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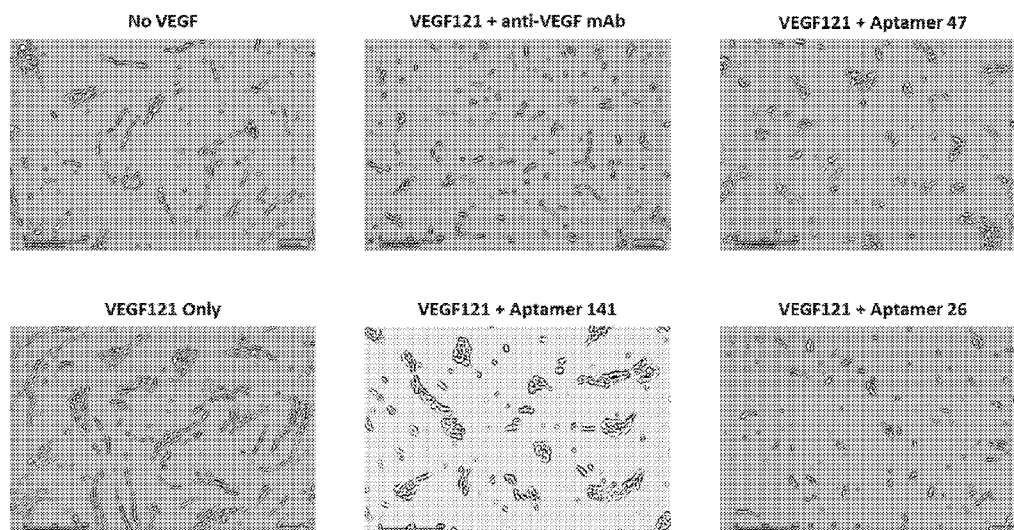


FIG. 16B

(57) Abstract: The application discloses methods and compositions for inhibiting functions associated with vascular endothelial growth factor-A (VEGF-A). The methods and compositions may involve the use of pan-variant specific aptamers for binding to VEGF-A, and preventing or reducing association of VEGF-A with Flt-1, KDR, or Nrp-1. The methods and compositions may include one or more aptamers that bind to receptor binding face of VEGF-A. The methods and compositions may include one or more aptamers that bind to a receptor binding domain of VEGF-A. The application further provides anti-VEGF-A aptamers for the treatment of ocular diseases or disorders. In some cases, the anti-VEGF-A aptamers may have a stem-loop secondary structure.



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STEM-LOOP COMPOSITIONS AND METHODS FOR INHIBITING VASCULAR ENDOTHELIAL GROWTH FACTOR

BACKGROUND OF THE INVENTION

[0001] Visual impairment is a national and global health concern that has a negative impact on physical and mental health. The number of people with visual impairment and blindness is increasing due to an overall aging population. Visual impairment and blindness can be caused by any one of a large number of eye diseases and disorders affecting people of all ages.

[0002] Vascular endothelial growth factor-A (VEGF-A) is thought to be the most significant regulator of angiogenesis in the VEGF family. VEGF-A promotes growth of vascular endothelial cells, which leads to the formation of capillary-like structures and may be necessary for the survival of newly formed blood vessels. VEGF-A is thought to play a role in various ocular diseases and disorders. Previous attempts at developing aptamers that inhibit VEGF-A have proven difficult because such aptamers have been unable to target multiple isoforms and variants of VEGF-A. Therefore, there is an un-met need for pan-variant specific aptamers that demonstrate high specificity and potency towards multiple isoforms and variants of VEGF-A. These needs may be met by the aptamers provided in the present disclosure.

SUMMARY OF THE INVENTION

[0003] In one aspect, an aptamer is provided having a nucleic acid sequence, wherein the aptamer comprises a stem-loop secondary structure which specifically binds to and inhibits at least one of VEGF-A₁₂₁ and VEGF-A₁₁₀. In some cases, the aptamer comprises up to five loops. In some cases, the aptamer comprises up to three stems. In some cases, the aptamer comprises up to one terminal stem. In some cases, the aptamer comprises up to two internal stems. In some cases, the aptamer comprises up to two terminal loops. In some cases, the aptamer comprises up to three internal loops. In some cases, the aptamer comprises, in a 5' to 3' direction: (i) a first side of a first base paired stem (S1); (ii) optionally, a first loop (L1); (iii) a first side of a second base paired stem (S2); (iv) a second loop (L2); (v) a second side of the second base paired stem (S2'); (vi) a third loop (L3); (vii) a first side of a third base paired stem (S3); (viii) a fourth loop (L4); (ix) a second side of the third base paired stem (S3'); (x) a fifth loop (L5); and (xi) a second side of the first base paired stem (S1'). In some cases, the S1' forms

at least one base pair with the S1. In some cases, the S1' forms from two to eight contiguous base pairs with the S1. In some cases, the S1' comprises from one to three nucleotides that are mismatched with the S1. In some cases, a 3' terminal nucleotide of the S1, and a 5' terminal nucleotide of the S1' form a base pair. In some cases, the base pair is C•G. In some cases, the base pair is separated from other base pairs in the S1 and the S1' by one or more mismatched nucleotides. In some cases, the S1 comprises a consensus nucleic acid sequence of 5'-HNBYHDCC-3', and the S1' comprises a consensus nucleic acid sequence of 5'-GKYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; K is G or U; V is A, C, or G; and W is A or U. In some cases, the S1 comprises a consensus nucleic acid sequence of 5'-HNBYHDNN-3', and the S1' comprises a consensus nucleic acid sequence of 5'-NNYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; and D is A, G, or U. In some cases, the L1 comprises up to one nucleotide. In some cases, the L1 comprises a consensus nucleic acid sequence of 5'-N*-3', wherein N* is A, C, G, U, a 3-carbon non-nucleotidyl spacer, two 3 carbon non-nucleotidyl spacers, a 6-carbon non-nucleotidyl spacer, or a 9-carbon non-nucleotidyl spacer. In some cases, the 3-carbon non-nucleotidyl spacer is 1,3-propanediol. In some cases, the 6-carbon non-nucleotidyl spacer is 1,6-hexanediol. In some cases, the 9-carbon non-nucleotidyl spacer is triethyleneglycol. In some cases, the S2' forms at least one base pair with the S2. In some cases, the S2' forms up to two base pairs with the S2. In some cases, the S2 comprises a consensus nucleic acid sequence of 5'-CC-3', and the S2' comprises a consensus nucleic acid sequence of 5'-GG-3'. In some cases, the S2 comprises a consensus nucleic acid sequence of 5'-NN-3', and the S2' comprises a consensus nucleic acid sequence of 5'-NN-3', where N is A, C, G, or U. In some cases, the L2 comprises up to four nucleotides. In some cases, the L2 comprises a consensus nucleic acid sequence of 5'-GCGC-3'. In some cases, the L2 comprises a consensus nucleic acid sequence of 5'-KNGC-3', where K is G or U; and N is A, C, G or U. In some cases, the S3' forms at least one base pair with the S3. In some cases, the S3' forms from four to six base pairs with the S3. In some cases, the S3' forms six base pairs with the S3, and the S3 has one mismatch nucleotide at a 3' terminal nucleotide of the S3. In some cases, the S3' forms four base pairs with the S3, and the L4 has six or eight nucleotides. In some cases, the S3' forms five base pairs with the S3, and the L4 has four or six nucleotides. In some cases, the S3' forms six base pairs with the S3, and the L4 has four nucleotides. In some cases, the S3' forms six base pairs with S3, the S3 has one mismatch

