	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	261
4149-8_327	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	262

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_328	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	263
4149-8_329	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn-A^1-MBn-A^1-G-C-G^1	264
4149-8_330	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn-A^1-MBn-A^1-G-C-G^1	265
4149-8_331	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Th-A^1-MBn-A^1-G-C-G^1	266
4149-8_332	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-C3-C^1-G-C-G-Bn-Pe-Bn-A^1-MBn-A^1-G-C-G^1	267
4149-8_333	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-C3-C^1-G-C-G-Bn-Pe-Th-A^1-MBn-A^1-G-C-G^1	268
4149-8_334	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-Heg-Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	269
4149-8_335	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-Heg-Heg-Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	270
4149-8_336	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-Heg-Heg-Heg-Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	271
4149-8_337	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-Heg-Heg-Heg-Heg-Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	272
4149-8_338	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-Heg-Heg-Heg-Heg-Heg-Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	273
4149-8_339	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-C3-(3'-Doubler)-T	274
4149-8_340	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-(3'-Doubler)-T	275
4149-8_341	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-Heg-(3'-Doubler)-T	276
4149-8_342	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1-Heg-Heg-Heg-(3'-Doubler)-T	277
4149-8_343	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	278
4149-8_344	Bn-Bn-A^1-C-Heg-Gº-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	279
4149-8_345	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-Gº-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	280
4149-8_346	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-Gº-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	281
4149-8_347	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-Gº-C-G^1	282
4149-8_349	Bn-Bn-A^1-Cº-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	283

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_352	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-Cº-G^1	284
4149-8_353	Bn-Bn-A^1-C-Heg-G-Bn-Bn-Aº-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	285
4149-8_354	2Nap-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	286
4149-8_355	FBn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	287
4149-8_356	NE-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	288
4149-8_357	2NE-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	289
4149-8_358	PP-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	290
4149-8_359	Ib-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	291
4149-8_360	Bn-MBn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	292
4149-8_361	T-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	293
4149-8_362	Bn-T-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	294
4149-8_363	Bn-Bn-A^1-C-Heg-G-T-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	295
4149-8_369	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A^2-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	296
4149-8_370	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	297
4149-8_371	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	298
4149-8_372	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	299
4149-8_373	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	300
4149-8_374	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	301
4149-8_375	FBn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	302
4149-8_376	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	303
4149-8_377	FBn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	304
4149-8_378	FBn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	305
4149-8_379	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	306
4149-8_380	FBn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	307

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_381	FBn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	308
4149-8_382	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	309
4149-8_383	FBn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	310
4149-8_384	FBn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	311
4149-8_385	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	312
4149-8_386	FBn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	313
4149-8_388	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	314
4149-8_389	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	315
4149-8_390	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	316
4149-8_391	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	317
4149-8_392	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	318
4149-8_393	FBn-Bn-A^1-C-Heg-G-Bn-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	319
4149-8_394	Bn-MBn-A^1-C-Heg-G-Bn-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	320
4149-8_395	FBn-MBn-A^1-C-Heg-G-Bn-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	321
4149-8_396	FBn-Bn-A^1-C-Heg-G-Bn-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	322
4149-8_397	Bn-MBn-A^1-C-Heg-G-Bn-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	323
4149-8_398	FBn-MBn-A^1-C-Heg-G-Bn-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	324
4149-8_399	FBn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	325
4149-8_400	FBn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-A^1-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	326
4149-8_402	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-G-C-G--Bn-Bn-Bn-A-Bn-A-G-C	327
4149-8_403	Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-A-G-C-G--Bn-Bn-Bn-A-Bn-A-G-C	328
4149-8_404	Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-C3-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C	329
4149-8_418	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G^1-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	330
4149-8_419	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G^1-Nap-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	331
4149-8_420	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G-C-G^1-2Nap-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	332

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_421	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G^1-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	333
4149-8_422	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G^1-C-G^1-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	334
4149-8_423	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G^1-C-G-Nap -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	335
4149-8_424	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A-C-C3-C^1-G^1-C^1-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	336
4149-8_425	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^1-C-C3-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	337
4149-8_431	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Nap-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	338
4149-8_432	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	339
4149-8_433	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Nap-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	340
4149-8_434	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	341
4149-8_435	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Nap-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	342
4149-8_436	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Bn-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	343
4149-8_437	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A^2-C-C3-C^1-G-C-G^2-Nap-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	344

No superscript indicates deoxyribose
Superscript o indicates 2'-fluoro
Superscript 1 indicates 2'-O-methyl
Superscript 2 indicates phosphorothioate (deoxyribose)

C3= three carbon linker
Heg= hexaethylene glycol linker
Nap= naphthyl-dU
Pe= phenethyl-dU
BT= benzothiophenyl-dU
Th= thiophenyl-dU
Ib= isobutyl-dU
Trp= tryptaminyl-dU
2Nap= 2-naphthyl-dU
2NE= 2-naphthylethyl-dU
NE= naphthylethyl-dU
MBn= methylenedioxybenzyl-dU
PP= phenpropyl-dU
Tyr= tyrosyl-dU
FBn= fluorobenzyl-dU
Bn= benzyl-dU

3'-Doubler=Symmetric Doubler Phosphoramidite (Glen Research, Cat# 10-1920-02)

Table 1a. Homodimers of PDGF aptamer 4149-8_260 (SL5):

			SOMAmer (2.5 nM) PDGF-BB (1nM) duplicates	
Seqi	**Kd (M)**	**Ratio to 4149-8 _38**	**Relative PDGFRb phosphorylation**	
4149-8_38	3.00E-11	1.0	5.4%	3.6%
4149-8_334	4.92E-11	1.6	1.7%	1.7%
4149-8_335	5.46E-11	1.8	1.5%	1.5%
4149-8_336	1.84E-11	0.61	1.7%	2.3%
4149-8_337	3.79E-12	0.1		
4149-8_338	2.84E-11	0.93	1.4%	1.3%
4149-8_339 3'DBLR	6.20E-12	0.2	2.7%	3.6%
4149-8_340 3'DBLR	1.89E-11	0.6	1.7%	3.2%
4149-8_341 3'DBLR	2.72E-11	0.9	1.5%	1.9%
4149-8_342 3'DBLR	1.81E-11	0.6	1.6%	1.5%

Table 2. Sequences Representative of the Truncated Variants with K_d values for PDGF of more than 10 nM

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_14	C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	345
4149-8_15	A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	346
4149-8_16	Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	347
4149-8_17	C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	348
4149-8_18	Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	349
4149-8_19	A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G-Bn-C-A-A-C-C-C-G-A-G-Bn-G-A-A-C-A	350
4149-8_33	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A	351
4149-8_34	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-T	352
4149-8_35	C-Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A	353
4149-8_41	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A	354
4149-8_42	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-T	355
4149-8_43	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A	356

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_65	Bn-C3-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	357
4149-8_66	Bn-Bn-C3-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	358
4149-8_74	Bn-Bn-A-C-G-A-C-Bn-A-C-C3-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	359
4149-8_75	Bn-Bn-A-C-G-A-C-Bn-A-C-G-C3-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	360
4149-8_76	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-C3-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	361
4149-8_77	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-C3-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	362
4149-8_81	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-C3-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	363
4149-8_82	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C3-G-Bn-Bn-Bn-A-Bn-A-G-C-G	364
4149-8_83	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-C3-Bn-Bn-Bn-A-Bn-A-G-C-G	365
4149-8_84	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-C3-Bn-Bn-A-Bn-A-G-C-G	366
4149-8_85	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-C3-Bn-A-Bn-A-G-C-G	367
4149-8_86	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-C3-A-Bn-A-G-C-G	368
4149-8_88	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-C3-A-G-C-G	369
4149-8_89	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-C3-G-C-G	370
4149-8_90	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-C3-C-G	371
4149-8_102	Bn-Bn-A^1-C-G-C3-C-C3-C3-C-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	372
4149-8_104	Bn-U^1-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	373
4149-8_107	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-U^1-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	374
4149-8_108	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-U^1-Bn-Bn-A-Bn-A-G-C-G	375
4149-8_109	Bn-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-U^1-Bn-A-Bn-A-G-C-G	376
4149-8_111	Nap-Bn-A-C-G-A-C-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-U^1-A-G-C-G	377
4149-8_127	Bn-Nap-A-C-Heg-Heg-G-U^1-Nap-A-C-A-C-G-C-G-Nap-Nap-Bn-A-Nap-A-G-C-G	378
4149-8_129	Bn-Nap-A^1-C-Heg-Heg-G-U^1-Nap-A-C-A^1-C-G-C-G-Nap-Nap-Bn-A-Nap-A^1-G-C-G^1	379
4149-8_134	Bn-Bn-A-C-G-Bn-Bn-A-C-A-C-G-C-G-Bn-Bn-Bn-A-Bn-A-G-C-G	380
4149-8_178	Bn-Bn-A^1-C-Heg-G-Bn-Trp-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	381

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_179	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Trp-Bn-Bn-A-Bn-A^1-G-C-G^1	382
4149-8_180	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Trp-Bn-A-Bn-A^1-G-C-G^1	383
4149-8_186	Bn-Bn-A^1-C-Heg-G-Bn-2Nap-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	384
4149-8_196	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-2NE-Bn-A-Bn-A^1-G-C-G^1	385
4149-8_244	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A^1-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	386
4149-8_250	Tyr-MBn-A^1-C-Heg-G-FBn-Ib-A-C-A^1-C-G-C-G-2Nap-Pe-Th-A-NE-A^1-G-C-G^1	387
4149-8_251	Tyr-MBn-A^1-C-Heg-G-FBn-Ib-A-C-A^1-C^1-G-C-G-2Nap-Pe-Th-A^1-NE-A^1-G^1-C-G^1	388
4149-8_252	Trp-Ib-A^1-C-Heg-G-NE-Ib-A-C-A^1-C-G-C-G-Th-Pe-Th-A-MBn-A^1-G-C-G^1	389
4149-8_253	Trp-Ib-A^1-C-Heg-G-NE-Ib-A-C-A^1-C^1-G-C-G-Th-Pe-Th-A^1-MBn-A^1-G^1-C-G^1	390
4149-8_258	Bn-Bn-A^1-C-Heg-G-U^1-Ib-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	391
4149-8_276	Bn-Bn-A^1-C-Heg-G-FBn-Ib-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	392
4149-8_277	Tyr-Bn-A^1-C-Heg-G-Bn-Ib-A-C-A^1-C-G-C-G-Bn-Pe-Th-A-Bn-A^1-G-C-G^1	393
4149-8_278	Tyr-Bn-A^1-C-Heg-G-FBn-Ib-A-C-A^1-C-G-C-G-2Nap-Pe-Th-A-Bn-A^1-G-C-G^1	394
4149-8_304	Bn-Bn-A^1-C-C3-G-Bn-Bn-A-C-C3--G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	395
4149-8_305	Bn^1-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	396
4149-8_306	Bn-Bn^1-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	397
4149-8_309	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn^1-Bn-Bn-A-Bn-A^1-G-C-G^1	398
4149-8_310	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn^1-Bn-A-Bn-A^1-G-C-G^1	399
4149-8_348	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-Aº-C-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	400
4149-8_350	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-Cº-A^1-C^1-G-C-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	401
4149-8_351	Bn-Bn-A^1-C-Heg-G-Bn^1-Bn-A-C-A^1-C^1-G-Cº-G-Bn-Pe-Bn^1-A^1-Bn-A^1-G-C-G^1	402
4149-8_364	Bn-Bn-A^1-C-Heg-G-Bn-T-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-Bn-A^1-G-C-G^1	403
4149-8_365	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-T-Bn-Bn-A-Bn-A^1-G-C-G^1	404
4149-8_366	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-T-Bn-A-Bn-A^1-G-C-G^1	405

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.
4149-8_367	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-T-A-Bn-A^1-G-C-G^1	406
4149-8_368	Bn-Bn-A^1-C-Heg-G-Bn-Bn-A-C-A^1-C-G-C-G-Bn-Bn-Bn-A-T-A^1-G-C-G^1	407
4149-8_405	Bn-Bn-A-C-Heg-G-Bn-Bn-A-C-C3-C-G-Bn-Bn-Bn-A-Bn-A-G	408
4149-8_426	Bn-MBn-A^1-C-Heg-G-Bn^1-Bn-A^1-C-C3-C^1-G-C-G^1-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	409

No superscript indicates deoxyribose
Superscript o indicates 2'-fluoro
Superscript 1 indicates 2'-O-methyl
Superscript 2 indicates phosphorothioate (deoxyribose)

C3= three carbon linker
Heg= hexaethylene glycol linker
Nap= naphthyl-dU
Pe= phenethyl-dU
BT= benzothiophenyl-dU
Th= thiophenyl-dU
Ib= isobutyl-dU
Trp= tryptaminyl-dU
2Nap= 2-naphthyl-dU
2NE= 2-naphthylethyl-dU
NE= naphthylethyl-dU
MBn= methylenedioxybenzyl-dU
PP= phenpropyl-dU
Tyr= tyrosyl-dU
FBn= fluorobenzyl-dU
Bn= benzyl-dU

Table 3. Truncations of PDGF aptamer Clone 4149-8_1.

Aptamer ID. No.	Sequence (5'→ 3')	Length	K_d (nM)	Seq. ID. NO.
4149-8_2	A-T-C-T-C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	50	0.05	2
4149-8_5	A-T-C-T-C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G	46	0.02	4
4149-8_7	A-T-C-T-C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G	42	0.02	6
4149-8_9	A-T-C-T-C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-	38	0.03	8

	C-A-A			
4149-8_10	A-T-C-T-C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C	36	0.05	9
4149-8_11	C-T-C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	48	0.03	10
4149-8_12	C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	46	0.04	11
4149-8_13	Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	44	0.8	12
4149-8_14	C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	42	13	345
4149-8_16	Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	38	22	347
4149-8_18	Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	34	13	349
4149-8_19	A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A-G-Z-G-A-A-C-A	32	74	350
4149-8_26	C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C-A-A-C-C-C-G-A	39	0.08	19
4149-8_27	C-T-C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-Z-C	34	0.09	20
4149-8_29	C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-T	31	0.07	22
4149-8_30	C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G	30	0.07	23
4149-8_31	C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C	29	0.30	24

4149-8_32	C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G	28	6	25
4149-8_33	C-Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A	27	>1000	351
4149-8_37	Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G-T	30	0.04	27
4149-8_38	Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C-G	29	0.05	28
4149-8_39	Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G-C	28	.11	29
4149-8_40	Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A-G	27	1.5	30
4149-8_41	Z-Z-A-C-G-A-C-Z-A-C-G-Z-Z-A-C-A-C-G-C-G-Z-Z-Z-A-Z-A	26	>1000	354

Table 4. Data-collection, refinement and modelstatistics.

DATA COLLECTION		
Ligand	**4149-8_260**	**4149-8_255**
Space group	$P4_12_12$	$P4_12_12$
Unit-cell parameters: a, b, c (Å)	59.10, 59.10, 167.01	59.40, 59.40 168.2
Wavelength (Å)	0.97918	0.97918
Resolution range (Å)	50.0-2.20 (2.26-2.20)	50.0-2.30 (2.36-2.30)
Unique reflections	15629	13750
Completeness (%)	96.8 (99.1)	97.2 (98.4)
R_{merge}	4.0 (57.8)	6.4 (57.2)
Mean $I/\sigma(I)$	25.3 (3.4)	19.3 (2.6)
REFINEMENT		
Ligand	**4149-8_260**	**4149-8_255**
Resolution range (Å)	50.0-2.20 (2.26-2.20)	50.0-2.30 (2.36-2.30)

R_{cryst}	0.225(0.329)	0.239(0.366)
R_{free}	0.262 (0.353)	0.279 (0.526)
R.m.s.d. bonds (Å)	0.018	0.018
R.m.s.d. angles (°)	1.882	2.017
Total atoms	1382	1355
Mean *B* factor (Å^2)	43.68	43.86
Residues in favored region (%)	98.9	98.9
Residues in allowed region (%)	100.0	100.0
Molprobity score (percentile)	1.45 (99th)	1.41 (99th)
PDB ID	4HQU	4HQX

Values in parenthesis indicate the values for the highest of twenty resolution shells

$R_{merge} = \Sigma_h\Sigma_i|I_i(h) - \langle I(h)\rangle|/\Sigma_h\Sigma_i I_i(h)$

$R_{free} = \Sigma_h||F_{obs}| - |F_{calc}||/\Sigma_h|F_{obs}|$.

The free R factor was calculated using 5% of the reflections omitted from the refinement (The CCP4 suite: programs for protein crystallography, 1994).

*Ligand B-factors are for ligands in the active sites of the protein monomers. Ligands from solvent (PEG, glycerol, etc.) were not included in the calculation.

Table 5. Base-pair parameters for the PDGF BB aptamer compared to B-form DNA.

Parameter	B-DNA	5'Stem loop		Miniknot S1		Miniknot S2	
Complementary base-pair parameters							
Buckle (deg.)	0.5 (6.7)	-5.67 (7.90)		0.32 (22.8)		-0.74 (15.3)	
Propeller (deg.)	-11.4 (5.3)	-9.84 (6.10)		7.43 (10.4)		2.42 (7.75)	
Opening (deg.)	0.60 (3.1)	-8.13 (25.2)		7.86 (12.5)		0.35 (0.40)	
Shear (Å)	0.00 (0.21)	1.12 (1.42)		1.29 (2.52)		-0.03 (0.42)	
Stretch (Å)	-0.15 (0.12)	-0.38 (0.66)		1.96 (3.55)		-0.12 (0.42)	
Stagger (Å)	0.09 (0.19)	0.04 (0.61)		-0.45 (0.69)		0.28 (0.05)	
Base-pair step parameters		**U2, A3/ U7, U8**	**A3, C4/ G6, U7**	**C10, A9/ U16, G15**	**A9, U20/ U17, U16**	**C12, G13/ C23, G24**	**G13, C14/ G22, C23**
Tilt (deg.)	-0.1 (2.5)	-0.80	-1.45	-4.65	-5.83	-2.39	3.86
Roll (deg.)	0.6 (5.2)	0.34	0.55	6.12	-5.64	4.19	3.94
Twist (deg.)	36 (6.8)	24.1	33.3	25.3	15.9	29	33.9
Shift (Å)	-0.02 (0.45)	3.20	-0.12	-0.90	-1.45	-0.17	0.78
Slide (Å)	0.23 (0.81)	0.25	-0.24	0.27	-4.49	0.21	-0.4
Rise (Å)	3.32 (0.19)	2.68	3.28	2.98	3.58	2.73	3.13
Local helical parameters							
Inclination (deg.)	2.1 (9.2)	0.81	0.96	13.6	-18.9	8.28	6.72
Tip (deg.)	0.0 (4.3)	1.92	2.52	10.4	19.5	4.72	-6.57
Helical twist (deg.)	36.5 (6.6)	24.1	33.4	26.3	17.8	29.4	34.3
x-displacement (Å)	0.05 (1.28)	0.52	-0.5	-0.97	-9.84	-0.34	-1.28
y-displacement (Å)	0.02 (0.87)	-7.89	-0.03	0.81	-0.12	-0.09	-0.73
Helical rise (Å)	3.29 (0.21)	2.58	3.28	3.07	5.08	2.73	3.13

Table 6. Truncations of PDGF aptamer clone 5169-4.

Aptamer ID. No.	Sequence (5'→3')	K_d (nM)	Seq. ID. NO.
5169-4_3	C-T-G-C-C-P-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A-P-G-A-G-G-A	0.029	410
5169-4_4	C-C-P-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A-P-G-A-G-G-A	0.027	411
5169-4_5	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A-P-G-A-G-G-A	0.031	412
5169-4_6	C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A-P-G-A-G-G-A	67	413
5169-4_7	C-T-G-C-C-P-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A-P-G-A	0.014	414
5169-4_8	C-T-G-C-C-P-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A	0.036	415
5169-4_9	C-T-G-C-C-P-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P	0.72	416
5169-4_10	C-T-G-C-C-P-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C	0.63	417
5169-4_11	C-T-G-C-C-P-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A	0.60	418

5169-4_12	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A	0.064	419
5169-4_13	G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A	0.027	420
5169-4_14	A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A-A	0.17	421
5169-4_15	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G-A	0.019	422
5169-4_16	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P-G	0.016	423
5169-4_17	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A-C-C-C-A-G-P	0.020	424
5169-4_18	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P-A	0.026	425
5169-4_19	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C-P	0.012	426
5169-4_20	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G-C	0.014	427
5169-4_21	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G-G	0.021	428
5169-4_22	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G	0.015	429
5169-4_23	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A	0.020	430
5169-4_24	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G	0.021	431
5169-4_25	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A	0.020	432
5169-4_26	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.017	433
5169-4_27	G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A	0.062	434

5169-4_28	G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G	0.048	435
5169-4_29	G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A	0.062	436
5169-4_30	G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.062	437
5169-4_32	G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-A-G-A-G	0.053	438

Table 7. C3 spacer substitutions at all positions in 5169-4_26.

Aptamer ID. No.	Sequence (5'→3')	K_d (nM)	Seq. ID. NO.
5169-4_59	C3-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.053	439
5169-4_60	C-C3-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	27	440
5169-4_61	C-G-C3-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	NB	441
5169-4_62	C-G-A-C3-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	42	442
5169-4_63	C-G-A-C-C3-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	29	443
5169-4_64	C-G-A-C-A-C3-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.054	444
5169-4_65	C-G-A-C-A-G-C3-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.074	445
5169-4_66	C-G-A-C-A-G-C-C3-P-G-P-A-P-G-C-A-C-A-P-C-P	8.5	446
5169-4_67	C-G-A-C-A-G-C-A-C3-G-P-A-P-G-C-A-C-A-P-C-P	NB	447
5169-4_68	C-G-A-C-A-G-C-A-P-C3-P-A-P-G-C-A-C-A-P-C-P	NB	448

5169-4_69	C-G-A-C-A-G-C-A-P-G-C3-A-P-G-C-A-C-A-P-C-P	NB	449
5169-4_70	C-G-A-C-A-G-C-A-P-G-P-C3-P-G-C-A-C-A-P-C-P	NB	450
5169-4_71	C-G-A-C-A-G-C-A-P-G-P-A-C3-G-C-A-C-A-P-C-P	NB	451
5169-4_72	C-G-A-C-A-G-C-A-P-G-P-A-P-C3-C-A-C-A-P-C-P	230	452
5169-4_73	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C3-A-C-A-P-C-P	0.015	453
5169-4_74	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-C3-C-A-P-C-P	0.37	454
5169-4_75	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C3-A-P-C-P	0.14	455
5169-4_76	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-C3-P-C-P	1.3	456
5169-4_77	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-C3-C-P	NB	457
5169-4_78	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C3-P	0.28	458
5169-4_79	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-C3	NB	459

Table 8. 2'O-methyl and deoxythymidine substitutions in 5169-4_26.