nucleotide at a 3' terminal nucleotide, and the L4 has three nucleotides. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GRGRWN3', and the S3' comprises a consensus nucleic acid sequence of 5'-NHYCYC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGRUN3', and the S3' comprises a consensus nucleic acid sequence of 5'-NWYCCC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGRUN3', and the S3' comprises a consensus nucleic acid sequence of 5'-NAYCCC-3', where R is A or G; N is A, C, G, or U; and Y is C or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGRUD-3', and the S3' comprises a consensus nucleic acid sequence of 5'-HWYCCC-3', wherein R is A or G; D is A, G, or U; H is A, C, or U; W is A or U; and Y is C or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GKGN-3', and the S3' comprises a consensus nucleic acid sequence of 5'-NSMC-3', where K is G or U; N is A, C, G or U and M is A or C. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGG-3', and the S3' comprises a consensus nucleic acid sequence of 5'-CCCC-3'. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGU-3', and the S3' comprises a consensus nucleic acid sequence of 5'-ACCC-3'. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGCU-3', and the S3' comprises a consensus nucleic acid sequence of 5'-AGCC-3'. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GBBNY-3', and the S3' comprises a consensus nucleic acid sequence of 5'-RNBNC-3', where B is C, G or U; R is A or G; N is A, C, G or U and Y is C or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGRU-3', and the S3' comprises a consensus nucleic acid sequence of 5'-AYCCC-3', where R is A or G; and Y is C or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-SVVVK-, and the S3' comprises a consensus nucleic acid sequence of 5'-MBBBS-3', where S is G or C; V is A, C, or G; K is G or U; M is A or C; and B is C, G, or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGGUD-3', and the S3' comprises a consensus nucleic acid sequence of 5'-HAUCCC-3', where D is A, G, or U; and H is A, C, or U. In some cases, the S3 comprises a consensus nucleic acid sequence of 5'-GGGRUUR-3', and the S3' comprises a consensus nucleic acid sequence of 5'-UAUCCC-3', where the underlined U is the single mismatched nucleotide, and R is A or G. In some cases, the L3 comprises up to one nucleotide. In

some cases, the L3 comprises a consensus nucleic acid sequence of 5'-A-3'. In some cases, the L3 comprises a consensus nucleic acid sequence of 5'-W-3', where W is A or U. In some cases, the L4 has three, four, six, or eight nucleotides. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-MAU-3', where M is A or C. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-CUA-3'. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-DNAH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U; or a consensus nucleic acid sequence of 5'-DNDH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U; or a consensus nucleic acid sequence of 5'-DNDN-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-UDNDHU-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-UDRGBU-3', where D is A, G, or U; R is A or G; N is A, C, G, or U; and B is G, C, or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-KNNNNW-3', where K is U or G; N is A, C, G, or U; and W is A, or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-UDUHRKYU-3', where D is A, G, or U; H is A, C, or U; R is A or G; K is G or U; and Y is C or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-UUUCAUUU-3'. In some cases, the L5 comprises up to four nucleotides. In some cases, the L5 comprises a consensus nucleic acid sequence of 5'-GYUU-3', where Y is C or U. In some cases, the L5 comprises a consensus nucleic acid sequence of GNNN-3', where N is A, C, G, or U. In some cases, the L5 comprises a consensus nucleic acid sequence of 5'-GNHW-3', where N is A, C, G, or U; H is A, C, or U; and W is A or U.

[0004] In another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGGGRUDDNDHHWYCCCGYUUGKYNKVNW-3' (**SEQ ID NO: 1**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G.

[0005] In yet another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-

HNBYHDCCUCCGCGCGGAGGGRUUDNDHUAYCCCGYUUGKYNKVNW-3' (**SEQ ID NO: 2**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; K is G or U; V is A, C, or G; and W is A or U.

[0006] In yet another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-

WNKYHDCCUCCGCGCGGAGGGGUDDNAHHAUCCCGUUUGGYBKMHW-3' (SEQ ID NO: 3), where W is A or U; N is A, C, G, or U; K is G or U; Y is C or U; H is A, C, or U; D is A, G, or U; B is C, G, or U; and M is A or C.

[0007] In yet another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-AUGCCGCCUCCGCGCGGAGGGGUUUCAUUCCCCGUUUGGCUGCAU-3' (SEQ ID NO: 4).

[0008] In another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-HNBYHDNNN*NNKNGCENNWGGGRUNDNDHNWYCCCGNNNNNNYNKVNW-3' (SEQ ID NO: 5), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; N* is A, C, G, U, deleted entirely, or a non-nucleotidyl spacer 3 modification, a 6 carbon alkyl linker (1,6-hexanediol), or a spacer 9 (triethyleneglycol) modification; D is A, G, or U; K is G or U; W is A or U; R is A or G; and V is A, C, or G.

[0009] In another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGDSBHDNNNNHNNBNCGYUUGKYNKVNW-3' (SEQ ID NO: 436), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G.

[0010] In another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGKBBHYDNNNKDBVCGYUUGKYNKVNW-3' (SEQ ID NO: 437), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G.

[0011] In another aspect, an aptamer is provided comprising a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGKSHUDRGBUDSMCGYUUGKYNKVNW-3' (SEQ ID NO: 438), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; M is A or C; and V is A, C, or G.

[0012] In some cases, less than 50% of pyrimidines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified pyrimidine. In some cases, less than 25% of pyrimidines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified pyrimidine. In some cases, less than 10% of pyrimidines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified pyrimidine. In some cases,

the nucleic acid sequence of any aptamer of the preceding does not comprise any C-5 modified pyrimidines. In some cases, the C-5 modified pyrimidine comprises a C-5 modified cytosine or a C-5 modified uridine. In some cases, the C-5 modified pyrimidine comprises a C-5 hydrophobic modification. In some cases, less than 100% of uridines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified uridine. In some cases, less than 50% of uridines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified uridine. In some cases, less than 25% of uridines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified uridine. In some cases, less than 10% of uridines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified uridine. In some cases, no uridines present in the nucleic acid sequence of any aptamer of the preceding comprise a C-5 modified uridine. In some cases, the C-5 modified uridine comprises a C-5 hydrophobic modification. In some cases, the C-5 modified uridine is selected from the group consisting of: 5-(N-benzylcarboxyamido)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamido)-2'-O-methyluridine, 5-(N-benzylcarboxyamido)-2'-fluorouridine, 5-(N-phenethylcarboxyamido)-2'-deoxyuridine (PEdU), 5-(N-thiophenylmethylcarboxyamido)-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxyamido)-2'-deoxyuridine (iBudU), 5-(N-tyrosylcarboxyamido)-2'-deoxyuridine (TyrdU), 5-(N-3,4-methylenedioxybenzylcarboxyamido)-2'-deoxyuridine (MBndU), 5-(N-4-fluorobenzylcarboxyamido)-2'-deoxyuridine (FBndU), 5-(N-3-phenylpropylcarboxyamido)-2'-deoxyuridine (PPdU), 5-(N-imidizolylethylcarboxyamido)-2'-deoxyuridine (ImdU), 5-(N-isobutylcarboxyamido)-2'-O-methyluridine, 5-(N-isobutylcarboxyamido)-2'-fluorouridine, 5-(N-tryptaminocarboxyamido)-2'-deoxyuridine (TrpdU), 5-(N-R-threoninylcarboxyamido)-2'-deoxyuridine (ThrdU), 5-(N-tryptaminocarboxyamido)-2'-O-methyluridine, 5-(N-tryptaminocarboxyamido)-2'-fluorouridine, 5-(N-[1-(3-trimethylamonium)propyl]carboxyamido)-2'-deoxyuridine chloride, 5-(N-naphthylmethylcarboxyamido)-2'-deoxyuridine (NapdU), 5-(N-naphthylmethylcarboxyamido)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamido)-2'-fluorouridine, 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamido)-2'-deoxyuridine), 5-(N-2-naphthylmethylcarboxyamido)-2'-deoxyuridine (2NapdU), 5-(N-2-naphthylmethylcarboxyamido)-2'-O-methyluridine, 5-(N-2-naphthylmethylcarboxyamido)-2'-fluorouridine, 5-(N-1-naphthylethylcarboxyamido)-2'-deoxyuridine (NEdU), 5-(N-1-naphthylethylcarboxyamido)-2'-O-methyluridine, 5-(N-1-

naphthylethylcarboxamide)-2'-fluorouridine, 5-(N-2-naphthylethylcarboxamide)-2'-deoxyuridine (2NEdU), 5-(N-2-naphthylethylcarboxamide)-2'-O-methyluridine, 5-(N-2-naphthylethylcarboxamide)-2'-fluorouridine, 5-(N-3-benzofuranylethylcarboxamide)-2'-deoxyuridine (BFdU), 5-(N-3-benzofuranylethylcarboxamide)-2'-O-methyluridine, 5-(N-3-benzofuranylethylcarboxamide)-2'-fluorouridine, 5-(N-3-benzothiophenylethylcarboxamide)-2'-deoxyuridine (BTdU), 5-(N-3-benzothiophenylethylcarboxamide)-2'-O-methyluridine, and 5-(N-3-benzothiophenylethylcarboxamide)-2'-fluorouridine. In some cases, the C-5 modified uridine is 5-(N-naphthylmethylcarboxamide)-2'-deoxyuridine (NapdU).

[0013] In some cases, any aptamer of the preceding selectively binds to a receptor binding face or receptor binding domain of VEGF-A₁₂₁ or VEGF-A₁₁₀. In some cases, the receptor binding domain comprises at least one of residues 1-109 of **SEQ ID NO: 6-10**. In some cases, the receptor binding domain comprises at least one of residues Phe17, Ile43, Ile46, Glu64, Gln79, Ile83, Lys84, Pro85, Arg82, His86, Asp63, and Glu67 of **SEQ ID NO: 6-10**. In some cases, any aptamer of the preceding inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis. In some cases, any aptamer of the preceding inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 25 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis. In some cases, any aptamer of the preceding inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 10 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis. In some cases, any aptamer of the preceding inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis. In some cases, any aptamer of the preceding inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis. In some cases, any aptamer of the preceding binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 50 nM as measured by surface plasmon resonance assay. In some cases, any aptamer of the preceding binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about

25 nM as measured by surface plasmon resonance assay. In some cases, any aptamer of the preceding binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 10 nM as measured by surface plasmon resonance assay. In some cases, any aptamer of the preceding binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 5 nM as measured by surface plasmon resonance assay. In some cases, any aptamer of the preceding binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 1 nM as measured by surface plasmon resonance assay. In some cases, any aptamer of the preceding selectively binds to and inhibits at least one of VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, any aptamer of the preceding inhibits or reduces an interaction of VEGF-A with KDR. In some cases, any aptamer of the preceding inhibits or reduces VEGF-A-induced KDR phosphorylation. In some cases, any aptamer of the preceding comprises RNA or sugar-modified RNA. In some cases, any aptamer of the preceding comprises DNA or sugar-modified DNA. In some cases, at least 50% of the nucleic acid sequence of any aptamer of the preceding comprises sugar-modified nucleotides. In some cases, 100% of the nucleic acid sequence of any aptamer of the preceding comprises sugar-modified nucleotides. In some cases, the sugar-modified nucleotides comprise a 2'F-modified nucleotide, a 2'OMe-modified nucleotide, or both. In some cases, the sugar-modified nucleotides are selected from the group consisting of: 2'F-G, 2'OMe-G, 2'OMe-U, 2'OMe-A, 2'OMe-C, and any combination thereof. In some cases, any aptamer of the preceding further comprises a 3' terminal inverted deoxythymidine. In some cases, any aptamer of the preceding comprises a nuclease-stabilized nucleic acid backbone. In some cases, the nucleic acid sequence of any aptamer of the preceding comprises from about 30 to about 90 nucleotides, wherein the nucleotides are unmodified nucleotides, modified nucleotides, or a combination of modified nucleotides and unmodified nucleotides. In some cases, any aptamer of the preceding is conjugated to a polyethylene glycol (PEG) molecule. In some cases, the PEG molecule has a molecular weight selected from the group consisting of: less than 5 kDa, less than 10 kDa, less than 20 kDa, less than 40 kDa, less than 60 kDa, and less than 80 kDa. In some cases, up to five nucleotides of any aptamer of the preceding comprise a phosphate-backbone modification. In some cases, the phosphate-backbone modification is a phosphorothioate substitution.