Aptamer ID. No.	Sequence (5'→3')	K_d (nM)	Seq. ID. NO.
5169-4_33	C^1-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.027	460
5169-4_34	C-G-A-C^1-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.033	461
5169-4_35	C-G-A-C-A-G-C^1-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.016	462

5169-4_36	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C^{1}-A-C-A-P-C-P	0.028	463
5169-4_37	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C^{1}-A-P-C-P	0.041	464
5169-4_38	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C^{1}-P	0.022	465
5169-4_39	C-G^{1}-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.058	466
5169-4_40	C-G-A-C-A-G^{1}-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.024	467
5169-4_41	C-G-A-C-A-G-C-A-P-G^{1}-P-A-P-G-C-A-C-A-P-C-P	0.097	468
5169-4_42	C-G-A-C-A-G-C-A-P-G-P-A-P-G^{1}-C-A-C-A-P-C-P	0.0069	469
5169-4_43	C-G-A^{1}-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.16	470
5169-4_44	C-G-A-C-A^{1}-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.53	471
5169-4_45	C-G-A-C-A-G-C-A^{1}-P-G-P-A-P-G-C-A-C-A-P-C-P	0.93	472
5169-4_46	C-G-A-C-A-G-C-A-P-G-P-A^{1}-P-G-C-A-C-A-P-C-P	0.093	473
5169-4_47	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A^{1}-C-A-P-C-P	0.016	474
5169-4_48	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A^{1}-P-C-P	0.014	475
5169-4_49	C-G-A-C-A-G-C-A-P^{1}-G-P-A-P-G-C-A-C-A-P-C-P	0.53	476
5169-4_50	C-G-A-C-A-G-C-A-P-G-P^{1}-A-P-G-C-A-C-A-P-C-P	0.047	477
5169-4_51	C-G-A-C-A-G-C-A-P-G-P-A-P^{1}-G-C-A-C-A-P-C-P	0.32	478
5169-4_52	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-	0.31	479

	P^1-C-P		
5169-4_53	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P^1	0.022	480
5169-4_54	C-G-A-C-A-G-C-A-T-G-P-A-P-G-C-A-C-A-P-C-P	44	481
5169-4_55	C-G-A-C-A-G-C-A-P-G-T-A-P-G-C-A-C-A-P-C-P	7.8	482
5169-4_56	C-G-A-C-A-G-C-A-P-G-P-A-T-G-C-A-C-A-P-C-P	NB	483
5169-4_57	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-T-C-P	120	484
5169-4_58	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-T	NB	485

Table 9. Multiple 2'-O-methyl substitutions in PDGF aptamer 5169-4_26.

Aptamer ID. No.	Sequence (5'→3')	K_d (nM)	Seq. ID. NO.
5169-4_80	C-G-A-C-A-G-C^1-A-P-G-P-A-P-G-C-A^1-C-A^1-P-C-P	0.0061	486
5169-4_81	C-G-A-C-A-G-C^1-A-P-G-P-A-P-G^1-C-A^1-C-A^1-P-C-P	0.0049	487
5169-4_82	C-G-A-C-A-G^1-C^1-A-P-G-P-A-P-G^1-C-A^1-C-A^1-P-C^1-P^1	0.0016	488
5169-4_83	C^1-G-A-C-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C-A^1-P-C^1-P^1	0.0021	489
5169-4_84	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C-A^1-P-C^1-P^1	0.0053	490
5169-4_85	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P^1-A-P-G^1-C^1-A^1-C^1-A^1-P-C^1-P^1	0.0062	491
5169-4_86	C^1-G^1-A-C^1-A-G^1-C^1-A-P-G-P^1-A-P-G^1-C^1-A^1-C^1-A^1-P-C^1-P^1	0.064	492
5169-4_87	C^1-G^1-A-C^1-A-G^1-C^1-A-P-G^1-P^1-A^1-P-G^1-C^1-A^1-C^1-A^1-P-C^1-P^1	NB	493
5169-4_88	C^1-G^1-A^1-C^1-A-G^1-C^1-A-P-G^1-P^1-A^1-P-G^1-C^1-A^1-C^1-A^1-P-C^1-P^1	NB	494
5169-4_89	C^1-G^1-A^1-C^1-A-G^1-C^1-A-P-G^1-P^1-A^1-P^1-G^1-C^1-A^1-C^1-A^1-P^1-C^1-P^1	NB	495
5169-4_90	C^1-G^1-A^1-C^1-A^1-G^1-C^1-A-P^1-G^1-P^1-A^1-P^1-G^1-C^1-A^1-C^1-A^1-P^1-C^1-P^1	NB	496
5169-4_91	C^1-G^1-A^1-C^1-A^1-G^1-C^1-A^1-P^1-G^1-P^1-A^1-P^1-G^1-C^1-A^1-C^1-A^1-P^1-C^1-P^1	NB	497
5169-4_92	C^1-G-A-C-A-G-C^1-A-P-G-P-A-P-G-C^1-A^1-C-A^1-P-C^1-P^1	0.0052	498
5169-4_105	C-G-A-C-A-G^1-C-A-P-G-P-A-P-G-C-A-C-A^1-P-C^1-P	0.0061	499
5169-4_106	C^1-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A^1-	0.016	517

	P-C^{1}-P		
5169-4_107	C^{1}-G-A-C-A-G^{1}-C-A-P-G-P-A-P-G-C-A-C-A^{1}-P-C-P	0.013	518
5169-4_108	C^{1}-G-A-C-A-G^{1}-C-A-P-G-P-A-P-G^{1}-C-A-C-A-P-C-P	0.012	519
5169-4_109	C^{1}-G-A-C-A-G^{1}-C-A-P-G-P-A-P-G-C-A^{1}-C-A-P-C-P	0.011	520
5169-4_110	C^{1}-G-A-C-A-G-C-A-P-G-P-A-P-G^{1}-C-A^{1}-C-A-P-C-P	0.0050	521
5169-4_111	C-G-A-C-A-G-C^{1}-A-P-G-P-A-P-G^{1}-C-A^{1}-C-A-P-C-P	0.0048	522
5169-4_112	C-G-A-C-A-G-C-A-P-G-P-A-P-G^{1}-C-A^{1}-C-A-P-C-P^{1}	0.0062	523
5169-4_113	C-G-A-C-A-G-C^{1}-A-P-G-P-A-P-G-C-A^{1}-C-A-P-C-P^{1}	0.012	524
5169-4_114	C-G-A-C-A-G-C^{1}-A-P-G-P-A-P-G-C-A-C^{1}-A-P-C-P^{1}	0.038	525
5169-4_115	C-G-A-C^{1}-A-G-C^{1}-A-P-G-P-A-P-G-C-A-C-A-P-C-P^{1}	0.024	526
5169-4_116	C-G-A-C^{1}-A-G-C-A-P-G-P-A-P-G-C-A-C^{1}-A-P-C-P^{1}	0.093	527
5169-4_117	C-G-A-C^{1}-A-G-C-A-P-G-P-A^{1}-P-G-C-A-C^{1}-A-P-C-P	0.71	528
5169-4_118	C-G^{1}-A-C^{1}-A-G-C-A-P-G-P-A-P-G-C-A-C^{1}-A-P-C-P	0.56	529
5169-4_119	C-G^{1}-A-C^{1}-A-G-C-A-P-G-P-A-P-G-C-A-C^{1}-A-P-C-P	NB	530
5169-4_120	C-G^{1}-A-C-A-G-C-A-P-G-P-A^{1}-P-G-C^{1}-A-C-A-P-C-P	NB	531
5169-4_121	C-G^{1}-A-C-A-G-C-A-P-G^{1}-P-A^{1}-P-G-C-A-C-A-P-C-P	NB	532

5169-4_122	C-G^{1}-A-C-A-G-C-A-P-G^{1}-P-A-P-G-C^{1}-A-C-A-P-C-P	NB	533
5169-4_123	C^{1}-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C^{1}-P^{1}	0.030	534
5169-4_124	C-G-A-C-A^{1}-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C^{1}-P^{1}	0.60	535
5169-4_125	C^{1}-G-A-C-A^{1}-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C^{1}-P	1.0	536
5169-4_126	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P^{1}-C^{1}-P^{1}	NB	537
5169-4_127	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A^{1}-C^{1}-A^{1}-P-C-P	0.010	538
5169-4_128	C-G-A-C-A-G-C-A-P-G-P-A-P^{1}-G^{1}-C^{1}-A-C-A-P-C-P	0.099	539
5169-4_129	C-G-A-C-A-G-C-A-P-G^{1}-P^{1}-A^{1}-P-G-C-A-C-A-P-C-P	NB	540
5169-4_130	C-G-A-C-A-G-C^{1}-A^{1}-P^{1}-G-P-A-P-G-C-A-C-A-P-C-P	4.5	541
5169-4_131	C-G-A-C^{1}-A^{1}-G^{1}-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.64	542
5169-4_132	C^{1}-G^{1}-A^{1}-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.42	543
5169-4_144	C^{1}-G-A-C-A-G-C^{1}-A-P-G-P-A-P-G^{1}-C-A^{1}-C-A^{1}-P-C-P^{1}	0.00050	544
5169-4_146	C^{1}-G-A-C^{1}-A-G^{1}-C^{1}-A-P-G-P-A-P-G^{1}-C^{1}-A^{1}-C^{1}-A^{1}-P-C^{1}-P^{1}	0.00060	545

Table 10. Truncations of VEGF aptamer Clone 4867-31.

Aptamer ID. No.	length	Sequence (5'→ 3')	K_d (nM) hVEGF165	K_d (nM) rVEGF164	K_d (nM) hVEGF121	Seq. ID. NO.
OH-4867-31_22	38	T-C-A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.26	0.54	0.82	546
OH-4867-31_23	37	T-C-A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	0.30	0.66	1.3	547
OH-4867-31_24	36	T-C-A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G	2.8	4.2	36	548
OH-4867-31_25	35	T-C-A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P	26	16	70	549
OH-4867-31-26	39	C-A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P	0.075	0.07	0.49	550
OH-4867-31-27	38	A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P	0.11	0.07	0.59	551
OH-4867-31-28	37	A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P	0.10	0.07	0.97	552
OH-4867-31-29	36	P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P	0.13	0.08	0.96	553
OH-4867-31-30	35	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P	0.20	0.15	1.4	554

CA 02920508 2016-02-04

OH-4867-31-31	34	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P	0.17	0.12	1.0	555
OH-4867-31-32	38	C-A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A	0.10	0.06	0.51	556
OH-4867-31-33	37	A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A	0.11	0.04	0.55	557
OH-4867-31-34	36	A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A	0.35	0.05	0.47	558
OH-4867-31-35	35	P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A	0.11	0.05	flat	559
OH-4867-31-36	34	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A	0.11	0.06	0.39	560
OH-4867-31-37	33	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A	0.092	0.04	0.21	561
OH-4867-31-38	37	C-A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.17	0.09	0.81	562
OH-4867-31-39	36	A-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.20	0.09	0.62	563
OH-4867-31-40	35	A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.14	0.10	0.70	564
OH-4867-31-41	34	P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-	0.13	0.08	0.63	565

CA 02920508 2016-02-04

		A-A-P-G-G-A				
OH-4867-31-42	33	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.071	0.05	0.20	566
OH-4867-31-43	32	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.050	0.05	0.29	567
OH-4867-31-44	32	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	0.13	0.38	0.14	568
OH-4867-31-45	31	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G	1.1	4.5	3.3	569
OH-4867-31-46	30	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P	5.9	9.6	NB	570
OH-4867-31-47	29	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A	NB	83	NB	571
OH-4867-31-48	28	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A	NB	142	NB	572
OH-4867-31-49	27	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A	NB	NB	NB	573
OH-4867-31-50	26	C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P	NB	NB	NB	574
OH-4867-31-51	31	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	0.11	0.074	0.20	575
OH-4867-31-52	30	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G	0.17	0.58	2.8	576

OH-4867-31-53	29	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P	3.2	NB	19	577
OH-4867-31-54	28	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A	NB	NB	NB	578
OH-4867-31-55	27	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A	NB	NB	NB	579
OH-4867-31-56	26	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A	NB	NB	NB	580
OH-4867-31-57	25	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P	NB	NB	NB	581
OH-4867-31-58	31	G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.10	0.21	0.15	582
OH-4867-31-59	30	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.16	0.20	0.14	583
OH-4867-31-60	29	C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.61	1.4	2.2	584
OH-4867-31-61	28	G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	2.9	13	33	585
OH-4867-31_143	29	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G			0.17	586
OH-4867-31_144	30	G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G			0.20	587

CA 02920508 2016-02-04

Table 11: C3 spacer substitutions at all positions in 4867-15_2.

Aptamer ID. No.	Sequence 5'→ 3'	K_d (nM)	ratio to parent	Seq. ID. NO.
OH-4867-31_2	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.15	1	588
OH-4867-15-24	V-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.11	0.72	589
OH-4867-15-25	C-V-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.13	0.84	590
OH-4867-15-26	C-C-V-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.20	1.3	591
OH-4867-15-27	C-C-C-V-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.40	2.7	592
OH-4867-15-28	C-C-C-T-V-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.34	2.3	593
OH-4867-15-29	C-C-C-T-C-V-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.20	1.4	594
OH-4867-15-30	C-C-C-T-C-C-V-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.11	0.76	595
OH-4867-15-31	C-C-C-T-C-C-A-V-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.095	0.64	596
OH-4867-15-32	C-C-C-T-C-C-A-P-V-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.079	0.53	597
OH-4867-15-33	C-C-C-T-C-C-A-P-C-V-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.12	0.83	598
OH-4867-15-34	C-C-C-T-C-C-A-P-C-A-V-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.14	0.95	599
OH-4867-15-35	C-C-C-T-C-C-A-P-C-A-G-V-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	3.4	23	600
OH-4867-15-36	C-C-C-T-C-C-A-P-C-A-G-C-V-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	23	153	601
OH-4867-15-37	C-C-C-T-C-C-A-P-C-A-G-C-C-V-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	30	205	602

OH-4867-15-38	C-C-C-T-C-C-A-P-C-A-G-C-C-G-V-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	603
OH-4867-15-39	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-V-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	604
OH-4867-15-40	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-V-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.17	1.1	605
OH-4867-15-41	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-V-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	13	85	606
OH-4867-15-42	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-V-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	31	207	607
OH-4867-15-43	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-V-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	9.2	62	608
OH-4867-15-44	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-V-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	609
OH-4867-15-45	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-V-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	3.5	24	610
OH-4867-15-46	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-V-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	611
OH-4867-15-47	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-V-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	612
OH-4867-15-48	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-V-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	613
OH-4867-15-49	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-V-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.079	0.54	614
OH-4867-15-50	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-V-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	3.5	24	615
OH-4867-15-51	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-V-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	1.6	11	616
OH-4867-15-52	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-V-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	0.20	1.3	617
OH-4867-15-53	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-V-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	23	157	618
OH-4867-15-54	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-V-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	619

CA 02920508 2016-02-04

OH-4867-15-55	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-V-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	620
OH-4867-15-56	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-V-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	621
OH-4867-15-57	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-V-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	622
OH-4867-15-58	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-V-A-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	623
OH-4867-15-59	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-V-A-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	624
OH-4867-15-60	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-V-P-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	625
OH-4867-15-61	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-V-G-G-A-A-P-P-G-G-A-G-G-A	NB	-	626
OH-4867-15-62	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-V-G-A-A-P-P-G-G-A-G-G-A	53	361	627
OH-4867-15-63	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-V-A-A-P-P-G-G-A-G-G-A	15	101	628
OH-4867-15-64	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-V-A-P-P-G-G-A-G-G-A	0.22	1.5	629
OH-4867-15-65	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-V-P-P-G-G-A-G-G-A	0.13	0.88	630
OH-4867-15-66	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-V-P-G-G-A-G-G-A	0.11	0.72	631
OH-4867-15-67	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-V-G-G-A-G-G-A	0.15	0.99	632
OH-4867-15-68	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-V-G-A-G-G-A	0.32	2.1	633
OH-4867-15-69	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-V-A-G-G-A	0.77	5.2	634
OH-4867-15-70	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-V-G-G-A	0.68	4.6	635
OH-4867-15-71	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-V-G-A	0.24	1.6	636

OH-4867-15-72	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-V-A	0.19	1.3	637
OH-4867-15-73	C-C-C-T-C-C-A-P-C-A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A-A-P-P-G-G-A-G-G-V	0.17	1.1	638

Table 12: 2'-O-methyl substitutions in VEGF aptamer 4867-31_43

Aptamer ID. No.	Sequence 5' → 3'	K_d (nM)	ratio to parent	Seq. ID. NO.
OH-4867-31-43	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.29	1.0	567
OH-4867-31_65	A[1]-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	639
OH-4867-31_66	A-G[1]-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.17	0.6	640
OH-4867-31_67	A-G-C[1]-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.33	1.2	641
OH-4867-31_68	A-G-C-C[1]-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	7.55	26.0	642
OH-4867-31_69	A-G-C-C-G[1]-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	643
OH-4867-31_70	A-G-C-C-G-U[1]-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	644
OH-4867-31_71	A-G-C-C-G-P-U[1]-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	645
OH-4867-31_72	A-G-C-C-G-P-P-C[1]-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.21	0.7	646
OH-4867-31_73	A-G-C-C-G-P-P-C-A[1]-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	5.99	20.7	647
OH-4867-31_74	A-G-C-C-G-P-P-C-A-A[1]-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	3.14	10.8	648
OH-4867-31_75	A-G-C-C-G-P-P-C-A-A-G[1]-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.15	0.5	649
OH-4867-31_76	A-G-C-C-G-P-P-C-A-A-G-U[1]-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	22.90	79.0	650
OH-4867-31_77	A-G-C-C-G-P-P-C-A-A-G-P-G[1]-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	6.64	22.9	651
OH-4867-31_78	A-G-C-C-G-P-P-C-A-A-G-P-G-C[1]-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.24	0.8	652
OH-4867-31_79	A-G-C-C-G-P-P-C-A-A-G-P-G-C-U[1]-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	653
OH-4867-31_80	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-U[1]-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	654
OH-4867-31_81	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G[1]-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.44	1.5	655
OH-4867-31_82	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-U[1]-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	656
OH-4867-31_83	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A[1]-G-G-A-P-P-P-A-A-A-P-G-G-A	0.10	0.3	657
OH-4867-31_84	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G[1]-G-A-P-P-P-A-A-A-P-G-G-A	0.21	0.7	658
OH-4867-31_85	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G[1]-A-P-P-P-A-A-A-P-G-G-A	25.80	89.0	659
OH-4867-31_86	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A[1]-P-P-P-A-A-A-P-G-G-A	6.21	21.4	660
OH-4867-31_87	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-U[1]-P-P-A-A-A-P-G-G-A	NB	-	661
OH-4867-31_88	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-	NB	-	662

	G-A-P-U[1]-P-A-A-A-P-G-G-A			
OH-4867-31_89	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-U[1]-A-A-A-P-G-G-A	NB	-	663
OH-4867-31_90	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A[1]-A-A-P-G-G-A	2.85	9.8	664
OH-4867-31_91	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A[1]-A-P-G-G-A	0.35	1.2	665
OH-4867-31_92	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A[1]-P-G-G-A	8.87	30.6	666
OH-4867-31_93	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-U[1]-G-G-A	NB	-	667
OH-4867-31_94	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G[1]-G-A	0.21	0.7	668
OH-4867-31_95	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G[1]-A	0.19	0.7	669
OH-4867-31_96	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A[1]	0.13	0.4	670
OH-4867-31_97	A-G-C-C-G-P-P-V-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	7.30	25.2	671
OH-4867-31_98	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-V-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.10	0.3	672
OH-4867-31_99	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-V-G-A-P-P-P-A-A-A-P-G-G-A	0.22	0.8	673
OH-4867-31_100	A-G-C-C-G-P-P-V-A-A-G-P-G-C-P-P-V-P-A-V-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	674
OH-4867-31_101	A-G-C-C-G-P-P-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	5.89	20.3	675
OH-4867-31_102	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	676
OH-4867-31_103	A-G-C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-A-P-P-P-A-A-A-P-G-G-A	NB	-	677
OH-4867-31_145	C-C-G-P-P-A-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	3.08	10.6	678
OH-4867-31_146	C-C-G-P-P-G-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	5.98	20.6	679
OH-4867-31_147	C-C-G-P-P-T-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	3.28	11.3	680
OH-4867-31_148	C-C-G-P-P-C-A-A-G-P-G-C-P-P-A-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.53	1.8	681
OH-4867-31_149	C-C-G-P-P-C-A-A-G-P-G-C-P-P-C-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.27	0.9	682
OH-4867-31_150	C-C-G-P-P-C-A-A-G-P-G-C-P-P-T-P-A-G-G-A-P-P-P-A-A-A-P-G-G-A	0.24	0.8	683
OH-4867-31_151	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-A-G-A-P-P-P-A-A-A-P-G-G-A	0.16	0.6	684
OH-4867-31_152	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-C-G-A-P-P-P-A-A-A-P-G-G-A	0.32	1.1	685
OH-4867-31_153	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-T-G-A-P-P-P-A-A-A-P-G-G-A	0.21	0.7	686

CA 02920508 2016-02-04

Table 13: Single 2'-O-methyl Nap-U Substitutions, and multiple 2'-O-methyl substitutions in VEGF aptamer 4867-31_143

Aptamer ID. No.	Sequence 5'→ 3'	K_d (M)	ratio to parent	% Activity Remaining	Seq. ID. NO.
4867-31_143	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	1.4E-10	1.0	12%, 7%	586
4867-31_154	C-C-G-P^1-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	5.9E-09	44		687
4867-31_155	C-C-G-P-P^1-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	3.3E-09	24		688
4867-31_156	C-C-G-P-P-C-A-A-G-P^1-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	6.8E-10	5.0	52%, 74%	689
4867-31_157	C-C-G-P-P-C-A-A-G-P-G-C-P^1-P-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	1.5E-09	11	54%, 101%	690
4867-31_158	C-C-G-P-P-C-A-A-G-P-G-C-P-P^1-G-P-A-G-G-A-P-P-P-A-A-A-P-G-G	1.7E-09	12	59%, 106%	691
4867-31_159	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P^1-A-G-G-A-P-P-P-A-A-A-P-G-G	5.6E-10	4.1	7%, 64%	692
4867-31_160	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P^1-P-P-A-A-A-P-G-G	1.5E-09	11	52%, 95%	693
4867-31_161	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P^1-P-A-A-A-P-G-G	6.4E-11	0.47	5%, 4%, -2%	694
4867-31_162	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P^1-A-A-A-P-G-G	8.9E-09	65	84%, 117%	695
4867-31_163	C-C-G-P-P-C-A-A-G-P-G-C-P-P-G-P-A-G-G-A-P-P-P-A-A-A-P^1-G-G	2.6E-10	1.9	53%, 45%	696
4867-31_183	C-C-G-P-P-C-A-A-G-P-G-C-P-P-V-P-A-V-G-A-P-P-P-A-A-A-P-G-G	9.1E-11	0.67	15%	697
4867-31_184	C-C-G-P-P-C-A-A-G^1-P-G-C-P-P-V-P-A^1-V-G-A-P-P-P-A-A-A-P-G-G	8.4E-11	0.62	7%	698
4867-31_185	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-V-P-A^1-V-G-A-P-P-P-A-A-A-P-G^1-G^1	2.6E-10	1.9	50%	699
4867-31_186	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-V-P-A^1-V-G-A-P-P-P-A-A^1-A-P-G^1-G^1	1.6E-09	12	77%	700
4867-31_187	C-C-G-P-P-C-A-A-G^1-P-G-C-P-P-V-P-A^1-G-G-A-P-P-P-A-A-	3.2E-11	0.24	-2%	701

	A-P-G-G				
4867-31_188	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-V-P-A^1-G^1-G-A-P-P-P-A-A-A-P-G^1-G^1	3.8E-11	0.28	-2%	702
4867-31_189	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-V-P-A^1-G^1-G-A-P-P-P-A-A^1-A-P-G^1-G^1	9.0E-10	6.6	13%	703

Table 14: Multiple 2'-O-methyl substitutions in VEGF aptamer 4867-31_143

Aptamer ID. No.	Sequence 5'→3'	K_d (M)	Ratio to 4867-31_188	Seq. ID. NO.
4867-31_188	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-V-P-A^1-G^1-G-A-P-P-P-A-A-A-P-G^1-G^1	4.8E-11	1.0	702
4867-31_190	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-V-P-A^1-G1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	8.9E-11	1.9	704
4867-31_191	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P-P-A-A-A-P-G^1-G^1	1.0E-10	2.1	705
4867-31_192	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	3.5E-11	0.7	706

Table 15. PDGF homodimers of 4149-8_379 and 5169-4_26.