[0014] In another aspect, an aptamer is provided according to any aptamer described in Table 1 of the specification.

[0015] In another aspect, an aptamer is provided having at least 70% sequence identity with any aptamer described in Table 1 of the specification. In some cases, the aptamer has at least 70% modification identity with any aptamer described in Table 1 of the specification.

[0016] In some cases, any aptamer of the preceding is provided for use in treating an ocular disease or disorder in a subject in need thereof. In some cases, one or more symptoms of the ocular disease or disorder are treated.

[0017] In another aspect, a method is provided for treating an ocular disease or disorder in a subject in need thereof, comprising administering to the subject any aptamer of the preceding, thereby treating the ocular disease or disorder. In some cases, the ocular disease or disorder is selected from the group consisting of: diabetic retinopathy, retinopathy of prematurity, central retinal vein occlusion, macular edema, choroidal neovascularization, neovascular age-related macular degeneration, myopic choroidal neovascularization, punctate inner choroidopathy, ocular histoplasmosis syndrome, familial exudative vitreoretinopathy, and retinoblastoma. In some cases, the ocular disease or disorder exhibits elevated levels of VEGF-A.

[0018] In yet another aspect, any aptamer of the preceding is provided for use in a formulation of a medicament for treatment of an ocular disease or disorder.

[0019] In yet another aspect, any aptamer of the preceding is provided for use for treatment of an ocular disease or disorder.

[0020] In yet another aspect, a method is provided for modulating vascular endothelial growth factor-A (VEGF-A) in a biological system, the method comprising: administering to the biological system any aptamer of the preceding, thereby modulating VEGF-A in the biological system. In some cases, biological system comprises a biological tissue or biological cells. In some cases, the biological system is a subject. In some cases, the subject is a human. In some cases, the modulating comprises inhibiting a function associated with VEGF-A. In some cases, the modulating comprises preventing or reducing an association of VEGF-A with one or more of Flt-1, KDR, or Nrp-1.

INCORPORATION BY REFERENCE

[0021] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entireties to the same extent as if each individual

publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0023] **FIG. 1A** depicts a non-limiting example of an aptamer library suitable for screening for aptamers that target VEGF-A, according to embodiments of the disclosure.

[0024] **FIG. 1B** depicts a non-limiting example of a reverse oligonucleotide hybridized to a portion of the aptamer library sequence depicted in **FIG. 1A**, according to embodiments of the disclosure.

[0025] **FIG. 1C** depicts non-limiting examples of structures of modified nucleotides that may be used to generate an aptamer library suitable for the selection of anti-VEGF-A aptamers according to embodiments of the disclosure.

[0026] **FIG. 2A** and **FIG. 2B** depict non-limiting examples of flow cytometry data demonstrating the ability of various aptamer selection rounds to bind to bead-immobilized VEGF-A₁₂₁ according to embodiments of the disclosure.

[0027] **FIG. 2C** depicts non-limiting examples of flow cytometry data demonstrating the ability of various aptamer selection rounds fluorescently labeled by hybridization to a fluorescently labeled primer to bind to bead-immobilized VEGF-A₁₁₀ in a dose-dependent fashion according to embodiments of the disclosure.

[0028] **FIG. 2D** depicts non-limiting examples of flow cytometry data demonstrating the ability of various aptamer selection rounds fluorescently labeled by hybridization to a fluorescently labeled primer to bind to bead-immobilized VEGF-A₁₂₁ in a dose-dependent fashion according to embodiments of the disclosure.

[0029] **FIG. 2E** depicts non-limiting examples of flow cytometry data demonstrating the ability of various aptamers from round 6 of the aptamer selection to bind to bead-immobilized VEGF-A₁₂₁ in the presence of soluble VEGF-A₁₂₁ according to embodiments of the disclosure.

[0030] FIG. 3 depicts non-limiting examples of flow cytometry analysis of Aptamer 4.2 fluorescently labeled by chemical conjugation interacting with VEGF-A₁₆₅- and VEGF-A₁₂₁-functionalized beads in a dose-dependent fashion according to embodiments of the disclosure.

[0031] FIG. 4A depicts non-limiting examples of inhibition of VEGF-A₁₆₅ binding to KDR by Aptamer 26, compared to Aptamer 7 or an anti-VEGF-A mAb. Compounds were tested in a dose-dependent fashion to determine an IC₅₀ against each isoform according to embodiments of the disclosure.

[0032] FIG. 4B depicts non-limiting examples of inhibition of VEGF-A₁₂₁ binding to KDR by Aptamer 26, compared to Aptamer 7 or an anti-VEGF-A mAb. Compounds were tested in a dose-dependent fashion to determine an IC₅₀ against each isoform according to embodiments of the disclosure.

[0033] FIG. 5A depicts non-limiting examples of inhibition of VEGF-A₁₆₅-stimulated KDR phosphorylation by Aptamers 26, compared to Aptamer 7 or an anti-VEGF-A mAb. Compounds were tested in a dose-dependent fashion to determine an IC₅₀ against each isoform according to embodiments of the disclosure.

[0034] FIG. 5B depicts non-limiting examples of inhibition of VEGF-A₁₂₁-stimulated KDR phosphorylation by Aptamer 26, compared an anti-VEGF-A mAb. Compounds were tested in a dose-dependent fashion to determine an IC₅₀ against each isoform according to embodiments of the disclosure.

[0035] FIG. 6 depicts a representation of nucleotide conservation within the top 250 stacks of sequences from round 6 of the degenerate selection conducted on Aptamer 26 weighted by the total number of sequences represented according to embodiments of the disclosure.