Aptamer ID. No.	Sequence (5' →3')	K_i (pM)	Seq. ID. NO.
4149-8_438	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1- Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1	4.2	707
4149-8_439	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1	3.5	708
4149-8_440	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1	1.4	709
4149-8_441	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-H-Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1	0.78	710
4149-8_442	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-H-H-Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1	0.51	711
4149-8_443	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-H-H-H-Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1	0.25	712
4149-8_444	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-H-H-H-H-Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1	0.61	713
4149-8_379D	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-G^1-C-G-A^1-Z-A^1-Z^1-Z-Z-G-C-G-C^1-C3-C-A-Z-Z^1-G-H-C-A^1-M-Z	11	714
4149-8_445	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-G^1-C-G-A^1-Z-A^1-Z^1-	1.1	715

	Z-Z-G-C-G-C^1-C3-C-A-Z-Z^1-G-H-C-A^1-M-Z		
4149-8_446	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-H-H-G^1-C-G-A^1-Z-A^1-Z^1-Z-Z-G-C-G-C^1-C3-C-A-Z-Z^1-G-H-C-A^1-M-Z	0.61	716
4149-8_447	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-H-H-H-H-G^1-C-G-A^1-Z-A^1-Z^1-Z-Z-G-C-G-C^1-C3-C-A-Z-Z^1-G-H-C-A^1-M-Z	0.88	717
5169-4_134	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P- C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	28	718
5169-4_135	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	9.3	719
5169-4_136	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	8.7	720
5169-4_137	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	5.1	721
5169-4_138	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	4.9	722
5169-4_139	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	3.6	723
5169-4_140	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	3.6	724
5169-4_26D	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-P-C-P-A-C-A-C-G-P-A-P-G-P-A-C-G-A-C-A-G-C	11	725

5169-4_141	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-P-C-P-A-C-A-C-G-P-A-P-G-P-A-C-G-A-C-A-G-C	13	726
5169-4_142	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-P-C-P-A-C-A-C-G-P-A-P-G-P-A-C-G-A-C-A-G-C	5.5	727
5169-4_143	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-H-H-P-C-P-A-C-A-C-G-P-A-P-G-P-A-C-G-A-C-A-G-C	2.0	728

Table 16. VEGF homodimers of 4867-31_192.

Aptamer ID. No.	Sequence (5'→ 3')	K_i (pM)	Seq. ID. NO.
4867-31_395	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1- C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	6.8	729
4867-31_396	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	50	730
4867-31_397	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	43	731
4867-31_398	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	29	732
4867-31_399	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	22	733
4867-31_400	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	14	734
4867-31_401	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	21	735
4867-31_192D	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-G^1-G^1-P-A-	32	736

	A-A-P-P^{1}-P-A-G-G^{1}-A^{1}-P-G^{1}-P-P-C^{1}-G-P-G^{1}-A-A-C^{1}-P-P-G-C-C		
4867-31_402	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}-H-H-G^{1}-G^{1}-P-A-A-A-P-P^{1}-P-A-G-G^{1}-A^{1}-P-G^{1}-P-P-C^{1}-G-P-G^{1}-A-A-C^{1}-P-P-G-C-C	27	737
4867-31_403	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}-H-H-H-H-G^{1}-G^{1}-P-A-A-A-P-P^{1}-P-A-G-G^{1}-A^{1}-P-G^{1}-P-P-C^{1}-G-P-G^{1}-A-A-C^{1}-P-P-G-C-C	20	738
4867-31_404	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}-H-H-H-H-H-H-G^{1}-G^{1}-P-A-A-A-P-P^{1}-P-A-G-G^{1}-A^{1}-P-G^{1}-P-P-C^{1}-G-P-G^{1}-A-A-C^{1}-P-P-G-C-C	33	739

CA 02920508 2016-02-04

Table 17: PDGF-VEGF aptamer constructs

Aptamer ID. No.	Sequence 5'→ 3'	Seq. ID. NO.
4149-8_313	C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G-G-Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C	740
4149-8_314	C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G-G-H-Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C	741
4149-8_315	C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G-G-H-H-Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C	742
4149-8_316	C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G-G-H-H-H-Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C	743
4149-8_317	Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C-G^1-C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G	744
4149-8_318	Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C-G^1-H-C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G	745
4149-8_319	Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C-G^1-H-H-C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G	746
4149-8_320	Z-Z-A^1-C-H-G-Z-Z-A-C-A^1-C^1-G-C-G-Z-Z-Z-A^1-Z-A^1-G-C-G^1-H-H-H-C-C-G-P-P-C-A-A-G-P-G-C-P-P-C3-P-A-C3-G-A-P-P-P-A-A-A-P-G	747

CA 02920508 2016-02-04

Table 18: Binding affinity and in vitro activity of PDGF-VEGF aptamer constructs.

		PDGF-BB	Hs27 PDGFRß	PDGF-AB	VEGF	VEGF121
Target	SeqID	Kd (M)	% Activity Remaining	Kd (M)	Kd (M)	Kd (M)
PDGF-BB	4149-8_39	8.75E-11		4.76E-11	1.00E-06	1.00E-06
PDGF-BB	4149-8_130	7.59E-11		1.41E-10	1.00E-06	1.00E-06
PDGF-BB	4149-8_273	6.14E-11		1.89E-10	1.00E-06	1.00E-06
VEGF121	4867-31_51	1.26E-07		1.48E-08	1.96E-10	7.28E-10
VEGF121	4867-31_183	1.95E-07		1.45E-08	1.38E-10	6.65E-10
Both	4149-8_313	9.33E-11	5%	1.65E-10	2.58E-10	4.31E-09
Both	4149-8_314	1.36E-10	2%	1.11E-10	1.43E-10	1.55E-09
Both	4149-8_315	1.19E-10	2%	1.23E-10	8.60E-11	7.58E-10
Both	4149-8_316	8.97E-11	2%	1.37E-10	1.57E-10	5.00E-10
Both	4149-8_317	6.38E-11	58%			8.09E-11
Both	4149-8_318	4.31E-11	43%	8.18E-11	2.70E-10	2.47E-09
Both	4149-8_319	2.42E-11	17%	3.02E-11	1.25E-10	1.07E-09
Both	4149-8_320	1.50E-11	13%	4.93E-11	7.68E-11	4.22E-10

Table 19. PDGF/VEGF aptamer construct 4149-8_401

Aptamer ID. No.	Sequence 5'→ 3'	Seq. ID. NO.
4149-8_401	Z-M-A^1-C-H-G-Z^1-Z-A-C-C3-C^1-G-C-G-Z-Z-Z^1-A^1-Z-A^1-G-C-G^1-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	748

Table 20. Binding affinity of PDGF/VEGF aptamer construct 4149-8_401

		PDGF-BB	PDGF-AB	VEGF165	VEGF121
Target	Seq ID	Kd (M)	Kd (M)	Kd (M)	Kd (M)
Both	4149-8_320	1.50E-11	4.93E-11	7.68E-11	4.22E-10
Both	4149-8_401	1.51E-11	1.93E-10	2.77E-10	3.74E-11

Table 21: Binding affinities of PDGF/VEGF aptamer constructs comprising 4149-8_379 and 4867-31_192

4149-8_401	PDGF_379	HHH	VEGF_192
4149-8_408	VEGF_192	H	PDGF_379
4149-8_409	VEGF_192	HH	PDGF_379
4149-8_410	VEGF_192	HHH	PDGF_379
4149-8_411	VEGF_192	HHHH	PDGF_379
4149-8_412	VEGF_192	HHHHH	PDGF_379
4149-8_413	VEGF_192	HHHHHH	PDGF_379
4149-8_414	PDGF_379	HH	VEGF_192
4149-8_415	PDGF_379	HHHH	VEGF_192
4149-8_416	PDGF_379	HHHHH	VEGF_192
4149-8_417	PDGF_379	HHHHHH	VEGF_192

	Kd (M)		
Aptamer ID. No.	**PDGF-BB**	**VEGF121**	**VEGF165**
E10030	4.7E-11	NB	NB
OH-4149-8_379	2.0E-11	NB	NB
OH-4867-31_192	5.7E-08	2.3E-11	4.1E-11
N-4149-8_401	5.6E-12	2.7E-11	2.5E-11
OH-4149-8_408	4.3E-11	4.4E-11	4.1E-11
OH-4149-8_409	6.0E-10	2.6E-11	2.9E-11
OH-4149-8_410	2.0E-11	2.6E-11	3.3E-11
OH-4149-8_411	7.2E-12	2.0E-11	4.5E-11
OH-4149-8_412	8.6E-12	2.0E-11	4.5E-11
OH-4149-8_413	1.0E-11	4.0E-11	6.3E-11
OH-4149-8_414	1.0E-11	2.3E-11	5.1E-11
OH-4149-8_415	8.1E-12	4.0E-11	3.0E-11
OH-4149-8_416	8.9E-12	4.1E-11	3.0E-11
OH-4149-8_417	1.1E-11	4.3E-11	6.5E-11

Table 22. Binding affinities of PDGF/VEGF aptamer constructs comprising 5169-4_26 and 4867-31_192.

Aptamer ID. No.	Sequence (5' →3')	K_d (nM) PDGF	K_d (nM) VEGF121	Seq. ID. NO.
5169-4_93	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.00020	0.052	749
5169-4_94	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.00050	0.066	750
5169-4_95	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.0012	0.043	751
5169-4_96	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.0045	0.076	752
5169-4_97	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.0037	0.10	753
5169-4_98	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1-H-C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P	0.0053	0.14	754

5169-4_99	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	0.0021	0.120	755
5169-4_100	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	0.0015	0.056	756
5169-4_101	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	0.00050	0.13	757
5169-4_102	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	0.011	0.14	758
5169-4_103	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	0.0061	0.12	759
5169-4_104	C-G-A-C-A-G-C-A-P-G-P-A-P-G-C-A-C-A-P-C-P-H-C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	0.0046	0.28	760

Table 23. Aptamers and aptamer constructs tested in Ocular PK Studies.

Test Article	Sequence ID	Target(s)	PEG
SL1010	4867-31_192	VEGF	40 kDa
SL1011	4149-8_379	PDGF	40 kDa
SL1012	4149-8_401	VEGF and PDGF	20 kDa
SL1013	4149-8_401	VEGF and PDGF	40 kDa

Table 24. Concentrations in the Vitreous Humor For Aptamer and Aptamer Constructs in the Ocular Pharmacokinetic Studies.

Time Point (hours)	EYE (OS or OD)	SL1010 Concentration (µg/mL)	SL1011 Concentration (µg/mL)	SL1012 Concentration (µg/mL)	SL1013 Concentration (µg/mL)
2	OS	296	280	538	563
2	OD	296	298	682	634
24	OS	195	164	330	520
24	OD	198	165	433	493
48	OS	188	131	359	376
48	OD	207	116	267	336
96	OS	146	96.3	227	307
96	OD	139	97.8	204	277
192	OS	70.2	16.8	92.8	145
192	OD	82.4	18.2	67.8	132

OS-Oculus Sinister; OD-Oculus Dexter

Table 25 Vitreous Humor Half-Life for Aptamer and Aptamer Constructs Following a Single Bilateral Intravitreous Dose to NZW Rabbits.

Test Article	Half-life (hours)	95% Confidence Interval
SL1010	105	90- 128
SL1011	47	42 - 55
SL1012	69	58- 85
SL1013	92	81- 106

Table 26. Phosphorothioate walk along aptamer 4867-31_192.

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.	K_d (M)	Ratio to parent
4867-31_192	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	706	1.1E-11	1
4867-31_409	C^2-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	763	1.1E-11	0.97
4867-31_410	C-C^2-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	764	1.6E-11	1.5
4867-31_411	C-C-G^2-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	765	3.2E-07	29,259
4867-31_412	C-C-G-P^2-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	766	2.2E-11	2.1
4867-31_413	C-C-G-P-P^2-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	767	1.5E-11	1.4
4867-31_414	C-C-G-P-P-C^1-A^2-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	768	1.5E-11	1.4
4867-31_415	C-C-G-P-P-C^1-A-A^2-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	769	1.1E-11	1.0
4867-31_416	C-C-G-P-P-C^1-A-A-G^1-P^2-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	770	9.3E-12	0.86
4867-31_417	C-C-G-P-P-C^1-A-A-G^1-P-G^2-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	771	9.4E-12	0.87
4867-31_419	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P^2-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	772	no data	no data
4867-31_420	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P^2-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	773	1.8E-11	1.6
4867-31_421	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P^2-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	774	1.5E-11	1.4
4867-31_422	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G^2-A-P-P^1-P-A-A-A-P-G^1-G^1	775	9.5E-12	0.88
4867-31_423	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A^2-P-P^1-P-A-A-A-P-G^1-G^1	776	1.8E-11	1.7
4867-31_424	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P^2-P^1-P-A-A-A-P-G^1-G^1	777	1.1E-11	1.1
4867-31_425	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P^2-A-A-A-P-G^1-G^1	778	1.6E-11	1.5

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.	K_d (M)	Ratio to parent
4867-31_426	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A^{2}-A-A-P-G^{1}-G^{1}	779	1.2E-11	1.1
4867-31_427	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A^{2}-A-P-G^{1}-G^{1}	780	8.1E-12	0.75
4867-31_428	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A^{2}-P-G^{1}-G^{1}	781	8.4E-12	0.78
4867-31_429	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P^{2}-G^{1}-G^{1}	782	1.2E-11	1.1
4867-31_438	C-C-G-P-P-C^{1,2}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}	783	1.3E-11	1.2
4867-31_439	C-C-G-P-P-C^{1}-A-A-G^{1,2}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}	784	2.4E-11	2.2
4867-31_440	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1,2}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}	785	9.9E-12	0.91
4867-31_441	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1,2}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}	786	1.7E-11	1.6
4867-31_442	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1,2}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}	787	1.5E-11	1.4
4867-31_443	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1,2}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1}	788	8.5E-12	0.79
4867-31_444	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1,2}-G^{1}	789	7.5E-12	0.70
4867-31_445	C-C-G-P-P-C^{1}-A-A-G^{1}-P-G-C^{1}-P-P-G^{1}-P-A^{1}-G^{1}-G-A-P-P^{1}-P-A-A-A-P-G^{1}-G^{1,2}	790	1.0E-11	0.96

Table 27. Sequences of the VEGF aptamers created in order to enhance nuclease resistance. The binding dissociation constant (K_d values) along with the number of 2'-OMe moieties (# OMe; superscript "1" indicates that the nucleotide has a 2'-O-methyl) and 3'-phosphorothioate linkages (# P=S; superscript "2" indicates that the nucleotide has a phosphorothioate linkage) are shown.

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.	K_d (M)	# OMe	# P=S
4867-31_192	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A-P-G^1-G^1	706	3.1E-11	9	0
4867-31_475	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A^2-A-P-G^1-G^1	791	2.6E-11	10	1
4867-31_476	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A-A^2-P-G^1-G^1	792	2.1-11	10	1
4867-31_477	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A^2-A^2-P-G^1-G^1	793	2.4E-11	10	2
4867-31_478	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P-A-A^2-A^2-P-G^1-G^1	794	1.9E-11	9	2
4867-31_479	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P- A^2-A-A-P-G^1-G^1	795	2.6E-11	10	1
4867-31_480	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P- A^2-A^2-A-P-G^1-G^1	796	2.8E-11	10	2
4867-31_481	C^1-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P- A^2-A^2-A^2-P-G^1-G^1	797	2.6E-11	10	3
4867-31_482	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P- A^2-A^2-A^2-P-G^1-G^1	798	2.4E-11	9	3
4867-31_483	C-C-G-P-P-C^1-A-A-G^1-P-G-C^1-P-P-G^1-P-A^1-G^1-G-A-P-P^1-P- A^2-A^2-A-P-G^1-G^1	799	3.2E-11	9	2

Table 28. A phosphorothioate walk along aptamers 5169-4_146.

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.	K_d(M)	Ratio to parent
5169-4_146	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	545	1.5E-11	1.0
5169-4_150	C^1-G^2-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	800	1.3E-11	0.89
5169-4_151	C^1-G-A^2-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	801	6.0E-12	0.42
5169-4_152	C^1-G-A-C^1-A^2-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	802	1.5E-11	1.0
5169-4_153	C^1-G-A-C^1-A-G^1-C^1-A^2-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	803	1.3E-11	0.92
5169-4_154	C^1-G-A-C^1-A-G^1-C^1-A-P^2-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	804	2.6E-11	1.8
5169-4_155	C^1-G-A-C^1-A-G^1-C^1-A-P-G^2-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	805	6.9E-12	0.48
5169-4_156	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P^2-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	806	7.8E-12	0.54
5169-4_157	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A^2-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	807	1.8E-11	1.2
5169-4_158	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P^2-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	808	1.9E-11	1.3
5169-4_159	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P^2- C^1-P^1	809	1.5E-11	1.0
5169-4_172	$C^{1,2}$-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	810	1.3E-11	0.90
5169-4_173	C^1-G-A-$C^{1,2}$-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	811	9.1E-12	0.63
5169-4_174	C^1-G-A-C^1-A-G^1-$C^{1,2}$-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	812	1.8E-11	1.2
5169-4_175	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-$C^{1,2}$-A^1-C^1-A^1-P- C^1-P^1	813	6.9E-12	0.48
5169-4_176	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-$C^{1,2}$-A^1-P- C^1-P^1	814	1.4E-11	0.93
5169-4_177	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- $C^{1,2}$-P^1	815	7.2E-12	0.50
5169-4_178	C^1-G-A-C^1-A-$G^{1,2}$-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	816	1.1E-11	0.76
5169-4_179	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-$G^{1,2}$-C^1-A^1-C^1-A^1-P- C^1-P^1	817	7.7E-12	0.53
5169-4_180	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-$A^{1,2}$-C^1-A^1-P- C^1-P^1	818	2.0E-11	1.4
5169-4_181	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-$A^{1,2}$-P- C^1-P^1	819	2.1E-11	1.4

Table 29. Sequences of the PDGFBB aptamers created in order to enhance nuclease resistance. The binding dissociation constant (K_d values) along with the number of 2'-OMe moieties (# OMe; superscript "1" indicates that the nucleotide has a 2'-O-methyl) and 3'-phosphorothioate linkages (# P=S; superscript "2" indicates that the nucleotide has a phosphorothioate linkage) are shown.

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.	Kd (M)	# OMe	# P=S
5169-4_146	C^1-G-A-C^1-A-G^1-C^1-A-P-G-P-A-P-G^1-C^1-A^1-C^1-A^1-P-C^1-P^1	545	2.6E-11	11	0
5169-4_182	C^1-G-A-C^1-A- G^1-C^1-A-P^2-G^2-P^2-A^2-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	820	3.9E-11	11	4
5169-4_188	C^1-G-A-$C^{1,2}$-A- G^1-C^1-A-P^2-G^2-P^2-A^2-P-G^1-C^1-A^1-C^1-A^1-P- C^1-P^1	821	8.6E-12*	11	5

*Ki value as determined using competition binding assay as described in Example 14.

Table 30. Sequences of the PDGF aptamers created in order to enhance nuclease resistance. The binding dissociation constant (K_d values) along with the number of 2'-OMe moieties (# OMe; superscript "1" indicates that the nucleotide has a 2'-O-methyl) and 3'-phosphorothioate linkages (# P=S; superscript "2" indicates that the nucleotide has a phosphorothioate linkage) are shown.

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.	K_d (M)	# OMe	# P=S
4149-8_379	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G-C-G-Bn -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	306	1.6E-11	7	0
4149-8_453	Bn-Bn-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G^1-C-G^2-P^2-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	822	2.0E-12	8	4
4149-8_454	Bn-Bn-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-P^2-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	823	1.3E-12	7	4
4149-8_455	Bn-Bn-A^1-C-H-G-Bn^1-Bn^2-A^2-C-V-C^1-G^1-C-G^2-P^2-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	824	1.7E-11	8	5
4149-8_456	Bn-Bn-A^1-C-H-G-Bn^1-Bn^2-A^2-C-V-C^1-G-C-G^2-P^2-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	825	2.7E-11	7	5
4149-8_457	Bn-Bn-A^1-C^2-H-G-Bn^1-Bn^2-A^2-C^2-V-C^1-G^1-C-G^2-P^2-Bn^2-$Bn^{1,2}$-A^1-Bn-A^1-G-C-G^1	826	4.3E-11*	7	5
4149-8_458	Bn-Bn-A^1-C^2-H-G-Bn^1-Bn^2-A^2-C^2-V-C^1-G^1-C^2-G^2-P^2-Bn^2-$Bn^{1,2}$-A^1-Bn-A^1-G-C-G^1	827	4.6E-11*	8	8
4149-8_459	Bn-Bn-A^1-C-H-G-Bn^1-Bn^2-A^2-C^2-V-C^1-G^1-C-G^2-P^2-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	828	1.3E-11*	8	6

*Ki value as determined using competition binding assay as described in Example 14.

Table 31. Sequences of the PDGF aptamers created in order to enhance nuclease resistance.