[0036] FIG. 7 depicts an exemplary secondary structure and consensus sequence for the top 250 stacks of sequences from the degenerate selection along with sequence variations observed within each domain of the aptamer. Dashed boxes indicate the variable lengths of stem S3 and loop L4. The statistics for the frequency of observed base pairing at each position are as indicated.

[0037] FIG. 8 depicts competitive TR-FRET data demonstrating the relative affinity of linker-containing anti-VEGF-A aptamers according to embodiments of the disclosure. Data is represented as the log of fold change in IC₅₀ as compared to parent aptamer.

[0038] **FIG. 9** depicts competitive TR-FRET data demonstrating the relative affinity of anti-VEGF-A aptamers according to embodiments of the disclosure. Data is represented as the log of fold change in IC_{50} as compared to parent aptamer.

[0039] **FIG. 10** depicts competitive TR-FRET data demonstrating the relative affinity of anti-VEGF-A aptamers according to embodiments of the disclosure. Data is represented as the log of fold change in IC_{50} as compared to parent aptamer.

[0040] **FIG. 11** depicts competitive TR-FRET data demonstrating the relative affinity of anti-VEGF-A aptamers according to embodiments of the disclosure. Data is represented as the log of fold change in IC_{50} as compared to parent aptamer.

[0041] **FIG. 12A** depicts non-limiting examples of TR-FRET data demonstrating the affinity of Aptamers 26, 47, 141, and the anti-VEGF-A mAb for VEGF-A₁₆₅. Compounds were tested in a dose-dependent fashion to determine a K_d against each isoform according to embodiments of the disclosure.

[0042] **FIG. 12B** depicts non-limiting examples of TR-FRET data demonstrating the affinity of Aptamers 26, 47, 141, and the anti-VEGF-A mAb for VEGF-A₁₂₁. Compounds were tested in a dose-dependent fashion to determine a K_d against each isoform according to embodiments of the disclosure.

[0043] **FIG. 13A** depicts non-limiting examples of AlphaLISA[®] data demonstrating inhibition VEGF-A₁₆₅ binding to KDR by Aptamers 26, 47, or 141, compared to an anti-VEGF-A mAb. Compounds were tested in a dose-dependent fashion to determine an IC_{50} against each isoform according to embodiments of the disclosure.

[0044] **FIG. 13B** depicts non-limiting examples of AlphaLISA[®] data demonstrating inhibition VEGF-A₁₂₁ binding to KDR by Aptamers 26, 47, or 141, compared to an anti-VEGF-A mAb. Compounds were tested in a dose-dependent fashion to determine an IC_{50} against each isoform according to embodiments of the disclosure.

[0045] **FIG. 14** depicts non-limiting examples of AlphaLISA[®] data demonstrating inhibition of VEGF-A₁₆₅-stimulated KDR phosphorylation by Aptamers 26, 47, or 141, compared to an anti-VEGF-A mAb. Compounds were tested in a dose-dependent fashion to determine an IC_{50} against each isoform according to embodiments of the disclosure.

[0046] **FIG. 15A** depicts non-limiting examples of tube formation data demonstrating inhibition of VEGF-A₁₆₅-stimulated angiogenesis by Aptamers 26, 47, or 141, compared to an anti-VEGF-

A mAb. Aptamers were tested in a dose-dependent fashion to determine an IC_{50} against each isoform according to embodiments of the disclosure.

[0047] **FIG. 15B** depicts non-limiting examples of tube formation data demonstrating inhibition of VEGF-A₁₂₁-stimulated angiogenesis by Aptamers 26, 47, or 141, compared to an anti-VEGF-A mAb. Aptamers were tested in a dose-dependent fashion to determine an IC_{50} against each isoform according to embodiments of the disclosure.

[0048] **FIG. 16A** depicts non-limiting examples of representative images of the inhibition of VEGF-A₁₆₅-stimulated angiogenesis by Aptamers 26, 47, or 141, compared to an anti-VEGF-A mAb. Images depict the VEGF-A-induced and inhibition of VEGF-A-induced tube formation of GFP-HUVEC cells according to embodiments of the disclosure.

[0049] **FIG. 16B** depicts non-limiting examples of representative images of the inhibition of VEGF-A₁₂₁-stimulated angiogenesis by Aptamers 26, 47, or 141, compared to an anti-VEGF-A mAb. Images depict the VEGF-A-induced and inhibition of VEGF-A-induced tube formation of GFP-HUVEC cells according to embodiments of the disclosure.

[0050] **FIG. 17** depicts competitive TR-FRET data demonstrating the relative affinity of anti-VEGF-A aptamers according to embodiments of the disclosure. Data is represented as the log of fold change in IC_{50} as compared to parent aptamer.

[0051] **FIG. 18** depicts competitive TR-FRET data demonstrating the relative affinity of anti-VEGF-A aptamers according to embodiments of the disclosure. Data is represented as the log of fold change in IC_{50} as compared to parent aptamer.

[0052] **FIG. 19** depicts a non-limiting example of secondary structures of stem S3 and loop L4 of anti-VEGF-A aptamers according to embodiments of the disclosure.

[0053] **FIG. 20** depicts competitive TR-FRET data demonstrating the relative affinity of anti-VEGF-A aptamers according to embodiments of the disclosure. Data is represented as the log of fold change in IC_{50} as compared to parent aptamer.

DETAILED DESCRIPTION OF THE INVENTION

[0054] The disclosure herein provides aptamer compositions that selectively bind to and inhibit vascular endothelial growth factor-A (VEGF-A) and methods of using said aptamer compositions. In various aspects, an aptamer composition of the disclosure may comprise an

anti-VEGF-A aptamer that binds to one or more isoforms or variants of VEGF-A. In various aspects, an aptamer composition of the disclosure may comprise a pan-variant specific anti-VEGF-A aptamer that binds to each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, the anti-VEGF-A aptamers may bind to the receptor binding face of VEGF-A, or a portion thereof. In some cases, the anti-VEGF-A aptamers may bind to the receptor binding domain (RBD) of VEGF-A, or a portion thereof. In some cases, an aptamer of the disclosure does not bind to the heparin-binding domain (HBD) of VEGF-A. Without wishing to be bound by theory, anti-VEGF-A aptamers of the disclosure may prevent or reduce binding of VEGF-A to a VEGF receptor (VEGFR). For example, an anti-VEGF-A aptamer of the disclosure may prevent or reduce binding of VEGF-A to VEGFR1 (also known as Fms-related tyrosine kinase 1 (Flt1)), VEGFR2 (also known as Kinase insert domain receptor (KDR) or Flk-1), Neuropilin-1 (Nrp-1), or any combination thereof. In some cases, an anti-VEGF-A aptamer of the disclosure may inhibit a function associated with VEGF-A (*e.g.*, engaging a VEGF receptor, a signaling pathway downstream of VEGF-A, or both).