Aptamer ID. No.	Sequence (5'→ 3')	SEQ ID NO.	K_d (M)
4149-8_379	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G-C-G-Bn -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	306	5.2E-11
4149-8_391	Bn-Bn-A^1-C-H-G-Bn-Bn-A^2-C-V-C^1-G-C-G^2-Bn -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	317	1.2E-11
4149-8_418	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G-C-G^1-Bn -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	330	2.1E-10
4149-8_419	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G-C-G^1-P-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	331	1.3E-9
4149-9_420	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G-C-G^1-i-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	829	1.4E-9
4149-8_421	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G^1-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	333	1.9E-11
4149-8_422	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G^1-C-G^1-Bn -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	334	2.7E-10
4149-8_423	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G^1-C-G-P -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	335	3.3E-11
4149-8_424	Bn-M-A^1-C-H-G-Bn^1-Bn-A-C-V-C^1-G^1-C^1-G-Bn -Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	336	6.6E-9
4149-8_425	Bn-M-A^1-C-H-G-Bn^1-Bn-A^1-C-V-C^1-G-C-G-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	337	9.1E-10
4149-8_426	Bn-M-A^1-C-H-G-Bn^1-Bn-A^1-C-V-C^1-G-C-G^1-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	409	4.2E-8
4149-8_431	Bn-M-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-P-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	338	4.3E-11
4149-8_432	Bn-M-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-Bn-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	339	2.9E-11
4149-8_433	Bn-M-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-P-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	340	6.6E-11
4149-8_434	Bn-M-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-Bn-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	341	3.2E-11
4149-8_435	Bn-Bn-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-P-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	342	1.6E-11
4149-8_436	Bn-Bn-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-Bn-Bn^2-Bn^1-A^1-Bn-A^1-G-C-G^1	343	7.7E-12
4149-8_437	Bn-Bn-A^1-C-H-G-Bn^1-Bn-A^2-C-V-C^1-G-C-G^2-P-Bn-Bn^1-A^1-Bn-A^1-G-C-G^1	344	7.4E-12

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1) A nucleic acid molecule comprising the nucleic acid sequence

5' - C-G-A-C-A-G-C-A-Z-G-Z-A-Z-G-C-A-C-A-Z-C-Z - 3' (SEQ ID NO: 830),

wherein Z is a C-5 modified pyrimidine, and at least one of positions 4, 9, 10, 11 and 12 of the nucleic acid molecule comprise a phosphorothioate linkage or moiety, wherein position 1 is the first nucleoside starting from the 5'-terminus of SEQ ID NO: 830.

2) The nucleic acid molecule of claim 1, wherein at least two, three, four or five of positions 4, 9, 10, 11 and 12 of the nucleic acid molecule comprise a phosphorothioate linkage or moiety, and wherein position 1 is the first nucleoside starting from the 5'-terminus of SEQ ID NO: 830.

3) The nucleic acid molecule of claim 1, wherein at least one of positions 1, 4, 6, 7, 14, 15, 16, 17, 18, 20 and 21 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of SEQ ID NO: 830..

4) The nucleic acid molecule of claim 1, wherein at least two, three, four, five, six, seven, eight, nine, ten or eleven of positions 1, 4, 6, 7, 14, 15, 16, 17, 18, 20 and 21 of the nucleic acid molecule comprise a 2'-O-methyl modification, and wherein position 1 is the first nucleoside starting from the 5'-terminus of SEQ ID NO: 830..

5) The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is selected from the group consisting of SEQ ID NOS: 545, 800- 820 and 821.

6) The nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises eleven 2'-O-methyl nucleosides and four or five phosphorothioate linkages or moieties.

7) The nucleic acid molecule of any one of claims 1 to 6, wherein the C-5 modified pyrimidine independently, and for each occurrence, is selected from the group consisting of a 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamide)-2'-O-

Date Reçue/Date Received 2023-02-07

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-isobutylcarboxyamide)-2'-O-methyluridine, 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-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine, and 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine).

8) The nucleic acid molecule of any one of claims 1 to 5, wherein the C-5 modified pyrimidine is a 5-(N-naphthylmethylcarboxyamide)-2′-deoxyuridine (NapdU).

9) The nucleic acid molecule of any one of claims 1 to 4, 6, 7 and 8, wherein the nucleic acid molecule binds a PDGF protein with a binding affinity of less than 10 nM, less than 5 nM, less than 2 nM, less than 1 nM or less than 0.1 nM.

10) The nucleic acid molecule of claim 8, wherein the nucleic acid molecule further comprises a nucleic acid sequence that binds a VEGF protein with a binding affinity of less than 10 nM, less than 5 nM, less than 2 nM, less than 1 nM or less than 0.1 nM.

11) The nucleic acid molecule of claim 10, wherein the nucleic acid sequence that binds the VEGF protein is 5'-CCGPP CAAGP GCPPG PAGGA PPPAA APGG-3'; where "P" is the single-letter designation for Nap-dU.

12) The nucleic acid molecule of claim 8, wherein the nucleic acid molecule comprises at least two, three, four, five, six, seven, eight or nine 2'-O-methyl nucleosides.

Date Reçue/Date Received 2023-02-07

13) The nucleic acid molecule of any one of claims 1 to 12, wherein the nucleic acid molecule comprises DNA, RNA or a combination thereof.

14) The nucleic acid molecule of any one of claims 1 to 13, further comprising an inverted deoxynucleotide at the 3' terminus of the nucleic acid molecule.

15) The nucleic acid molecule of claim 14, wherein the deoxynucleotide is a deoxythymidine.

16) The nucleic acid molecule of any one of claims 1 to 15, wherein the nucleic acid molecule is less sensitive to a nuclease compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety.

17) The nucleic acid molecule of claim 16, wherein the nuclease is a DNase enzyme.

18) The nucleic acid molecule of claim 17, wherein the DNase enzyme is a DNase I or a DNase II enzyme.

19) The nucleic acid molecule of any one of claims 1 to 18, wherein the nucleic acid molecule is more stable in vitreous humor from New Zealand White Rabbits compared to the same nucleic acid molecule without a phosphorothioate linkage or moiety.

20) A nucleic acid molecule comprising the nucleic acid sequence

5' - C^1-G-A-$C^{1,2}$-A-G^1-C^1-A-Z^2-G^2-Z^2-A^2-Z-G^1-C^1-A^1-C^1-A^1-Z-C^1-Z^1 - 3' (SEQ ID NO: 833),

wherein,

C^1 is independently, for each occurrence, selected from the group consisting of a cytidine, a deoxycytidine and a 2'-O-methylcytidine;

$C^{1,2}$ is independently, for each occurrence, selected from the group consisting of a cytidine, a cytidine comprising a phosphorothioate linkage, a deoxycytidine, a deoxycytidine comprising a phosphorothioate linkage, a 2'-O-methylcytidine and a 2'-O-methylcytidine comprising a phosphorothioate linkage;

Date Reçue/Date Received 2023-02-07

G^1 is independently, for each occurrence, selected from the group consisting of a guanosine, a deoxyguanosine and a 2'-O-methylguanosine;

G^2 is independently, for each occurrence, selected from the group consisting of a guanosine, a guanosine comprising a phosphorothioate linkage, a deoxyguanosine and a deoxyguanosine comprising a phosphorothioate linkage;

Z^1 is independently, for each occurrence, selected from the group consisting of a uridine, a deoxyuridine, a 2'-O-methyluridine, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group;

Z^2 is independently, for each occurrence, selected from the group consisting of a uridine, a uridine comprising a phosphorothioate linkage, a deoxyuridine, a deoxyuridine comprising a phosphorothioate linkage, a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU)comprising a phosphorothioate linkage;

Z is independently, for each occurrence, selected from the group consisting of a uridine, a deoxyuridine and a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU);

A^1 is independently, for each occurrence, selected from the group consisting of an adenosine, a deoxyadenosine and a 2'-O-methyladenosine;

A^2 is independently, for each occurrence, selected from the group consisting of an adenosine, an adenosine comprising a phosphorothioate linkage, a deoxyadenosine and a deoxyadenosine comprising a phosphorothioate linkage; and

wherein, the nucleic acid molecule comprises at least one, two, three, four or five phosphorothioate linkages.

21) The nucleic acid molecule of claim 20, wherein $C^{1,2}$ is a 2'-O-methylcytidine comprising a phosphorothioate linkage.

22) The nucleic acid molecule of claim 21, wherein Z^1 is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a 2'-O-methyl group.

Date Reçue/Date Received 2023-02-07

23) The nucleic acid molecule of claim 20, wherein Z^2 is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU) comprising a phosphorothioate linkage.

24) The nucleic acid molecule of claim 20, wherein Z is a 5-(N-naphthylmethylcarboxyamide)-2'-deoxyuridine (NapdU).

25) The nucleic acid molecule of claim 20, wherein C^1 is a 2'-O-methylcytidine.

26) The nucleic acid molecule of claim 20, wherein G^1 is a 2'-O-methylguanosine.

27) The nucleic acid molecule of claim 20, wherein G^2 is a deoxyguanosine comprising a phosphorothioate linkage.

28) The nucleic acid molecule of claim 20, wherein A^1 is a 2'-O-methyladenosine.

29) The nucleic acid molecule of claim 20, wherein A^2 is a deoxyadenosine comprising a phosphorothioate linkage.

30) A composition comprising two different nucleic acid molecules selected from the group consisting of the nucleic acid molecule of any one of claims 1 to 29.

31) The composition of claim 30, wherein the composition is in the form selected from the group consisting of a solution, a powder, a gel and an emulsion.

32) A combination of two different nucleic acid molecules selected from the group consisting of the nucleic acid molecule of any one of claims 1 to 29, wherein the two nucleic acid molecules are covalently linked or non-covalently linked.

33) The nucleic acid molecule of any one of claims 1 to 6, 9, 20 to 23 and 27, wherein the nucleic acid molecule inhibits PDGF-mediated phosphorylation of a PDGF receptor.

Date Reçue/Date Received 2023-02-07

34) A pharmaceutical composition comprising at least one nucleic acid molecule of any one of claims 1 to 29 and 33 and a pharmaceutically acceptable carrier.

35) The pharmaceutical composition of claim 34, wherein the pharmaceutical composition is for intravitreal injection.

36) Use of a therapeutically effective amount of the pharmaceutical composition of claim 34 to treat macular degeneration.

37) Use of a therapeutically effective amount of the pharmaceutical composition of claim 34 to prevent macular degeneration.

38) Use of a therapeutically effective amount of the pharmaceutical composition of claim 34 to treat an ophthalmic condition.

39) The use according to claim 38, wherein the ophthalmic condition is selected from the group consisting of retinitis, macular degeneration, choroiditis, retinopathy, hypertensive retinopathy, diabetic retinopathy, chronic dry eye, AIDS-related vision loss, amblyopia, hemianopia, retinal vein occlusions, trachoma, keratoconus, chorioretinal inflammation, central serous retinopathy, uveitis, retinal dystrophy, edema, glaucoma, and cataract.

40) The use according to claim 36 or 37, wherein the macular degeneration is age-related macular degeneration.

41) The use according to claim 40, wherein the macular degeneration is dry age-related macular degeneration or wet age-related macular degeneration.

42) Use of a therapeutically effective amount of the pharmaceutical composition of claim 34 to treat fibrosis.

Date Reçue/Date Received 2023-02-07

43) The use of claim 42, wherein the fibrosis is selected from pulmonary fibrosis, renal fibrosis, occular fibrosis, corneal fibrosis, posterior segment fibrotic eye disease, fibrovascular retinal scarring and cystic fibrosis.

44) Use of a therapeutically effective amount of the pharmaceutical composition of claim 34 to treat a cardiovascular disease.

45) The use according to claim 44, wherein the cardiovascular disease is selected from atherosclerosis, restenosis, a cardiac hypertrophy related condition, and a vascular disorder.

46) Use of a therapeutically effective amount of the pharmaceutical composition of claim 34 to treat cancer.

47) The use according to claim 46, wherein the cancer is selected from bladder cancer, lung cancer, breast cancer, melanoma, colon cancer, rectal cancer, occular cancer, lymphoma, endometrial cancer, pancreatic cancer, liver cancer, renal cancer, prostate cancer, leukemia, and thyroid cancer.

48) The nucleic acid molecule of any one of claims 1 to 29, wherein the nucleic acid molecule is an aptamer.

49) Use of at least one nucleic acid molecule of any one of claims 1 to 29 and 33 or the composition of claim 30 or 31 or the combination of claim 32 in the manufacture of a medicament to treat macular degeneration.

50) Use of at least one nucleic acid molecule of any one of claims 1 to 29 and 33 or the composition of claim 30 or 31 or the combination of claim 32 in the manufacture of a medicament to prevent macular degeneration.

Date Reçue/Date Received 2023-02-07

51) Use of at least one nucleic acid molecule of any one of claims 1 to 29 and 33 or the composition of claim 30 or 31 or the combination of claim 32 in the manufacture of a medicament to treat an ophthalmic condition.

52) The use according to claim 51, wherein the ophthalmic condition is selected from the group consisting of retinitis, macular degeneration, choroiditis, retinopathy, hypertensive retinopathy, diabetic retinopathy, chronic dry eye, AIDS-related vision loss, amblyopia, hemianopia, retinal vein occlusions, trachoma, keratoconus, chorioretinal inflammation, central serous retinopathy, uveitis, retinal dystrophy, edema, glaucoma, and cataract.

53) The use according to claim 49 or 50, wherein the macular degeneration is age-related macular degeneration.

54) The use according to claim 52, wherein the macular degeneration is dry age-related macular degeneration or wet age-related macular degeneration.

55) Use of at least one nucleic acid molecule of any one of claims 1 to 29 and 33 or the composition of claim 30 or 31 or the combination of claim 32 in the manufacture of a medicament to treat fibrosis.

56) The use of claim 55, wherein the fibrosis is selected from pulmonary fibrosis, renal fibrosis, occular fibrosis, corneal fibrosis, posterior segment fibrotic eye disease, fibrovascular retinal scarring and cystic fibrosis.

57) Use of at least one nucleic acid molecule of any one of claims 1 to 29 and 33 or the composition of claim 30 or 31 or the combination of claim 32 in the manufacture of a medicament to treat a cardiovascular disease.

58) The use according to claim 57, wherein the cardiovascular disease is selected from atherosclerosis, restenosis, a cardiac hypertrophy related condition, and a vascular disorder.

Date Reçue/Date Received 2023-02-07

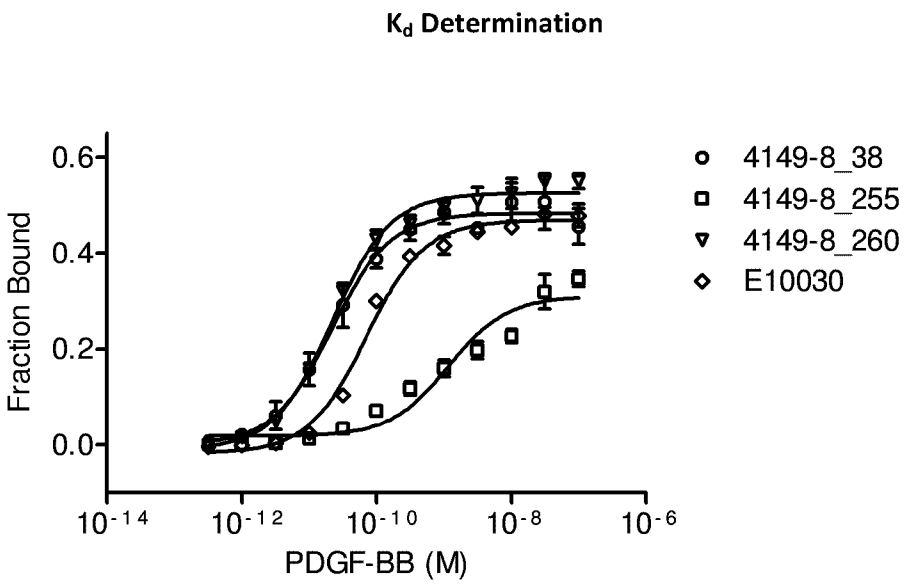
59) Use of at least one nucleic acid molecule of any one of claims 1 to 29 and 33 or the composition of claim 30 or 31 or the combination of claim 32 in the manufacture of a medicament to treat cancer.

60) The use according to claim 59, wherein the cancer is selected from bladder cancer, lung cancer, breast cancer, melanoma, colon cancer, rectal cancer, occular cancer, lymphoma, endometrial cancer, pancreatic cancer, liver cancer, renal cancer, prostate cancer, leukemia, and thyroid cancer.

Date Reçue/Date Received 2023-02-07

Figure 1

A



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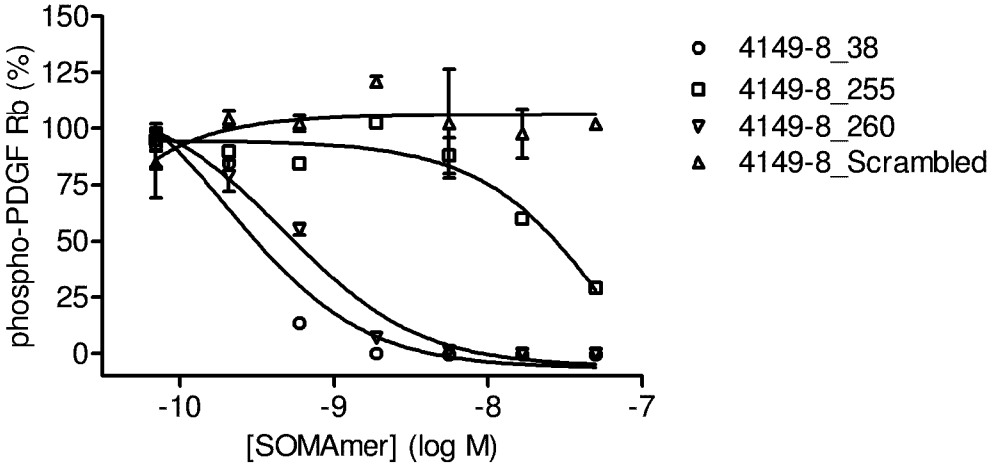

Date Reçue/Date Received 2021-04-30

Figure 1

C K_d Ratio

5-dU Modification	Bn-dU1	Bn-dU2	Bn-dU7	Bn-dU8	Bn-dU16	Bn-dU17	Bn-dU18	Bn-dU20
Bn-dU	1	1	1	1	1	1	1	1
iB-dU	0.4	0.7	0.8	0.4	0.3	16	6.2	8.7
Th-dU	0.5	1.5	1.2	0.9	0.5	0.7	0.5	0.9
FBn-dU	0.3	0.5	0.3	0.7	0.8	3.1	1	0.5
Pe-dU	1.7	1.8	0.8	2.1	1.5	0.5	1.1	1.5
PP-dU	0.3	1.1	0.8	1.9	0.9	13	106	1.9
Tyr-dU	0.2	2.5	1	33	1.2	34	4.4	1.9
MBn-dU	0.6	0.3	0.9	3.6	1.4	7.1	7.6	0.5
Nap-dU	0.9	1.3	1.4	3.3	1.1	4.6	27	0.9
2Nap-dU	0.3	0.4	0.5	601	0.2	106	34	2.4
NE-dU	0.3	3.1	0.6	17	1.4	38	51	0.3
2NE-dU	0.3	3.8	0.6	121	1.3	188	21	3.4
Trp-dU	0.3	3.1	1.1	233	153	307	61	0.8
BT-dU	0.6	2.9	1.9	35	4	30	113	1.8

D Relative PDGFRβ Phosphorylation

5-dU Modification	BndU1	BndU2	BndU7	BndU8	BndU16	BndU17	BndU18	BndU20
Bn-dU	1	1	1	1	1	1	1	1
iB-dU	1	3	1	44	1	81	21	25
Th-dU	1	3	1	2	1	2	1	3
FBn-dU	2	2	3	2	2	4	2	2
Pe-dU	1	1	1	9	5	1	4	1
PP-dU	4	2	1	17	1	42	57	5
Tyr-dU	1	22	2	59	4	59	28	3
MBn-dU	1	1	2	9	3	21	32	10
Nap-dU	1	3	3	24	4	5	51	1
2Nap-dU	1	1	1	90	3	82	60	8
NE-dU	4	29	3	66	8	76	63	1
2NE-dU	3	41	3	82	10	92	68	16
Trp-dU	1	3	1	63	9	100	21	2
BT-dU	2	12	1	54	7	74	72	4

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Figure 2

A

Nucleotide	SOMAmer Intramolecular Contacts	SOMAmer-PDGF Contacts	# Contact Atoms
BndU1	ππ: U1-Bn2 vW: Bn2-Bn6; Bn2-Bn7 H-bonds: U1-V39; U1-R56 (2)	vW, chain 1: Glu24, Arg27, Leu38, Val39, Trp40, Pro42 vW, chain 2: Cys52* , Cys53, Asn54, Asn55, Arg56 H-bonds (3): U1-Val39 (1) & U1-Arg56 (2)	24
BndU2	ππ: U2-A3; Bn2-U1 vW: Bn2-Bn6; Bn2-Bn7 H-bonds: U2-BndU8 (2)	vW, chain 1: Arg27, Ala35, Asn36, Phe37, Leu38, Ile77 vW, chain 2: Asn54	12
2'OMe A3	ππ: A3-C4, A3-U2 H-bonds: A3-U12 basepair	vW, chain 1: Asn36	1
BndU7	ππ: U7-G6, U7-U8 vW: Bn7-Bn2; Bn7-Bn16 H-bonds: U7-A3 basepair	vW, chain 2: Asn54, Asn55	3
BndU8	ππ: U8-Bn20, Bn8-Bn16, Bn8- Bn20 H-bonds: U8-BndU2 (2)	vW, chain 1: Leu38, Ile75, Lys80	3
BndU16	ππ: U16-G15, U16-U17 vW: Bn16-Bn7, Bn16-Bn8, Bn16-PE17 H-bonds: U16-A9 basepair	ππ: Bn16-Trp40 vW, chain 1: Arg73	4
PEdU17	ππ: U17-U16 vW: PE17-Bn8, PE17-Th18 H-bonds: U17-BndU20 linker	vW, chain 1: Leu38, Val39, Trp40, Arg73, Ile75, Phe84 H-bond (1): PEdU17 linker-Arg73 Charge-charge (1): non-bridging O to Arg73	18
ThdU18	vW: Th18-PE17, Th18-U17	vW, chain 1: Lys85, Lys86, Arg73, Phe84, Pro82, Lys74, Ile75	15
dA19	ππ: A19-sugar18 vW: sugar19-U20 backbone	vW, chain 1: Ile83, Pro82, Phe84 H-bonds (2): A19 N3-Phe84 NH & A19 N6H-Phe84 C=O	5
BndU20	ππ: U20-A9, Bn20-U8, Bn20-Bn8 H-bonds: BndU20 linker-U17 N3	vW, chain 1: Ile77, Lys80, Pro82, Phe84	11