[0055] The disclosure herein further provides aptamer compositions having unique stem-loop secondary structures that selectively bind to and inhibit a function associated with one or more isoforms or variants of VEGF-A and methods of using such aptamer compositions. In some cases, the aptamers of the disclosure may have, in a 5' to 3' direction, a first side of a first base paired stem (*e.g.*, stem S1); optionally, a first loop (*e.g.*, loop L1); a first side of a second base paired stem (*e.g.*, stem S2); a second loop (*e.g.*, loop L2); a second, complementary side of the second base paired stem (*e.g.*, stem S2'); a third loop (*e.g.*, loop L3); a first side of a third base paired stem (*e.g.*, stem S3), a fourth loop (*e.g.*, loop L4), a second, complementary side of the third base paired stem (*e.g.*, stem S3'), a fifth loop (*e.g.*, loop L5), and a second, complementary side of the first base paired stem (*e.g.*, stem S1'). Put another way, an anti-VEGF-A aptamer of the disclosure may have the following stem and loop structure: 5'-S1-L1-S2-L2-S2'-L3-S3-L4-S3'-L5-S1'-3'. In some cases, an anti-VEGF-A aptamer of the disclosure may have the following stem and loop structure: 5'-S1-S2-L2-S2'-L3-S3-L4-S3'-L5-S1'-3'.

[0056] The aptamers disclosed herein may also include one or more further elements (*e.g.*, additional stem(s) or loop(s)). In some cases, additional elements (*e.g.*, additional stem(s), loop(s), one or more nucleotides, etc.) may be located before (*e.g.*, 5' side) the first side of the first base paired stem, after (*e.g.*, 3' side) the second, complementary side of the first base paired

stem, or both. In some cases, additional elements may be located interspersed between other elements of the aptamer. Additional elements may include additional stem structures, loop structures, non-nucleotidyl linkers, or any number of overhanging, unpaired nucleotides.

[0057] In some embodiments, each element may be adjacent to each other. For example, the anti-VEGF-A aptamers of the disclosure may have, in a 5' to 3' direction, a first side of a first base paired stem. The 3' terminal end of the first side of the first base paired stem may be connected to the 5' terminal end of the first loop. The first loop may be connected at its 5' terminal end to the 3' terminal end of the first side of the first base paired stem, and the first loop may be connected at its 3' terminal end to the 5' terminal end of the first side of the second base paired stem. The first side of the second base paired stem may be connected at its 5' terminal end to the 3' terminal end of the first loop, and the first side of the second base paired stem may be connected at its 3' terminal end to the 5' terminal end of the second loop. The second loop may be connected at its 5' terminal end to the 3' terminal end of the first side of the second base paired stem, and the second loop may be connected at its 3' terminal end to the 5' terminal end of the second, complementary side of the second base paired stem. The second, complementary side of the second base paired stem may be connected at its 5' terminal end to the 3' terminal end of the second loop, and the second, complementary side of the second base paired stem may be connected at its 3' terminal end to the 5' terminal end of the third loop. The third loop may be connected at its 5' terminal end to the 3' terminal end of the second, complementary side of the second base paired stem, and the third loop may be connected at its 3' terminal end to the 5' terminal end of the first side of the third base paired stem. The first side of the third base paired stem may be connected at its 5' terminal end to the 3' terminal end of the third loop, and the first side of the third base paired stem may be connected at its 3' terminal end to the 5' terminal end of the fourth loop. The fourth loop may be connected at its 5' terminal end to the 3' terminal end of the first side of the third base paired stem, and the fourth loop may be connected at its 3' terminal end to the 5' terminal end of the second, complementary side of the third base paired stem. The second, complementary side of the third base paired stem may be connected at its 5' terminal end to the 3' terminal end of the fourth loop, and the second, complementary side of the third base paired stem may be connected at its 3' terminal end to the 5' terminal end of the fifth loop. The fifth loop may be connected at its 5' terminal end to the 3' terminal end of the second, complementary side of the third base paired stem, and the fifth loop may be connected at its 3'

terminal end to the 5' terminal end of the second, complementary side of the first base paired stem. The second, complementary side of the first base paired stem may be connected at its 5' terminal end to the 3' terminal end of the fifth loop. In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a terminal stem. In some cases, the terminal stem may be the first base paired stem (*e.g.*, S1). In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a plurality of terminal loops. In some cases, a terminal loop may include the second loop (*e.g.*, L2), and/or the fourth loop (*e.g.*, L4). In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a plurality of internal stems. In some cases, an internal stem may include the second base paired stem (*e.g.*, S2), and/or the third base paired stem (*e.g.*, S3). In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a plurality of internal loops. In some cases, an internal loop may include the first loop (*e.g.*, L1), the third loop (*e.g.*, L3), and/or the fifth loop (*e.g.*, L5). Non-limiting examples of stem-loop aptamers that may be used to inhibit VEGF-A are described throughout.

[0058] As described above, in some cases, an anti-VEGF-A aptamer of the disclosure may have the following stem and loop structure: 5'-S1-L1-S2-L2-S2'-L3-S3-L4-S3'-L5-S1'-3'. In other cases, an anti-VEGF-A aptamer of the disclosure may have the following stem and loop structure: 5'-S1-S2-L2-S2'-L3-S3-L4-S3'-L5-S1'-3'. In some cases, S1/S1', S3/S3', L4, and/or L5 may comprise any combination of nucleotide sequences provided in Tables 10-13.