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Figure 2

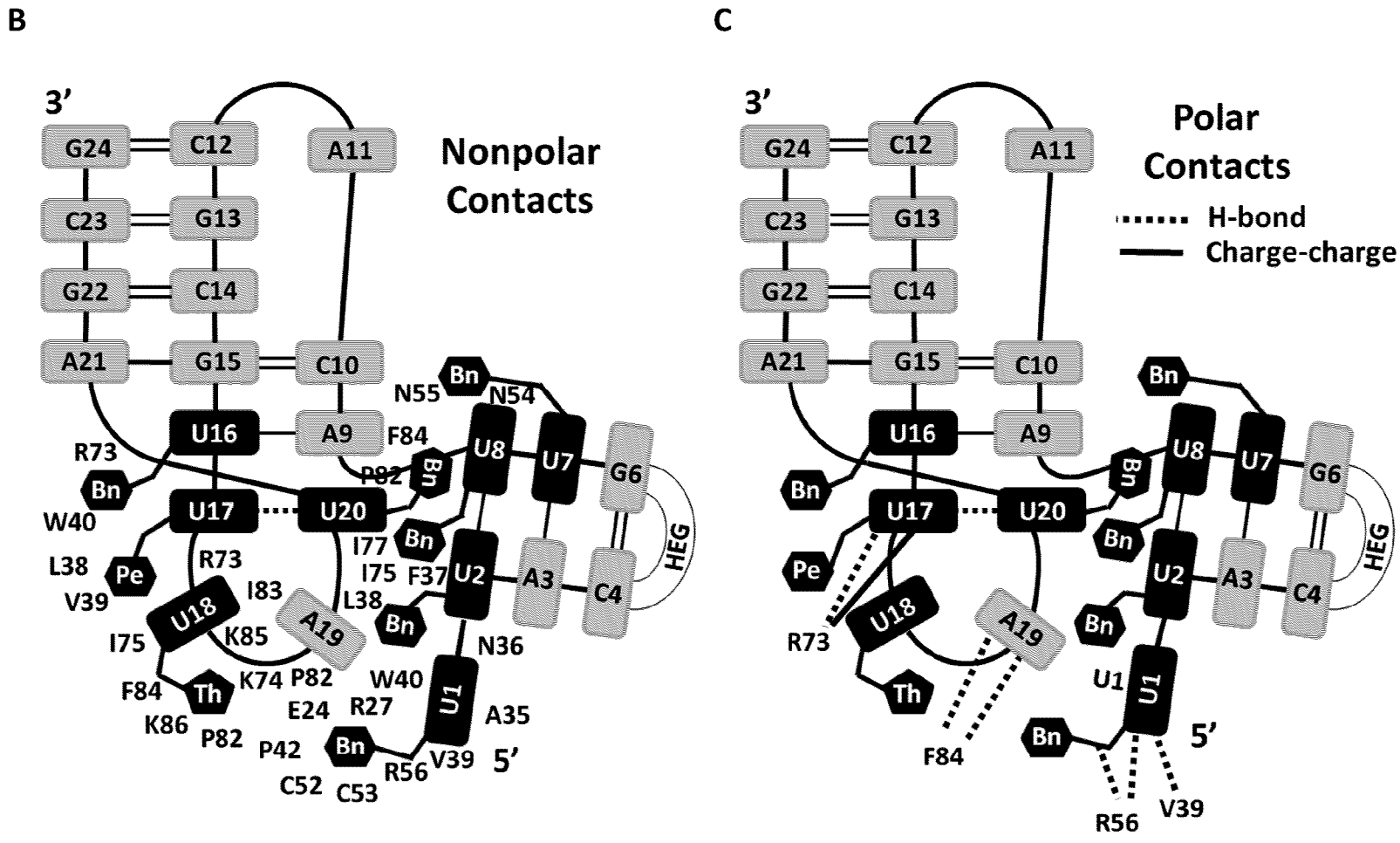

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Figure 3

A

	Bn	Bn	A	C	Linker	G	Bn	Bn	A	C	A	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C	G
A	0.00	0.00	0.57	0.05		0.01	0.00	0.00	0.99	0.00	0.38	0.13	0.01	0.00	0.01	0.00	0.01	0.00	1.00	0.01	0.99	0.01	0.01	0.01
C	0.00	0.00	0.11	0.82		0.12	0.32	0.00	0.00	0.99	0.31	0.84	0.00	0.98	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00	0.96	0.01
G	0.00	0.00	0.32	0.12		0.82	0.11	0.00	0.01	0.00	0.10	0.01	0.98	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.00	0.97
BndU	1.00	1.00	0.00	0.01		0.05	0.57	0.99	0.00	0.01	0.21	0.02	0.00	0.01	0.00	1.00	0.98	0.99	0.00	0.97	0.00	0.01	0.03	0.00
Consensus:	Bn	Bn	A/G	C	X_n	G	Bn	Bn	A	C	N	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C	G

```
Z Z A C G A C Z A - - - - - - - - - - - C G Z Z A C A C G C G Z Z Z A Z A G C G
Z Z A C G G A A - - - - - - - - - - - - C G Z Z A C G C G C G Z Z Z A Z A G C G
Z Z G G C Z C Z Z A G - - - - - - - - - G C C Z A C C C G C G Z Z Z A Z A G C G
Z Z A A C C C G A C G Z G A A - - - G G G Z Z Z A C C C G C G Z Z Z A Z A G C G
Z Z G C A C C - - - - - - - - - - - - - G C Z A C Z C G C G Z Z Z A Z A G C G
Z Z A C C C C G Z Z Z Z A Z Z G G C G G G G Z Z A C Z A G C G Z Z Z A Z A G C G
```

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Figure 3

B

																															PDGF-BB K_d (nM)
SL1	Bn	Bn	A	C	G	A	C	Bn	A	C	G	Bn	Bn	A	C	A	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C	G		0.022
SL6	Bn	Bn	A	C	G	A	C	Bn	A	C	G	Bn	Bn	A	C	A	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C			0.035
SL7	Bn	Bn	A	C	G	HEG linker				C	G	Bn	Bn	A	C	A	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C	G		0.018
SL8	Bn	Bn	A	C	HEG linker						G	Bn	Bn	A	C	A	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C	G		0.016
SL9	Bn	Bn	A	C	G	A	C	Bn	A	C	G	Bn	Bn	A	C	A		G	C	G	Bn	Bn	Bn	A	Bn	A	G	C			0.022
SL10	Bn	Bn	A	C	HEG linker						G	Bn	Bn	A	C	A		G	C	G	Bn	Bn	Bn	A	Bn	A	G	C			0.053
SL11	Bn	Bn	A	C	HEG linker						G	Bn	Bn	A	C	V		G	C	G	Bn	Bn	Bn	A	Bn	A	G	C			0.039
SL12	Bn	Bn	A	C	HEG linker						G	Bn	Bn	A	C	V			C	G	Bn	Bn	Bn	A	Bn	A	G				>1000
Nt #:	1	2	3	4				5			6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		

HEG Hexaethylene glycol linker
V Three carbon "C3" linker
BndU-BndU base pair
Canonical base pair
Base triple
Non-essential base pair

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Figure 4

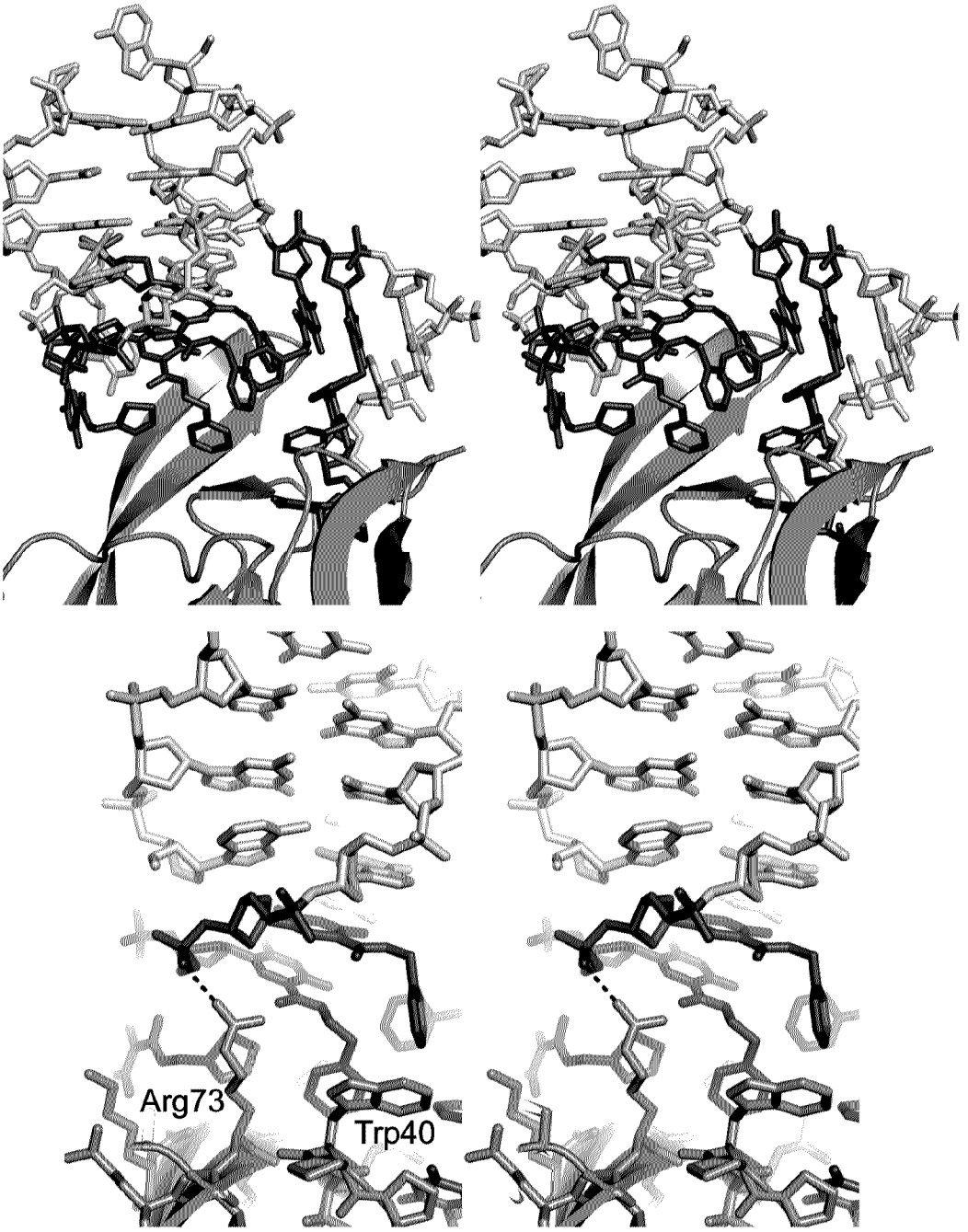

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Figure 5

A

SOMAmer	SOMAmer	PDGF-BB	PDGF-BB +tRNA	PDGF-AB	PDGF-AB +tRNA	PDGF-AA	PDGF-AA +tRNA	PDGF-CC	PDGF-CC +tRNA	PDGF-DD	PDGF-DD +tRNA
		K_d (nM)									
4149-8_38	SL1	0.02	0.06	0.08	6.0	1.6	>1000	>1000	>1000	100	>1000
4149-8_130	SL3	0.02	0.05	0.24	1.6	8.7	>1000	>1000	>1000	290	>1000
4149-8_255	SL4	0.43	1.2	2.0	25	3.4	460	>1000	>1000	>1000	>1000
4149-8_260	SL5	0.02	0.13	0.08	3.9	8.0	420	>1000	>1000	710	>1000

B

```
PDGF-B        SLGSLTIAEPAMIAECKTRTEVFEISRRLIDRTNANFLVWPPCVEVQRCSGCCNNRNVQCRPTQVQLRPV
PDGF-A              SIEEAVPAVCKTRTVIYEIPRSQVDPTSANFLIWPPCVEVKRCTGCCNTSSVKCQPSRVHHRSV
PDGF-C        VVDLNLLTEEVRLYSCTPRNFSVSIR   EELKRTDTIFWPGCLLVKRCGGNCACCLHNCNECQCVPSKV
PDGF-D SYHDRKSKVDLDRLNDDAKRYSCTPRNYSVNIR   EELKLANVVFFPRCLLVQRCGGNCGCGTVNWRSCTCNSGKT

PDGF-B QVRKIEIVRKKP          IFKKATVTLEDHLACKCETVAAARPVTRSPGGSQEQRAKTPQTRVTIRT
PDGF-A KVAKVEYVRKKP          KLKEVQVRLEEHLECACATTSLN      PDYREEDTGRPRES
PDGF-C TKKYHEVLQLRP   KTGVRGLHKSLTDVALEHHEECDCVCRGST      GG
PDGF-D VKKYHEVLQFEPGHIKRRGRAKTMALVDIQLDHHERCDCICSSRP      PR

PDGF-B VRVRRPPKGKHRKFKHTHDKTALKETLGA
PDGF-A         GKKRKRKR      LKPT
```

PDGF-A: bold: residues involved in propeptide binding

PDGF-B: bold: residues involved in receptor binding

Figure 6

A

C3 Linker Substitution (K_d ratio)	32	5700	1200	96	0.8	0.8	0.9	0.8	1.1	0.9	390	10000	13000	30000	280	2.2	3.1	610	4300	30000	30000	9200	30000	34	7600	30000	4600	190	1.5		PDGF BB K_d(nM)	PDGF AB K_d(nM)	Cellular IC_{50} (nM)
Position#:5′	1	2	3	4	5						6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	3′			
SL1	Bn	Bn	A	C	G	A	C	Bn	A	C	G	Bn	Bn	A	C	A	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C	G		0.02	0.06	0.2
SL2	Bn	Bn	A	C	HEG linker						G	Bn	Bn	A	C	A	C	G	C	G	Bn	Bn	Bn	A	Bn	A	G	C	G		0.02	ND	0.3
SL3	Bn	Bn	mA	C	HEG linker						G	Bn	Bn	A	C	mA	C	G	C	G	Bn	Bn	Bn	A	Bn	mA	G	C	mG		0.05	0.19	0.3

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Figure 6

B

Position#: 5′	1	2	3 4	5	6	7	8	9 10 11 12 13 14 15	16	17	18	19	20	21 22 23 24	3′	PDGF BB K_d(nM)	PDGF AB K_d(nM)	Cellular IC_{50}(nM)
4149-8_255	Bn	Bn	mA C	HEG linker	G	Bn	iB	A C mA C G C G	Bn	Pe	Th	A	Bn	mA G C mG		1.20	1.40	77.0
4149-8_260	Bn	Bn	mA C	HEG linker	G	Bn	Bn	A C mA C G C G	Bn	Pe	Th	A	Bn	mA G C mG		0.02	0.06	0.5

5-dU modification	R group	1	2	7	8	16	17	18	20
Bn-dU		1	1	1	1	1	1	1	1
dT*	CH_3	**2.3**	**830**	**0.6**	**3200**	**2600**	**760**	**2000**	**890**
iB-dU		**0.4**	**0.7**	**0.8**	**0.4**	**0.3**	**16**	**6.2**	**8.7**
Th-dU	S	**0.5**	**1.5**	**1.2**	**0.9**	**0.5**	**0.7**	**0.5**	**0.9**
FBn-dU	F	**0.3**	**0.5**	**0.3**	**0.7**	**0.8**	**3.1**	**1**	**0.5**
PE-dU		**1.7**	**1.8**	**0.8**	**2.1**	**1.5**	**0.5**	**1.1**	**1.5**
PP-dU		**0.3**	**1.1**	**0.8**	**1.9**	**0.9**	**13**	**106**	**1.9**
Tyr-dU	OH	**0.2**	**2.5**	**1**	**33**	**1.2**	**34**	**4.4**	**1.9**
MBn-dU	O O	**0.6**	**0.3**	**0.9**	**3.6**	**1.4**	**7.1**	**7.6**	**0.5**
Nap-dU		**0.9**	**1.3**	**1.4**	**3.3**	**1.1**	**4.6**	**27**	**0.9**
2Nap-dU		**0.3**	**0.4**	**0.5**	**601**	**0.2**	**106**	**34**	**2.4**
NE-dU		**0.3**	**3.1**	**0.6**	**17**	**1.4**	**38**	**51**	**0.3**
2NE-dU		**0.3**	**3.8**	**0.6**	**121**	**1.3**	**188**	**21**	**3.4**
Trp-dU	NH	**0.3**	**3.1**	**1.1**	**233**	**153**	**307**	**61**	**0.8**
BT-dU	S	**0.6**	**2.9**	**1.9**	**35**	**4**	**30**	**113**	**1.8**