[0059] The disclosure further provides anti-VEGF-A aptamers comprising consensus nucleic acid sequences. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a first base paired stem having a consensus nucleic acid sequence of 5'-HNBYHDCC-3', and a second, complementary side of the first base paired stem having a consensus nucleic acid sequence of 5'-GKYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; K is G or U; V is A, C, or G, and W is A or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a first base paired stem having a consensus nucleic acid sequence of 5'-HNBYHDNN-3', and a second, complementary side of the first base paired stem having a consensus nucleic acid sequence of 5'-NNYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; K is G or U; V is A, C, or G, and W is A or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first loop having a nucleic acid sequence of 5'-U-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a nucleic acid sequence of 5'-N*-3', where N

is A, C, G, or U, a non-nucleotidyl spacer 3 modification (Sp3), two non-nucleotidyl spacer 3 modifications (Sp3-Sp3), a 6 carbon alkyl linker (1,6-hexanediol), a spacer 9 (triethyleneglycol) modification, or may be deleted. In some cases, the first loop is optional. In such cases, the aptamer may have the following structure: 5'-S1-S2-L2-S2'-L3-S3-L4-S3'-L5-S1'-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a second base paired stem having a consensus nucleic acid sequence of 5'-CC-3', and a second, complementary side of a second base paired stem having a consensus nucleic acid sequence of 5'-GG-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a second base paired stem having a consensus nucleic acid sequence of 5'-NN-3', and a second, complementary side of a second base paired stem having a consensus nucleic acid sequence of 5'-NN-3', where N is A, C, G, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a second loop having a nucleic acid sequence of 5'-GCGC-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a second loop having a consensus nucleic acid sequence of 5'-GYGC-3', where Y is C or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a second loop having a consensus nucleic acid sequence of 5'-KNGC-3', where K is G or U; and N is A, G, C, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a third loop having a nucleic acid sequence of 5'-A-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a third loop having a consensus nucleic acid sequence of 5'-W-3', where W is A or U.

[0060] In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a consensus nucleic acid sequence of 5'-GRGRWN-3', and a second, complementary side of a third base paired stem having a consensus nucleic acid sequence of 5'-NHYYC-3', where R is A or G; D is A, G, or U; H is A, C, or U; W is A or U; and Y is C or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GKGN-3', and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-NSMC-3' where K is G or U; N is A, C, G or U and M is A or C. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GBBNY-3' and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-RNBNC-3', where B is C, G or U; R is A or G; N is A, C, G or U and Y is C or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base

paired stem having a consensus nucleic acid sequence of 5'-GGGRUD-3', and a second, complementary side of a third base paired stem having a consensus nucleic acid sequence of 5'-HWYCCC-3', where R is A or G; D is A, G, or U; H is A, C, or U; W is A or U; and Y is C or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GGGG-3', and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-CCCC-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GGGU-3', and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-ACCC-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GGCU-3', and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-AGCC-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GGGRU-3' and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-AYCCC-3', where R is A or G; and Y is C or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GGGGUD-3', and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-HAUCCC-3', where D is A, G, or U; and H is A, C, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a nucleic acid sequence of 5'-GGGRUUR-3', and a second, complementary side of a third base paired stem having a nucleic acid sequence of 5'-UAUCCC-3', where R is A or G. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a consensus nucleic acid sequence of 5'-SVVVK-3', and a second, complementary side of a third base paired stem having a consensus nucleic acid sequence of 5'-MBBBS-3', where S is G or C; V is A, C, or G; K is G or U; M is A or C; and B is C, G, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a consensus nucleic acid sequence of 5'-GGGRUN'-3, and a second, complementary side of a third base paired stem having a consensus nucleic acid sequence of 5'-NWYCCC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a first side of a third base paired stem having a consensus nucleic acid sequence of 5'-GGGRUN'-3, and a second,

complementary side of a third base paired stem having a consensus nucleic acid sequence of 5'-NAYCCC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U.

[0061] In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fourth loop having a nucleic acid sequence of 5'-MAU-3', where M is A or C. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fourth loop having a nucleic acid sequence of 5'-DNAH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fourth loop having a nucleic acid sequence of 5'-DNDH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fourth loop having a nucleic acid sequence of 5'-UDNDHU-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fourth loop having a nucleic acid sequence of 5'-UUUCAUUU-3'. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fifth loop having a nucleic acid sequence of 5'-GYUU-3', where Y is C or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fifth loop having a consensus nucleic acid sequence of 5'-GNNN-3', where N is A, C, G, or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a fifth loop having a consensus nucleic acid sequence of 5'-GNHW-3', where N is A, C, G, or U; H is A, C, or U; and W is A or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGGGRUDDNDHHWYCCCGYUUGKYNKVNW-3' (**SEQ ID NO: 1**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGGGRUUDNDHUAYCCCGYUUGKYNKVNW-3' (**SEQ ID NO: 2**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; K is G or U; V is A, C, or G, and W is A or U. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-WNKYHDCCUCCGCGCGGAGGGGUDDNAHHAUCCCGUUUGGYBKMHW-3' (**SEQ ID NO: 3**), where W is A or U; N is A, C, G, or U; K is G or U; Y is C or U; H is A, C, or U; D is A, G, or U; B is C, G, or U; and M is A or C. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a nucleic acid sequence of 5'-AUGCCGCCUCCGCGCGGAGGGGUUUCAUUUCCCGUUUGGCUGCAU-3' (**SEQ ID**

NO: 4). In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-

HNBYHDNNN*NNKNGCNNWGGGRUNDNDHNWYCCCGNNNNNNYNKVNW-3' (**SEQ ID NO: 5**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; N* is A, C, G, U, can be deleted entirely, or is a non-nucleotidyl spacer 3 modification, a 6 carbon alkyl linker (1,6-hexanediol), or a spacer 9 (triethyleneglycol) modification; D is A, G, or U; K is G or U; W is A or U; R is A or G; and V is A, C, or G. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-

HNBYHDCCUCCGCGCGGAGDSBHDNNNNHNNBNCGYUUGKYNKVNW-3' (**SEQ ID NO: 436**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G.

[0062] In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-

HNBYHDCCUCCGCGCGGAGKKBHYDNNNKDBVCGYUUGKYNKVNW-3' (**SEQ ID NO: 437**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-

HNBYHDCCUCCGCGCGGAGKSHUDRGRBUDSMCGYUUGKYNKVNW -3' (**SEQ ID NO: 438**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; M is A or C; and V is A, C, or G.