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Figure 7

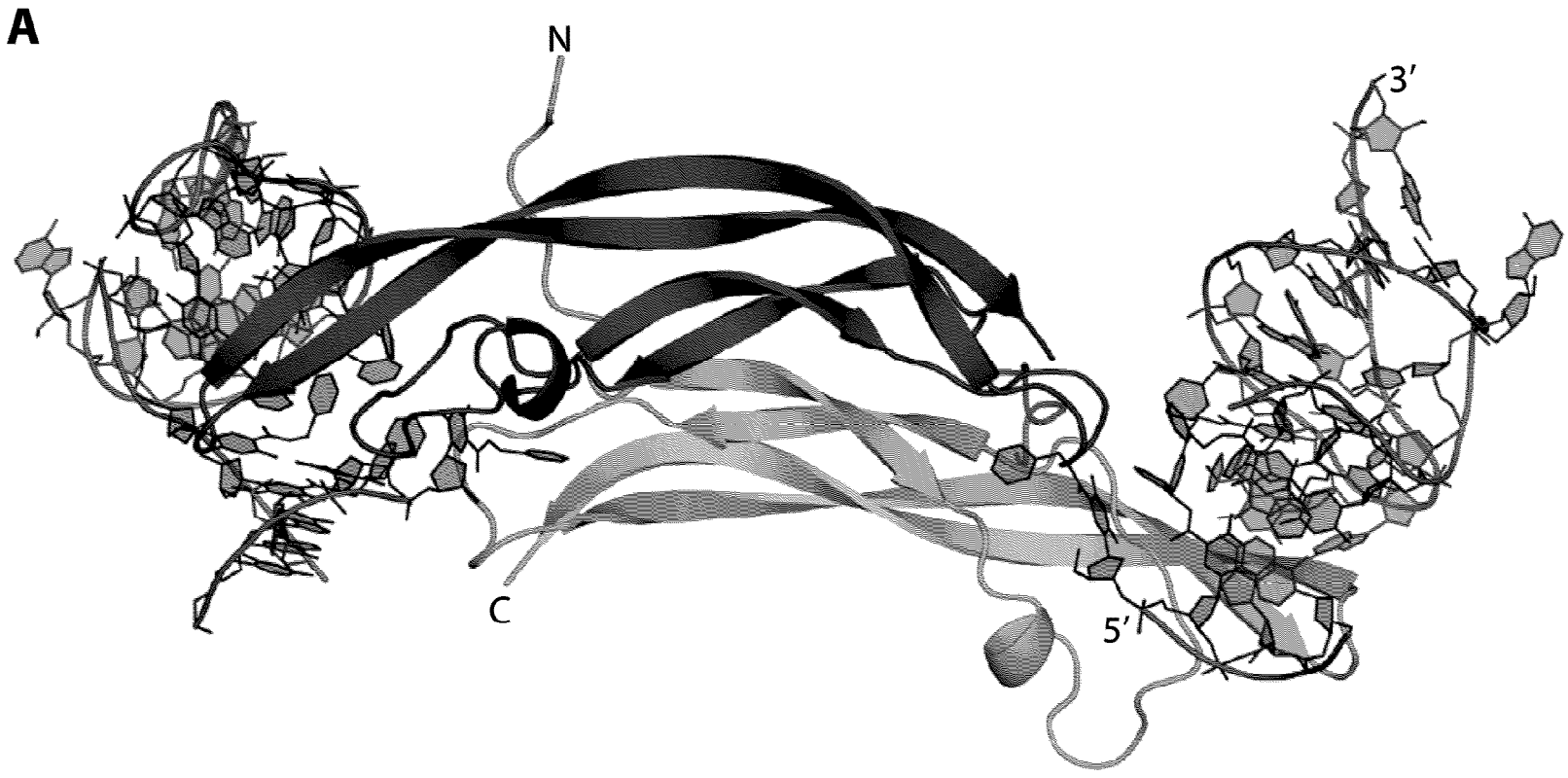

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Figure 7

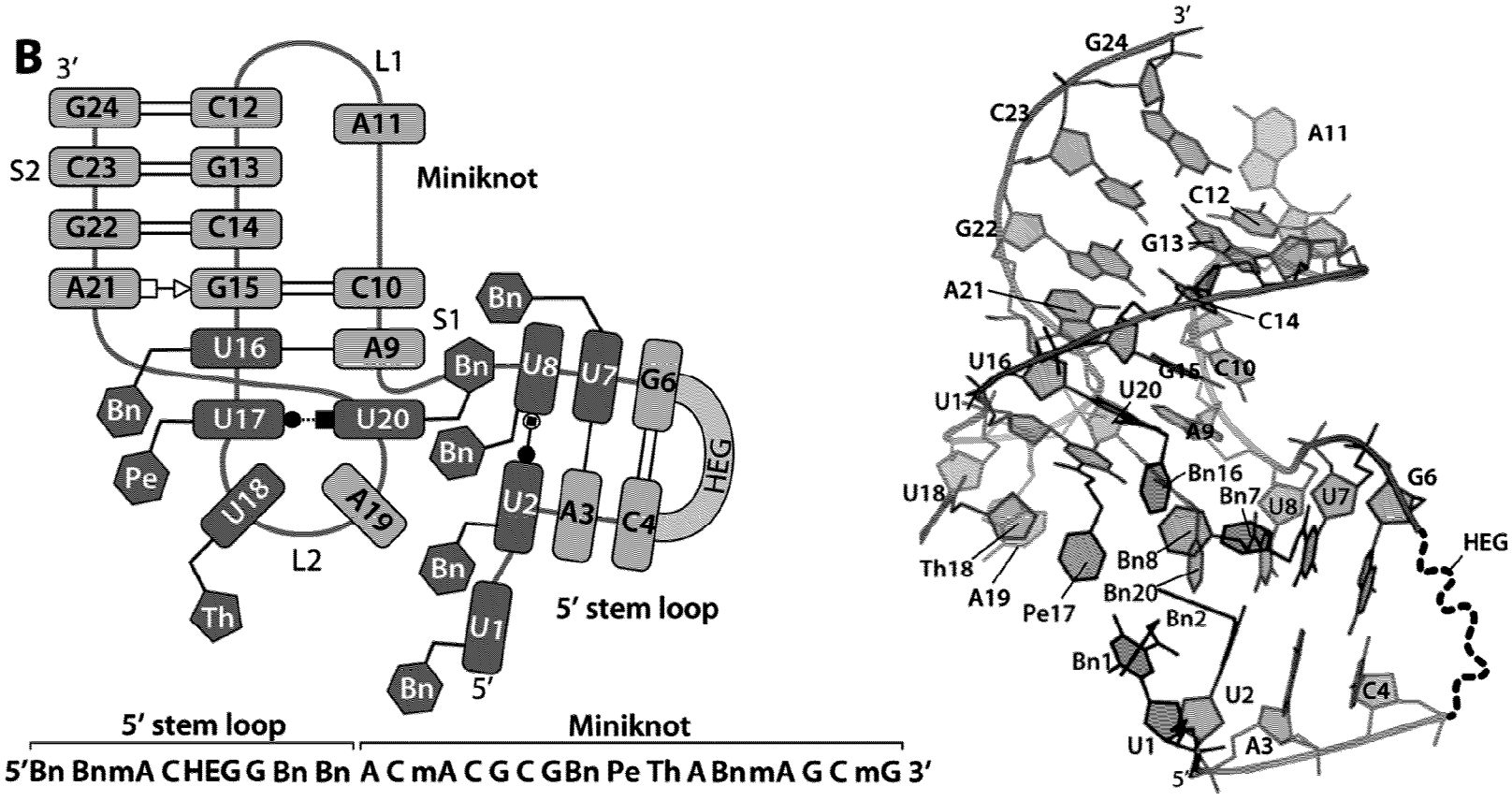

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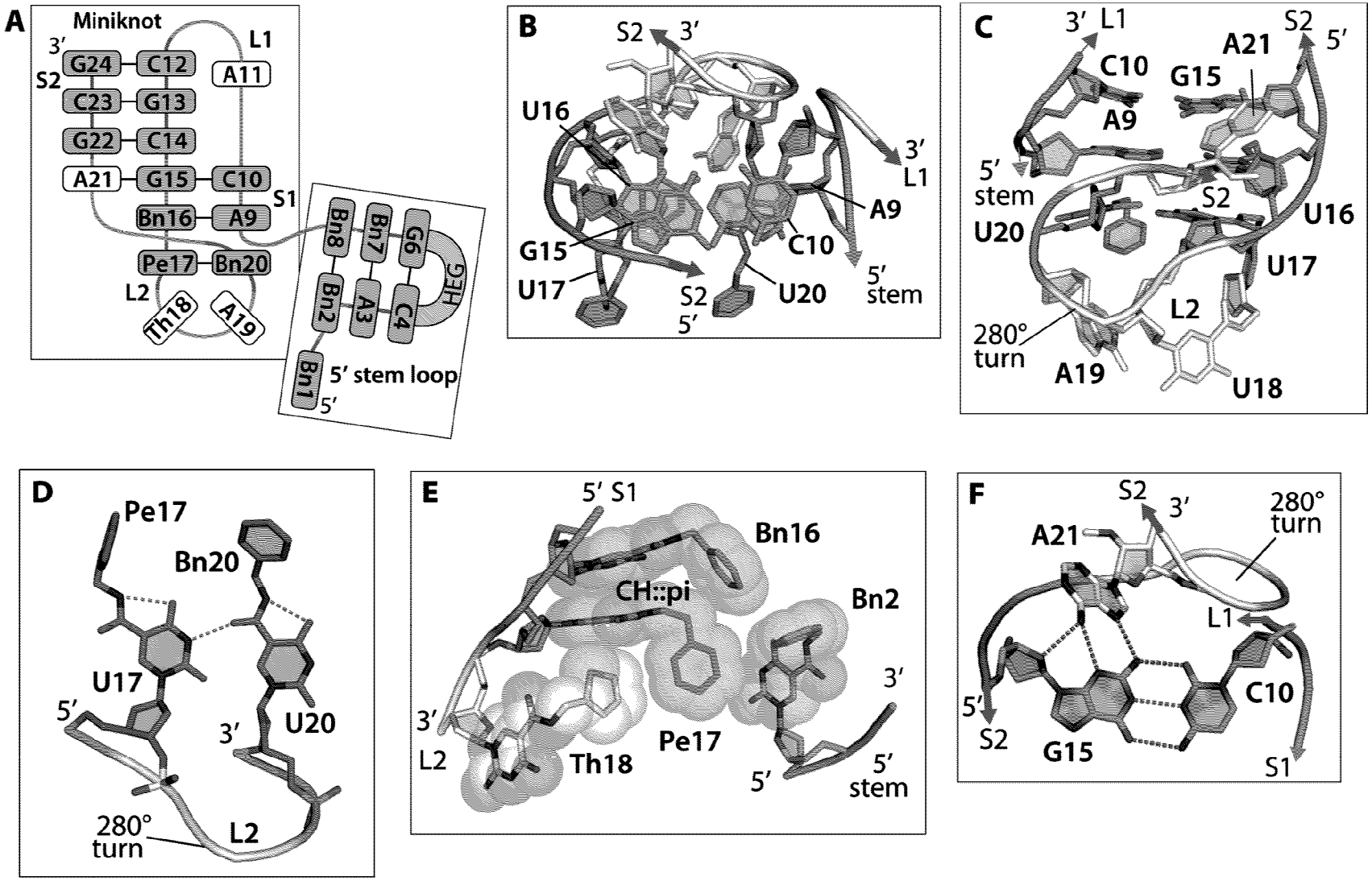
Figure 8



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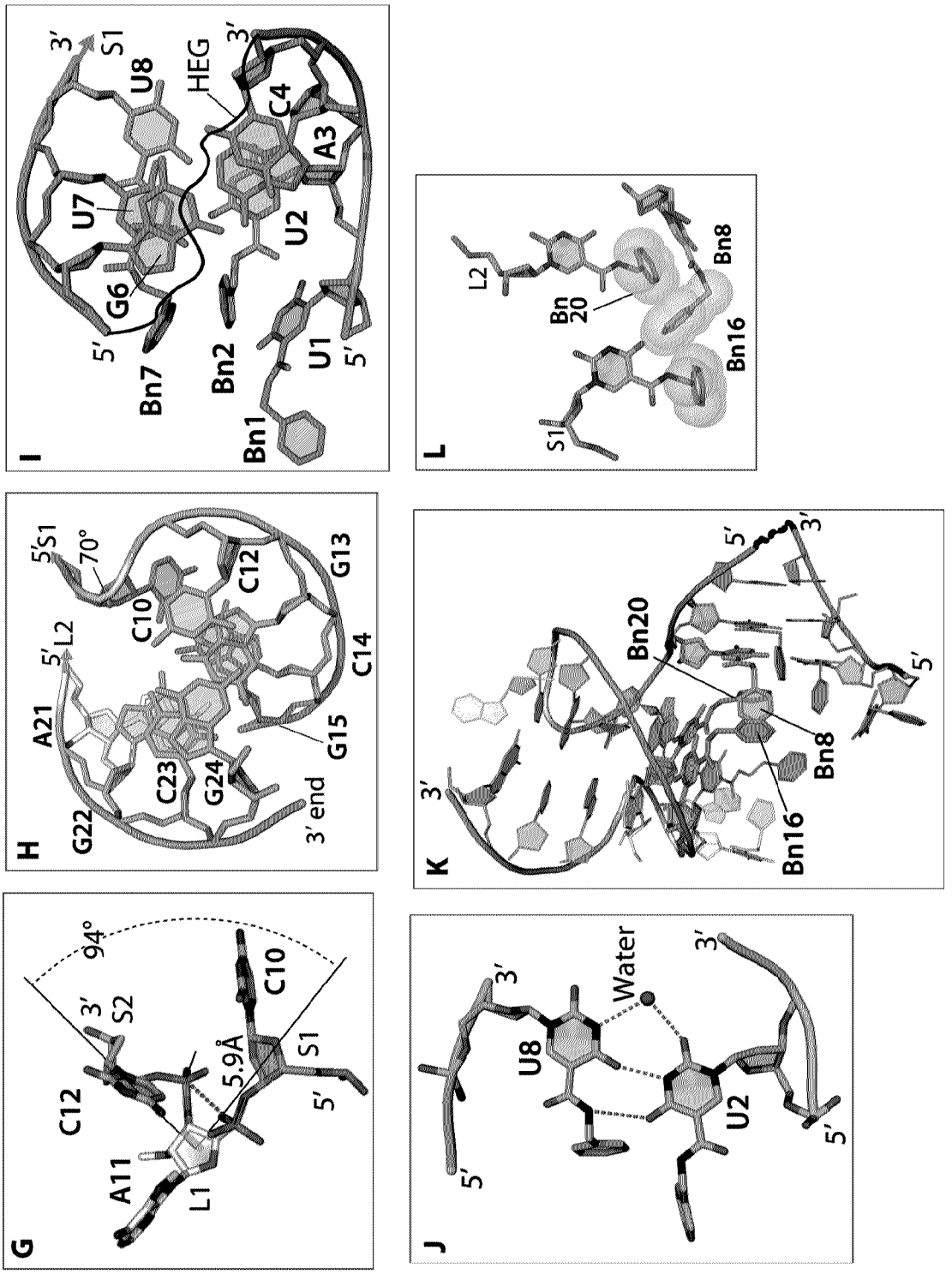
G
A11
C12
L1
3′
S2
94°
5.9Å
C10
S1
5′
H
G22
A21
5′
L2
5′S1
70°
C23
G24
C10
C12
G13
G15
C14
3′ end
I
5′
Bn7
G6
U7
U8
3′
S1
HEG
Bn2
U2
C4
A3
3′
Bn1
U1
5′
J
5′
3′
U8
Water
U2
3′
5′
K
3′
Bn20
Bn16
Bn8
5′
3′
5′
L
S1
L2
Bn
20
Bn16
Bn8

Figure 8

Figure 8

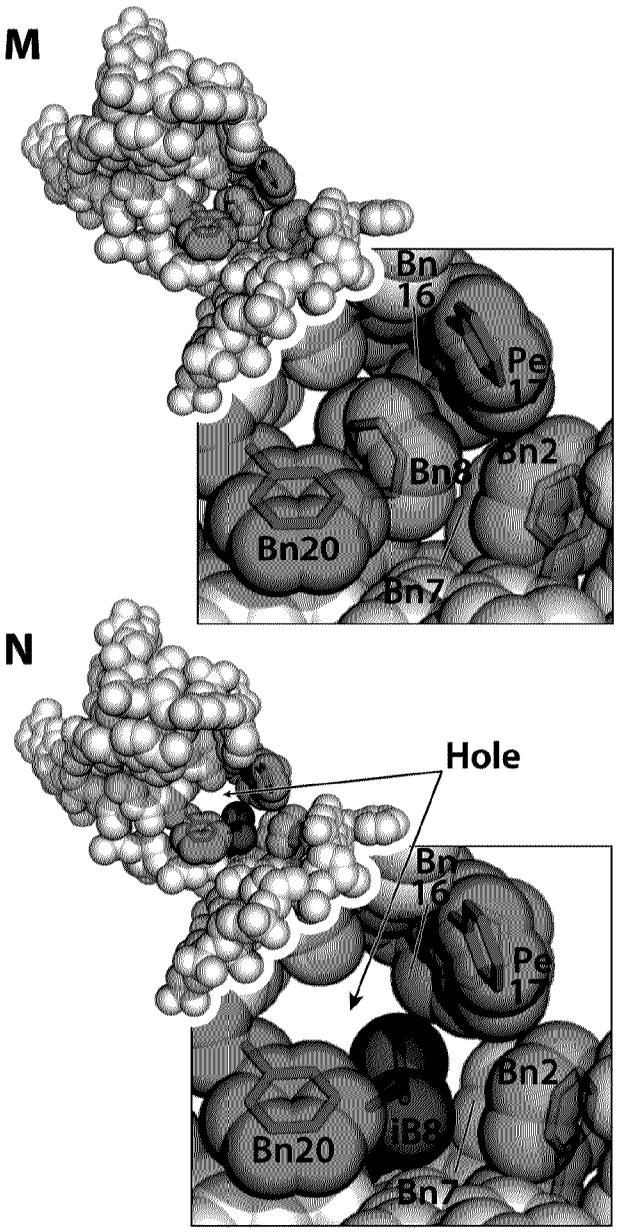

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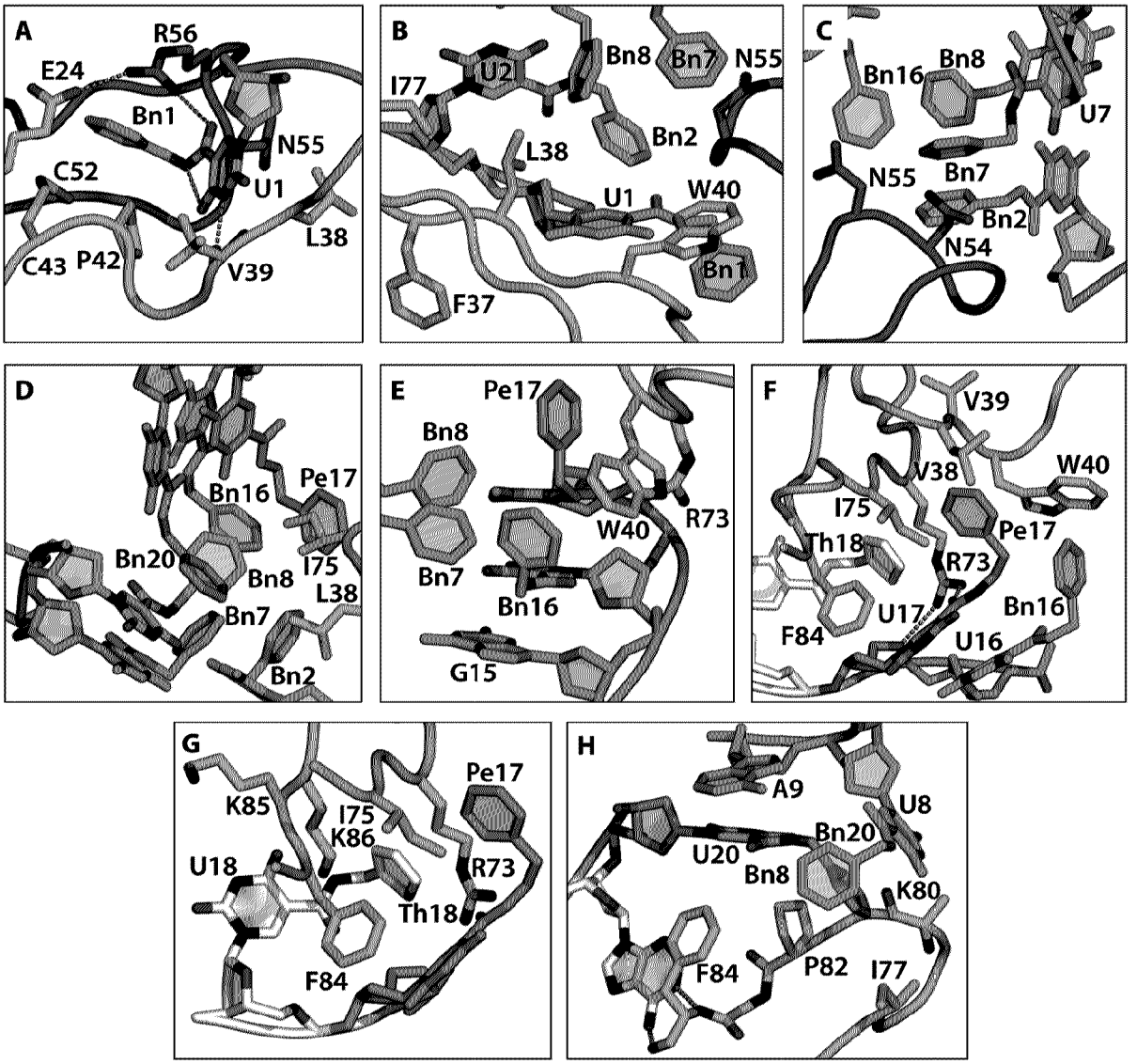
Figure 9


A
R56
E24
Bn1
N55
C52
U1
L38
C43
P42
V39
B
U2
Bn8
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N55
I77
L38
Bn2
U1
W40
Bn1
F37
C
Bn16
Bn8
U7
N55
Bn7
Bn2
N54
D
Pe17
Bn16
Bn20
Bn8
I75
L38
Bn7
Bn2
E
Pe17
Bn8
W40
R73
Bn7
Bn16
G15
F
V39
V38
W40
I75
Pe17
Th18
R73
U17
Bn16
F84
U16
G
Pe17
K85
I75
K86
U18
R73
Th18
F84
H
A9
U8
Bn20
U20
Bn8
K80
F84
P82
I77

Figure 10

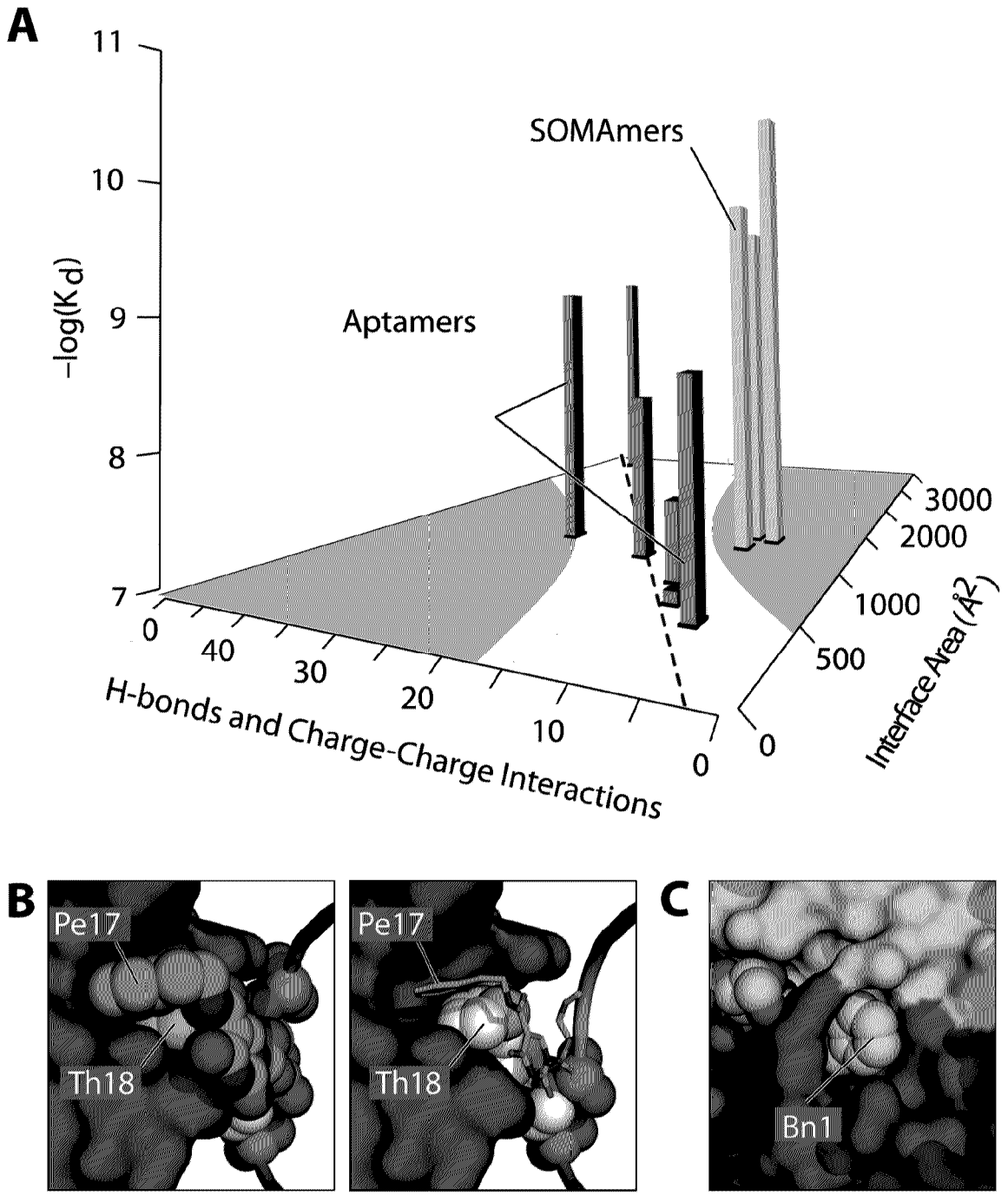

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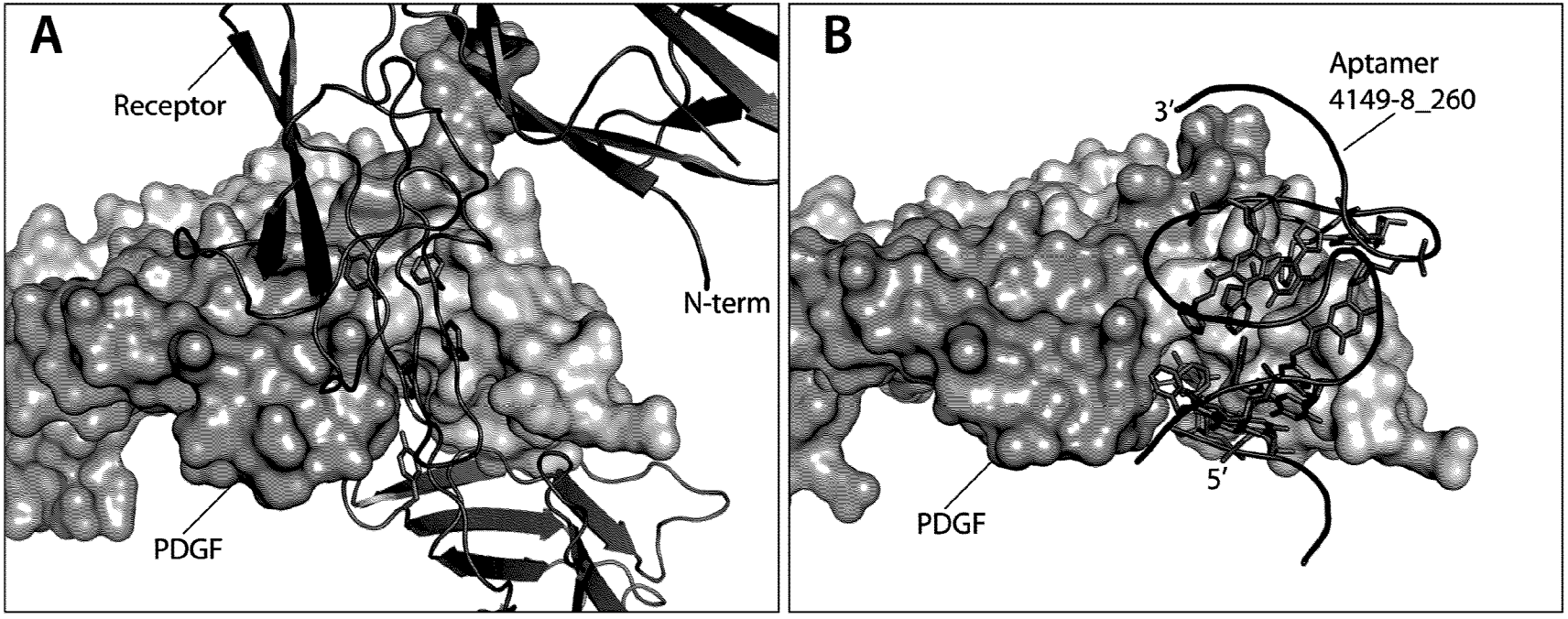
Figure 11



C PDGF-B Sequence:

```
1         10        20        30        40        50        60        70        80
SLGSLTIAEPAMIAECKTRTEVFEISRRLIDRTNANFLVWPPCVEVQRCSGCCNNRNVQCRPTQVQLRPVQVRKIEIVRK
KPIFKKATVTLEDHLACKCETVAAARPVTRSPGGSQEQRAKTPQTRVTIRTVRVRRPPKGKHRKFKHTHDKTALKETLGA
81        90        100       110       120       130       140       150       160
```

Residues within 4Å of: SOMAmer SOMAmer and receptor Receptor

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Figure 12

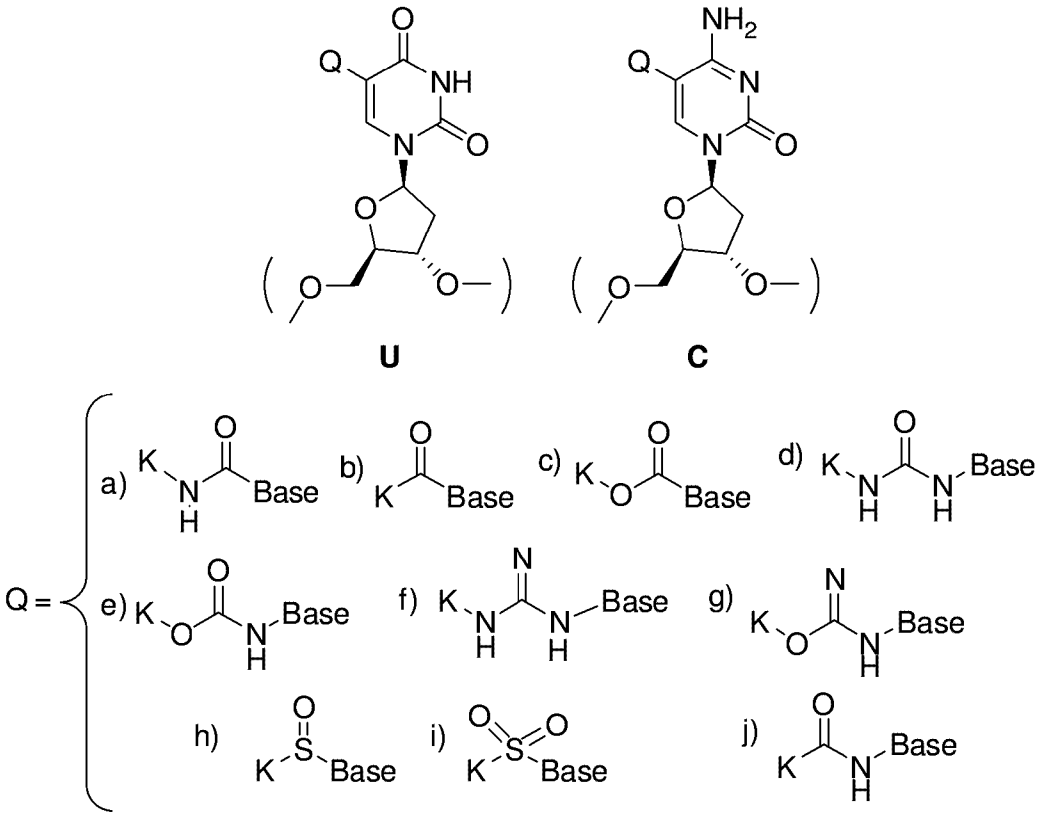

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Figure 12 Continued

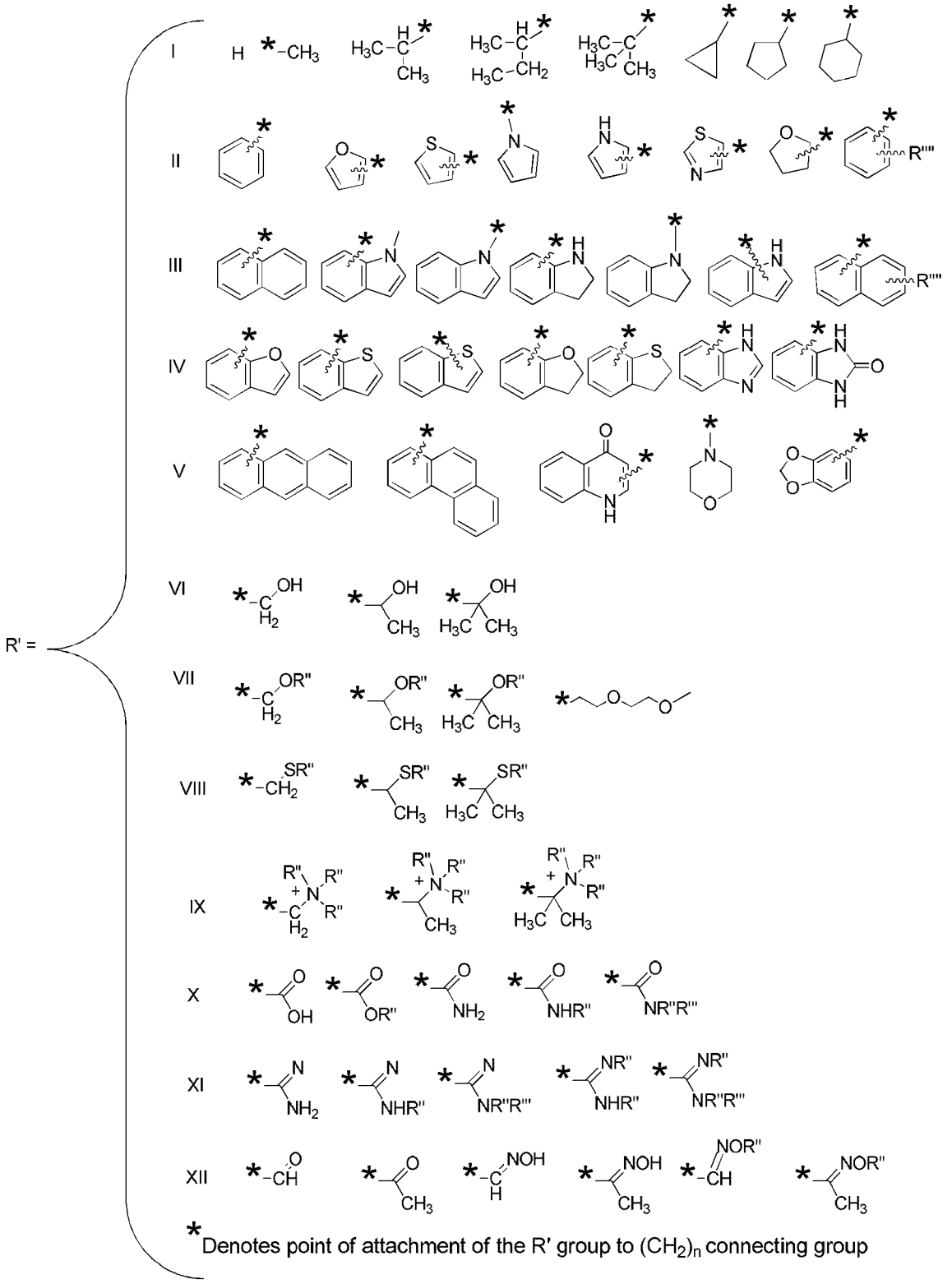

Figure 13

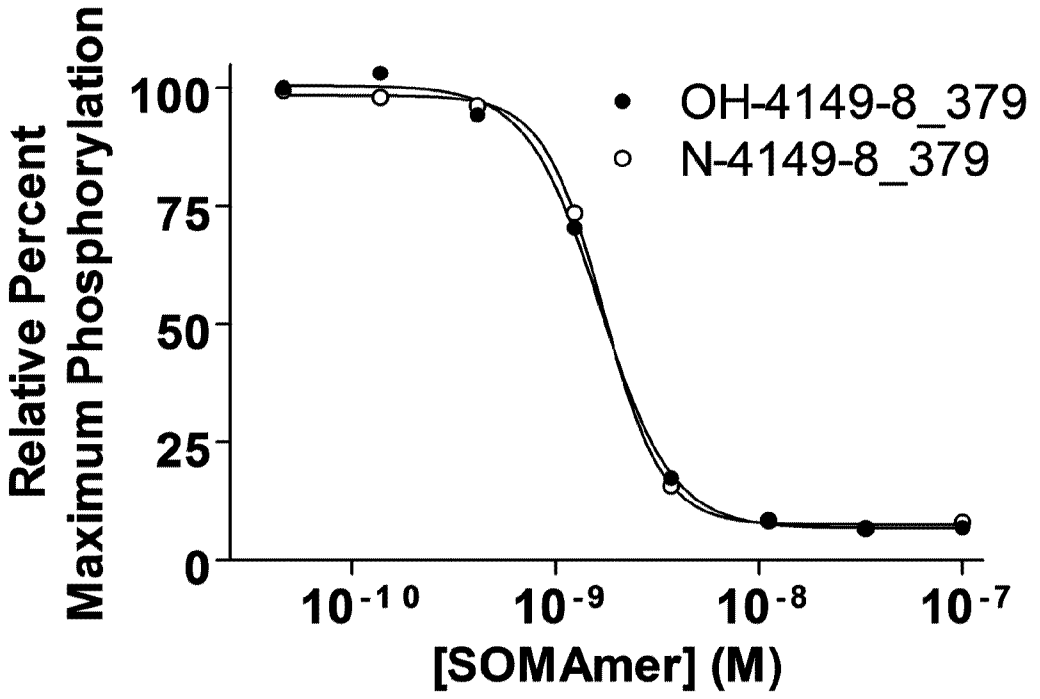

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Figure 14

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Consensus	V	R	M	S	N	N	V	K	P	G	P	A	P	G	V	A	V	R	P	C	P
Frequencies																					
A	0.18	0.14	0.80	0.04	0.67	0.20	0.35	0.78	0.00	0.00	0.00	1.00	0.00	0.00	0.29	0.92	0.14	0.86	0.00	0.00	0.04
C	0.39	0.02	0.10	0.78	0.10	0.12	0.51	0.12	0.02	0.02	0.00	0.00	0.00	0.00	0.55	0.08	0.63	0.00	0.02	0.98	0.00
G	0.27	0.78	0.04	0.10	0.14	0.59	0.10	0.06	0.06	0.98	0.00	0.00	0.00	0.96	0.10	0.00	0.18	0.10	0.00	0.00	0.00
P	0.04	0.00	0.02	0.06	0.10	0.10	0.04	0.04	0.92	0.00	1.00	0.00	1.00	0.04	0.06	0.00	0.06	0.04	0.98	0.02	0.96

Date Reçue/Date Received 2021-04-30

Figure 15

Mod	Nap-dU4	Nap-dU5	Nap-dU10	Nap-dU13	Nap-dU14	Nap-dU16	Nap-dU21	Nap-dU22	Nap-d23	Nap-dU27
Nap-dU	1	1	1	1	1	1	1	1	1	1
iBu-dU	1700	1900	110	28	20800	13	25	20800	1050	20800
Trp-dU	135	45	18	20800	870	15	180	81	16	1.4
FBn-dU	28	4	8.3	12	130	3.9	28	210	42	20800
2Nap-dU	13	20800	12	37	63	72	nd	20	27	420
PE-dU	340	130	57	44	170	7.3	210	34	350	240
Tyr-dU	20800	13000	42	210	2900	50	570	1600	38	64
NE-dU	85	10	8	17	23	1.3	51	34	30	1850
2NE-dU	59	97	164	91	490	13	1020	190	48	330
MBn-dU	140	78	1	9.6	56	4.1	20800	2.4	19	540
PP-dU	110	59	67	20	337	34	57	1800	20800	20800
Th-dU	300	370	8.9	67	510	4.6	169	157	23	20800
BT-dU	14	23	4.8	26	28	1.3	170	nd	16	130
Bn-dU	nd	nd	nd	8.3	nd	nd	34	74	61	310
MOE-dU	20800	20800	20	1440	20800	38	140	586	1040	20800
R(-)THF-dU	840	110	300	6090	24	110	1700	960	20800	nd
S(+)THF-dU	20800	3700	290	120	20800	40	440	20800	740	20800
BF-dU	1600	210	20800	180	225	104	3600	41	75	7900

Date Reçue/Date Received 2021-04-30

Figure 15 Continued

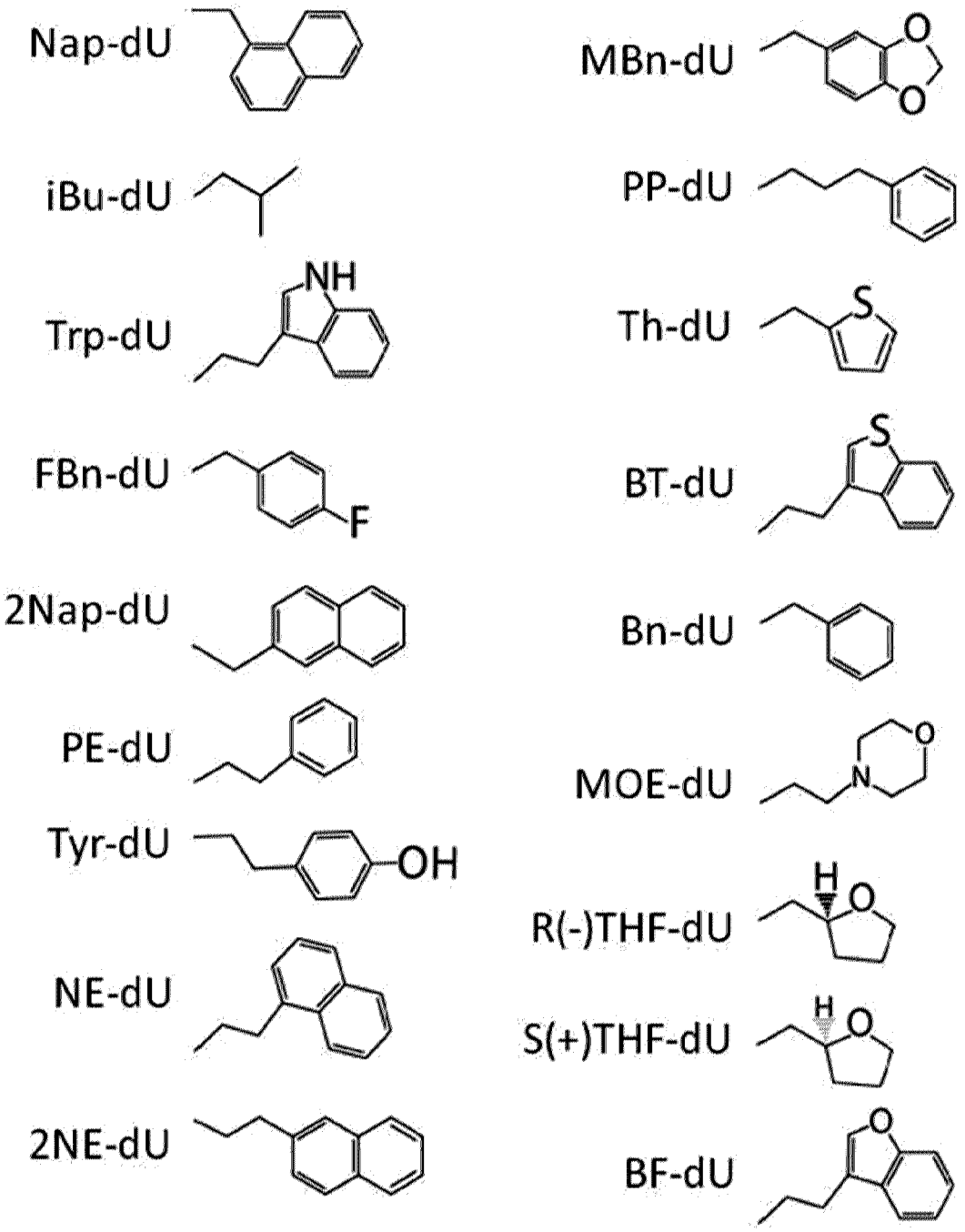

Figure 16

Position	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
Consensus	C	C	G	Z	Z	C	A	A	G	Z	G	C	Z	Z	G	Z	A	G	G	A	Z	Z	Z	A	A	A	Z	G	G
Frequencies																													
A	0.00	0.00	0.01	0.00	0.00	0.00	0.99	1.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00
C	0.99	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.01	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
G	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.99	0.00	0.00	0.00	0.99	0.00	0.01	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
T/Z	0.01	0.00	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	1.00	0.99	0.00	1.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00	0.99	0.00	0.00

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Figure 17

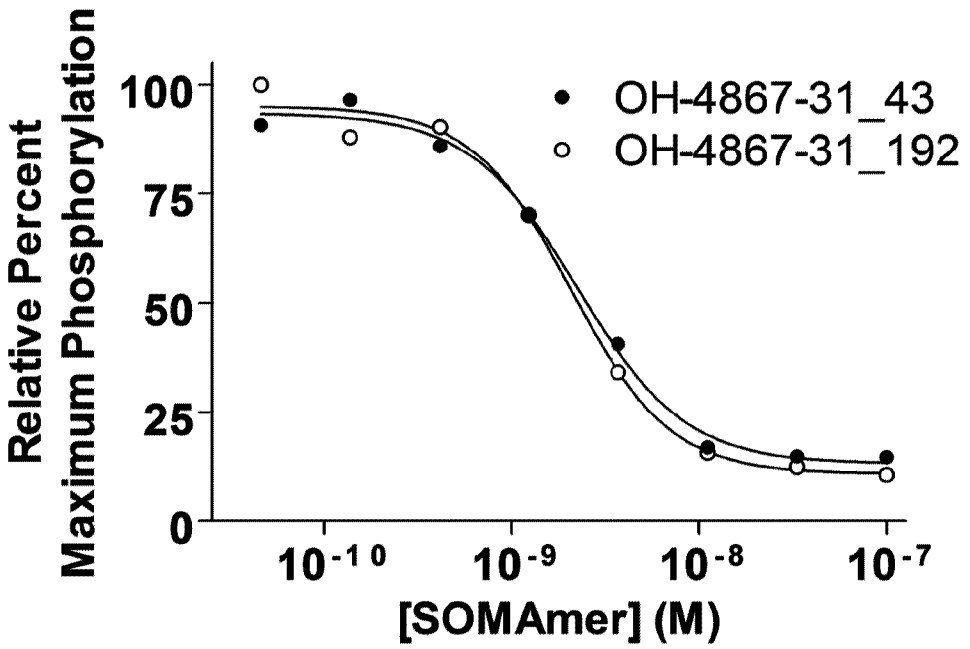

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Figure 18

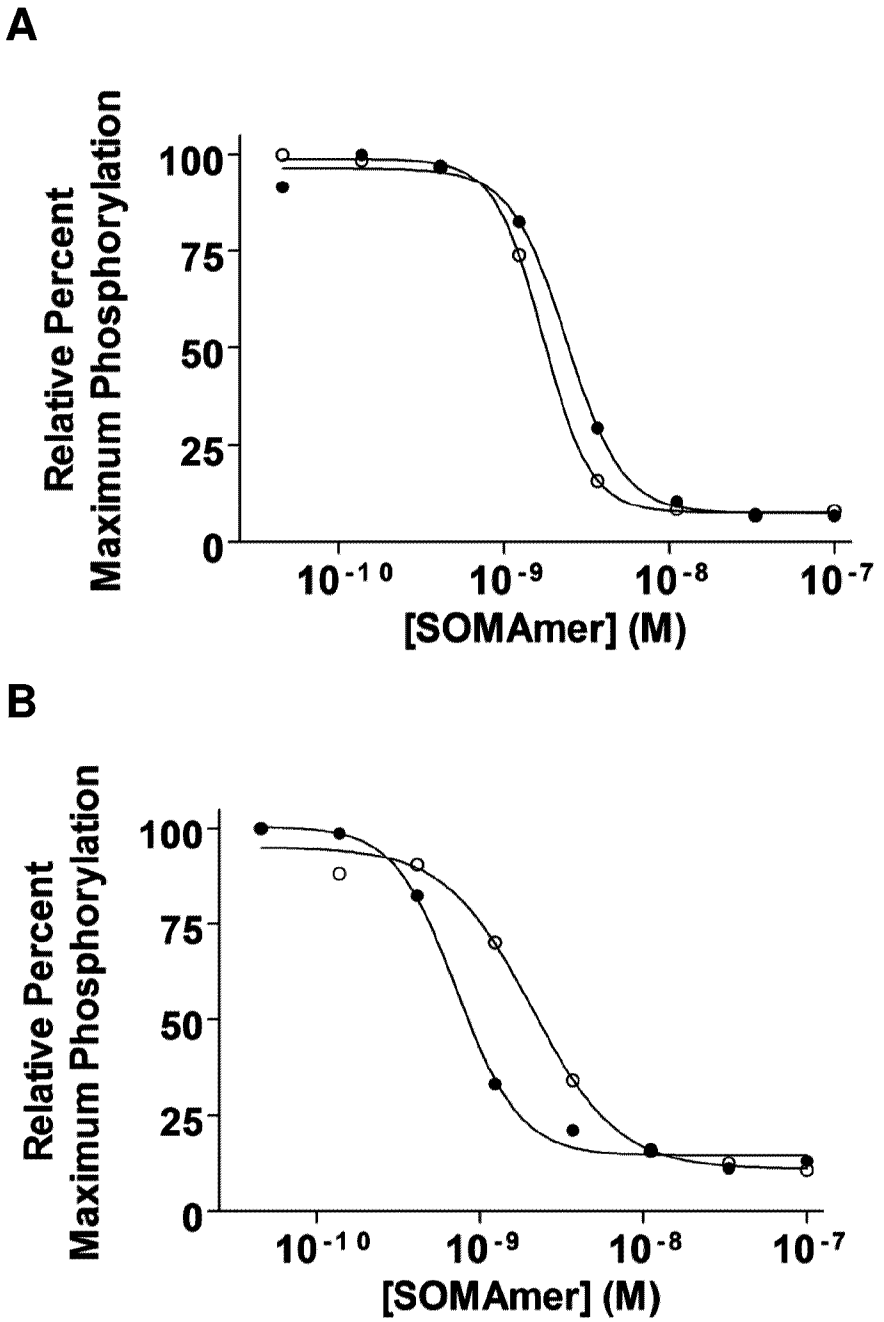

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Figure 19

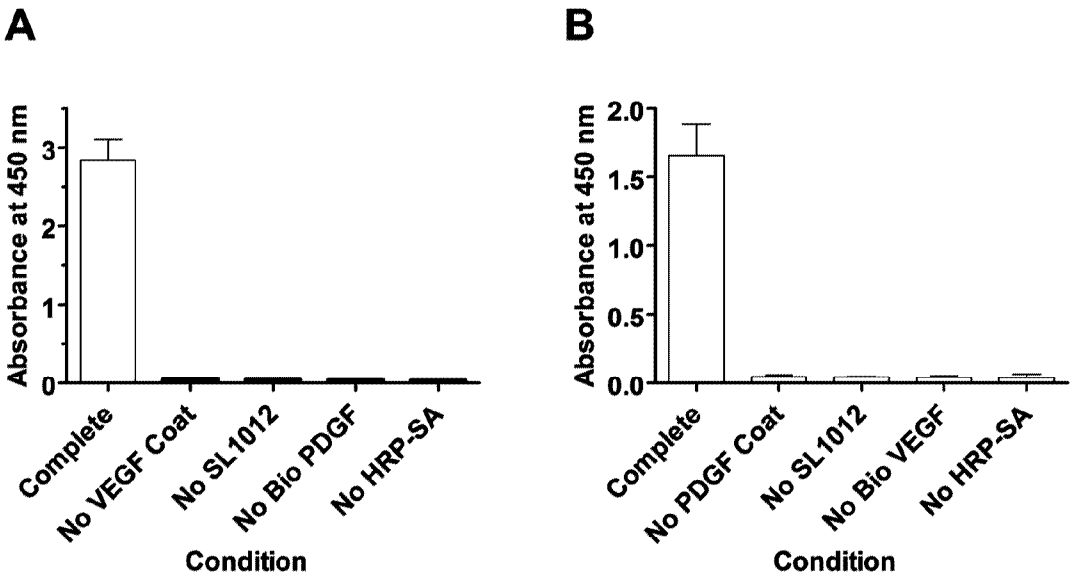

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Figure 20

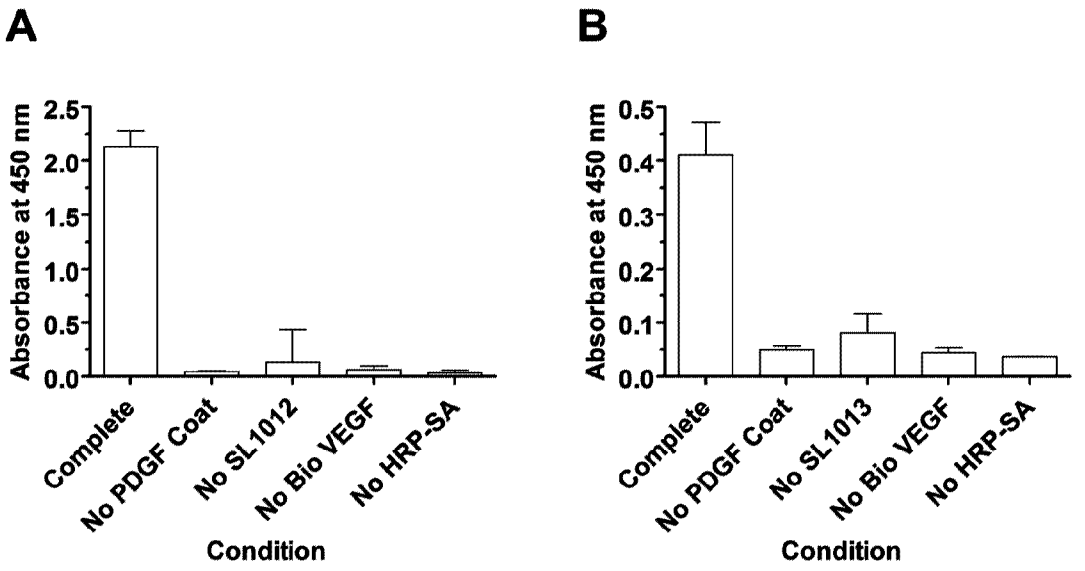

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Figure 21

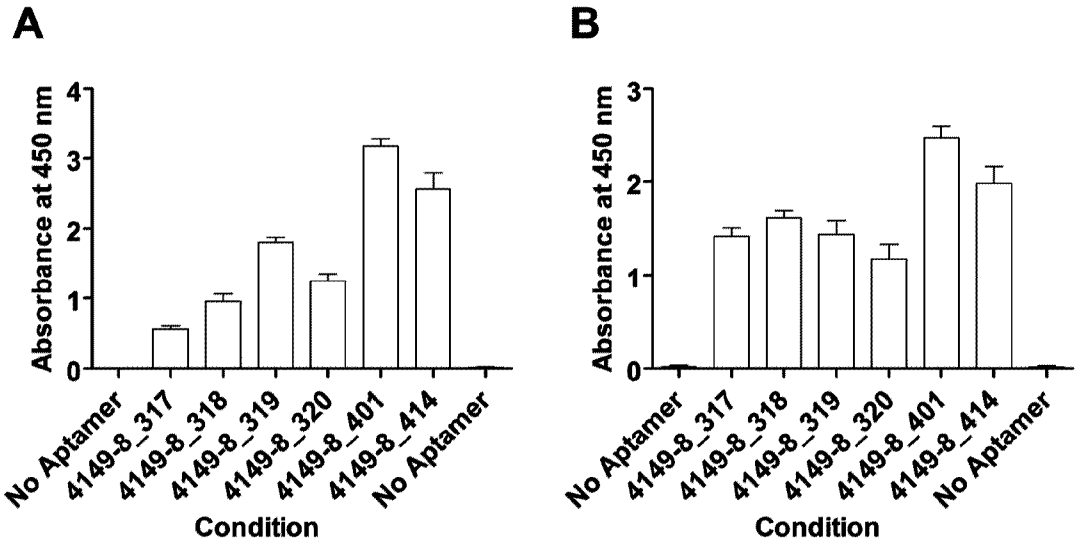

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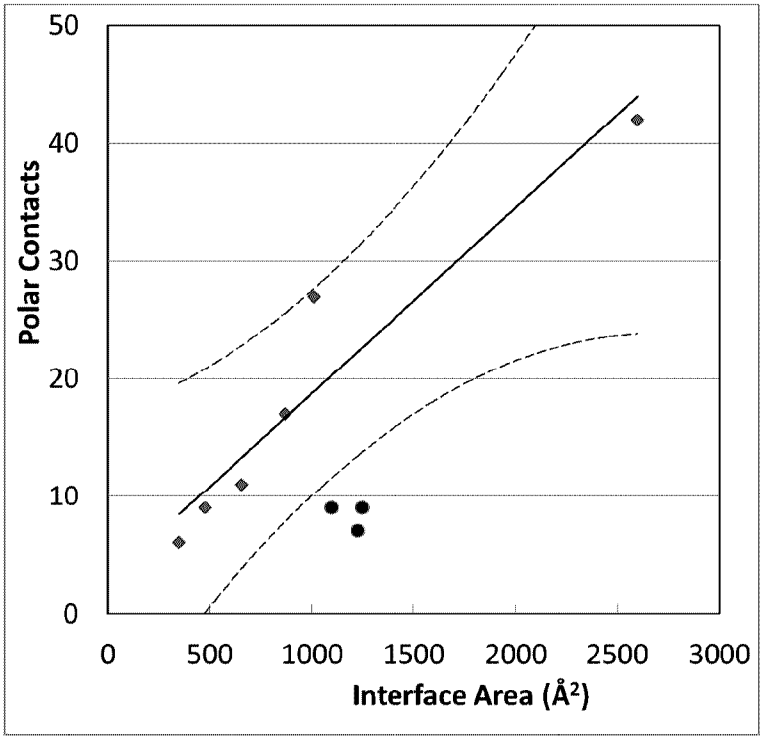
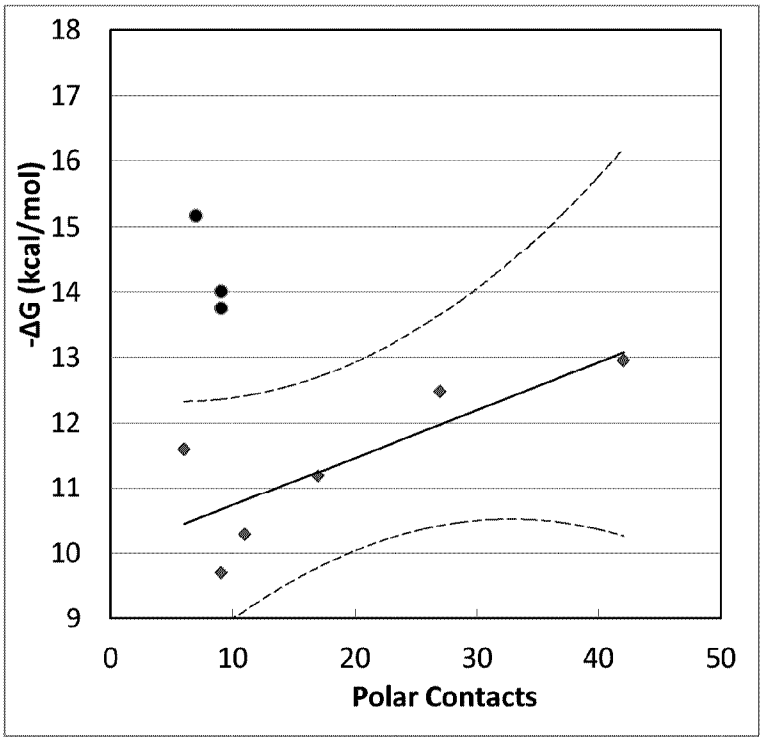
Figure 22

A



B



Date Reçue/Date Received 2021-04-30

Figure 22

C

	Protein Target	-Log (K_d)	-ΔG (kcal/mol)	Contact Atoms	Polar Contacts	Interface Area (Å^2)	Ligand Efficiency (kcal/mol per contact atom)	References	PDB Code
Aptamers	MS2 coat protein	8.6	11.6	25	6	348	0.464	(a)	6MSF
	IgG	7.1	9.7	37	9	477	0.262	(b)	3AGV
	Thrombin	7.6	10.3	62	11	657	0.166	(c)	3QLP
	NFκB	8.3	11.2	57	17	870	0.196	(d)	1OOA
	vWF	9.2	12.5	109	27	1011	0.115	(e)	3HXO
	GlnRS	9.6	13.0	203	42	2599	0.064	(f)	1EXD
SOMAmers	PDGF-BB	10.7	15.2	131	7	1225	0.116	(g)	4HQU
	Target 1	9.9	14.0	78	9	1097	0.180	(g)	
	Target 2	9.7	13.7	75	9	1248	0.183	(g)	

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Figure 23

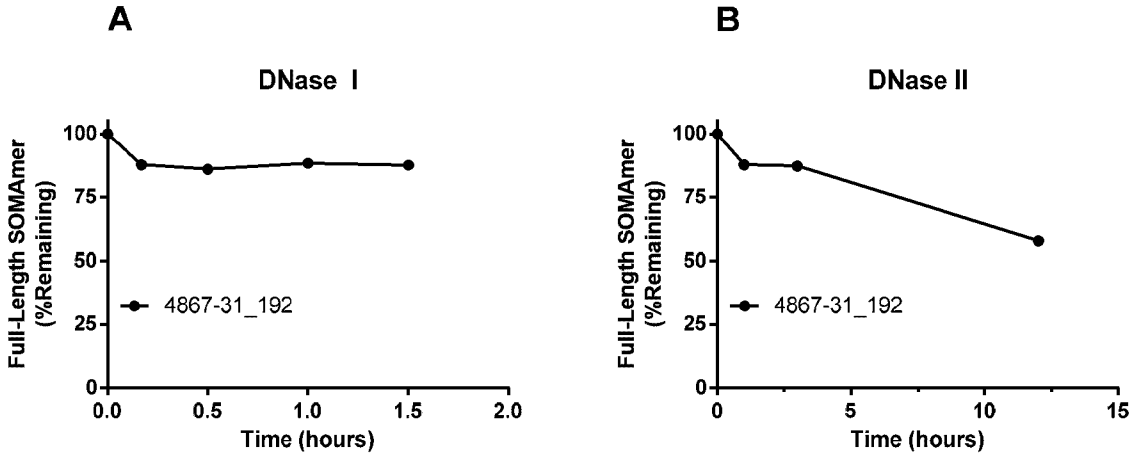

Figure 24

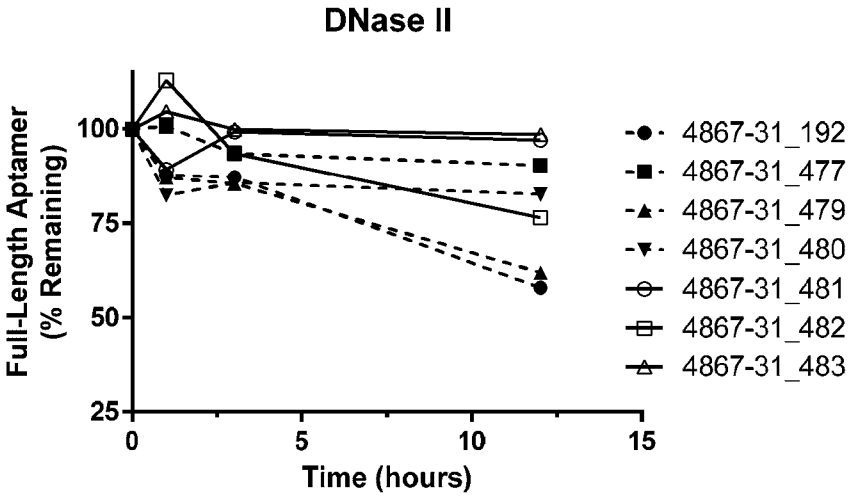

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Figure 25

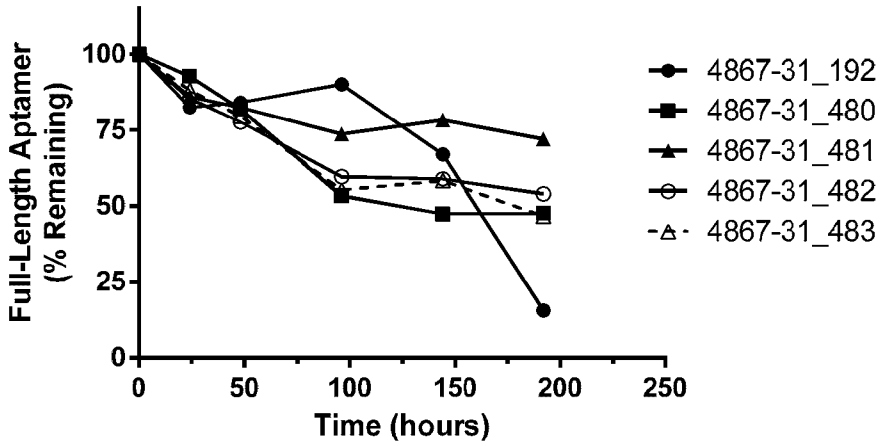

Figure 26

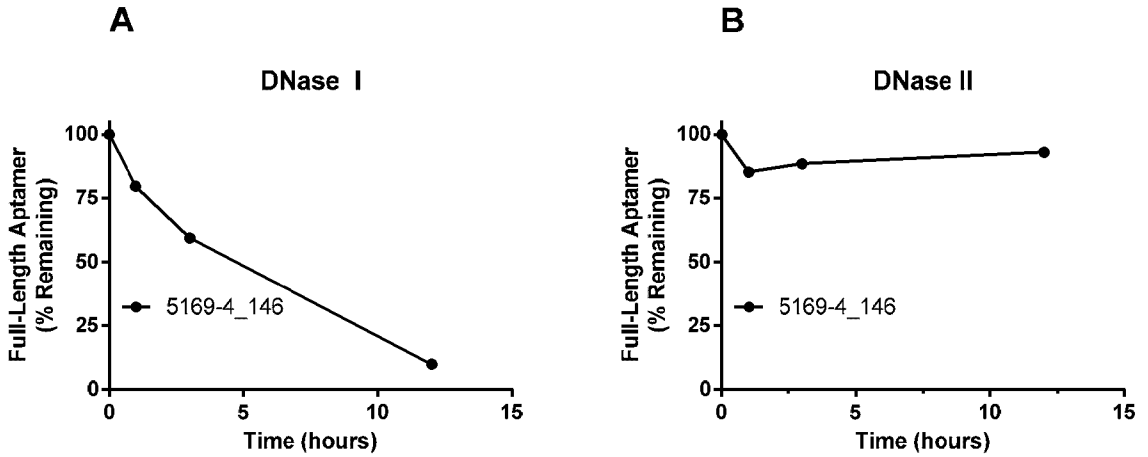

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Figure 27

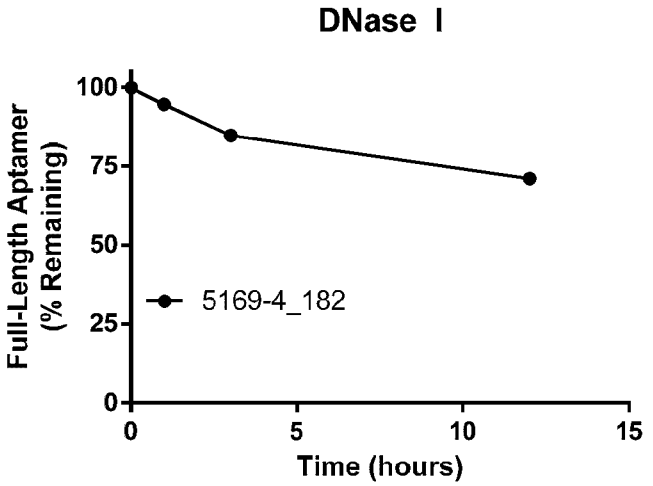

Figure 28

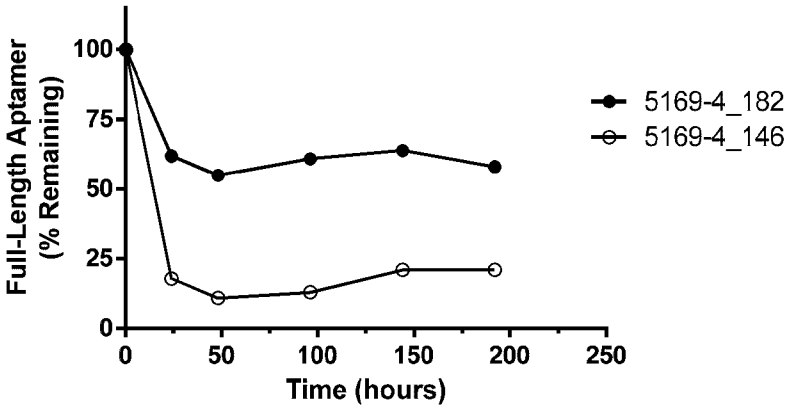

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Figure 29

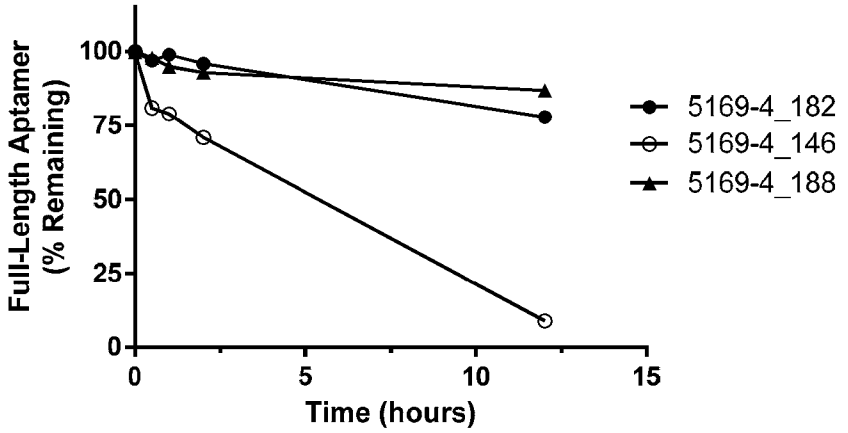
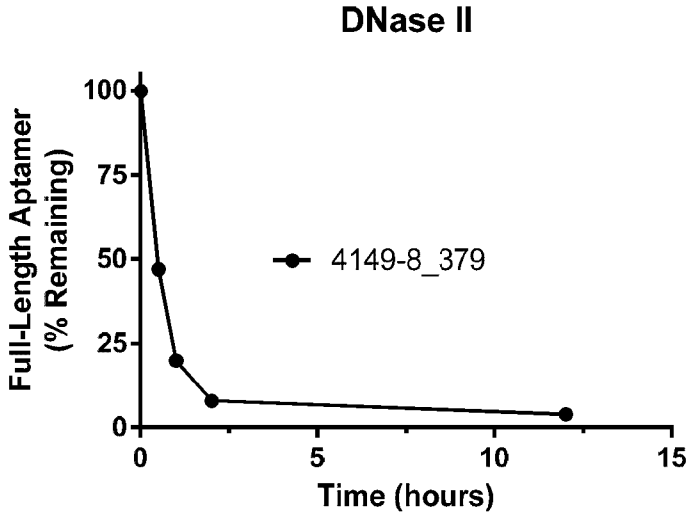


Figure 30



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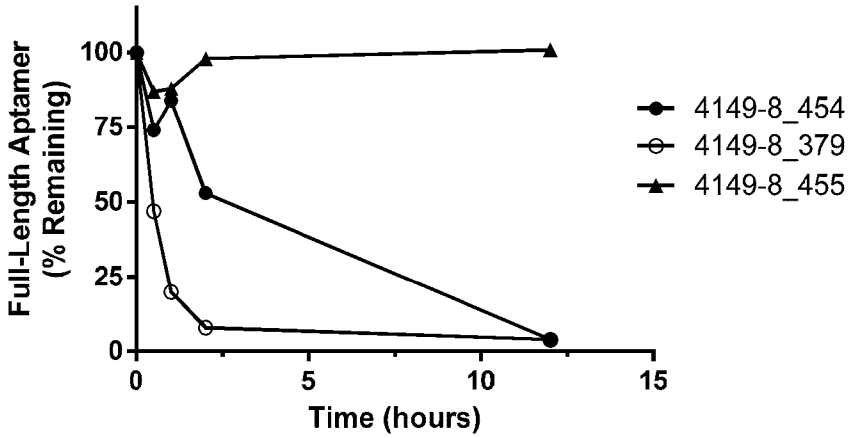

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