[0063] The disclosure herein further provides methods for inhibiting VEGF-A (and/or a downstream signaling pathway of VEGF-A). In some cases, the methods include administering an anti-VEGF-A aptamer (or a composition comprising said aptamer) to a biological system (*e.g.*, biological cells, biological tissue, a subject, and the like). The disclosure further provides methods for treating ocular diseases or disorders including administering an anti-VEGF-A aptamer (or a pharmaceutical composition comprising said aptamer) to a subject having, suspected of having, or at risk of developing, an ocular disease or disorder. In some cases, the anti-VEGF-A aptamer is an aptamer having a stem-loop structure as described herein. In some cases, the ocular disease or disorder may be diabetic retinopathy. In some cases, the ocular disease or disorder may be retinopathy of prematurity. In some cases, the ocular disease or disorder may be central retinal vein occlusion. In some cases, the ocular disease or disorder may

be macular edema. In some cases, the ocular disease or disorder may be choroidal neovascularization. In some cases, the ocular disease or disorder may be neovascular (or wet) age-related macular degeneration. In some cases, the ocular disease or disorder may be myopic choroidal neovascularization. In some cases, the ocular disease or disorder may be punctate inner choroidopathy. In some cases, the ocular disease or disorder may be presumed ocular histoplasmosis syndrome. In some cases, the ocular disease or disorder may be familial exudative vitreoretinopathy. In some cases, the ocular disease or disorder may be retinoblastoma. In some cases, a subject having, suspected of having, or at risk of developing, an ocular disease or disorder may exhibit elevated levels of one or more variants or isoforms of VEGF-A. For example, a subject having, suspected of having, or at risk of developing, an ocular disease or disorder may exhibit elevated levels of one or more of VEGF-A₂₀₆, VEGF-A₁₈₉, VEGF-A₁₆₅, VEGF-A₁₂₁, and VEGF-A₁₁₀.

[0064] In some aspects of the disclosure, the methods and compositions may involve the inhibition of a function associated with VEGF-A. In some cases, the methods and compositions may involve preventing or reducing VEGF-A binding to or interaction with one or more VEGF receptors. For example, the methods and compositions may involve preventing or reducing VEGF-A binding to or interaction with Flt-1, KDR, Nrp-1, or any combination thereof. In some cases, the methods and compositions may involve preventing or reducing downstream signaling associated with Flt-1, KDR, Nrp-1, or any combination thereof. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of ocular diseases or disorders. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of diabetic retinopathy. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of retinopathy of prematurity. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of central retinal vein occlusion. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of macular edema. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of choroidal neovascularization. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of neovascular (or wet) age-related macular degeneration. In some cases, the methods and

compositions may involve the inhibition of a function associated with VEGF-A for the treatment of myopic choroidal neovascularization. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of punctate inner choroidopathy. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of presumed ocular histoplasmosis syndrome. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of familial exudative vitreoretinopathy. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of retinoblastoma. In some cases, the methods and compositions may involve the inhibition of a function associated with VEGF-A for the treatment of an ocular disease or disorder exhibiting elevated levels of one or more isoforms or variants of VEGF-A.

[0065] In various aspects, the compositions may include one or more aptamers that selectively bind to and inhibit a function associated with VEGF-A. In some cases, the compositions may include one or more aptamers that bind to the receptor binding face of VEGF-A. In some cases, the compositions may include one or more aptamers that bind to the receptor binding domain of VEGF-A. In some cases, the compositions may include one or more aptamers that bind to a region of VEGF-A other than the heparin binding domain of VEGF-A. Put another way, the compositions may include one or more aptamers that do not bind to the heparin binding domain of VEGF-A. In some cases, the compositions may include one or more aptamers that bind to one or more variants or isoforms of VEGF-A. For example, the compositions may include one or more aptamers that bind to one or more of VEGF-A₂₀₆, VEGF-A₁₈₉, VEGF-A₁₆₅, VEGF-A₁₂₁, and VEGF-A₁₁₀. In some cases, the compositions may include one or more pan-variant specific anti-VEGF-A aptamers. In particular cases, the compositions may include pan-variant specific aptamers that bind to each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. Generally, a pan-variant specific aptamer disclosed herein may bind to a structural feature of VEGF-A which is shared amongst VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, the structural feature is the receptor binding face or the receptor binding domain. In some cases, the aptamers have a stem-loop secondary structure as described herein.

[0066] In some cases, the compositions may include one or more aptamers that prevent or reduce binding of VEGF-A to Flt-1, KDR, Nrp-1, or any combination thereof. In some cases, the

compositions may include one or more aptamers that prevent or reduce downstream signaling pathways associated with Flt-1, KDR, Nrp-1, or any combination thereof. In some cases, the aptamers may be RNA aptamers, DNA aptamers, modified RNA aptamers, or modified DNA aptamers.

[0067] In some cases, the aptamers do not contain non-naturally occurring hydrophobic modifications. In some aspects, less than 100% of the pyrimidines (*e.g.*, C, T, or U) present in a nucleic acid sequence of an aptamer herein comprise a C-5 modified pyrimidine. For example, less than 100%, less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% of the pyrimidines present in a nucleic acid sequence of an aptamer herein comprise a C-5 modified pyrimidine. In particular aspects, none of the pyrimidines present in a nucleic acid sequence of an aptamer herein comprise a C-5 modified pyrimidine. In another particular aspect, none of the bases in an aptamer sequence herein comprise a C-5 modification. In some aspects, less than 100% of the uridines present in a nucleic acid sequence of an aptamer herein comprise a C-5 modified uridine. For example, less than 100%, less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, or less than 5% of the uridines present in a nucleic acid sequence of an aptamer herein comprise a C-5 modified uridine. In particular aspects, none of the uridines present in a nucleic acid sequence of an aptamer herein comprise a C-5 modified uridine. In some cases, the C-5 modified pyrimidine or C-5 modified uridine is selected from the group consisting of: 5-(N-benzylcarboxyamido)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamido)-2'-O-methyluridine, 5-(N-benzylcarboxyamido)-2'-fluorouridine, 5-(N-phenethylcarboxyamido)-2'-deoxyuridine (PEdU), 5-(N-thiophenylmethylcarboxyamido)-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxyamido)-2'-deoxyuridine (iBudU), 5-(N-tyrosylcarboxyamido)-2'-deoxyuridine (TyrdU), 5-(N-3,4-methylenedioxybenzylcarboxyamido)-2'-deoxyuridine (MBndU), 5-(N-4-fluorobenzylcarboxyamido)-2'-deoxyuridine (FBndU), 5-(N-3-phenylpropylcarboxyamido)-2'-deoxyuridine (PPdU), 5-(N-imidizolyethylcarboxyamido)-2'-deoxyuridine (ImdU), 5-(N-isobutylcarboxyamido)-2'-O-methyluridine, 5-(N-isobutylcarboxyamido)-2'-fluorouridine, 5-(N-tryptaminocarboxyamido)-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. In particular aspects, none of the aptamers provided herein comprise NapdU. In other particular cases, the aptamers are not SOMAmers.

[0068] In general, “sequence identity” refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. Two or more sequences (polynucleotide or amino acid) can be compared by determining their “percent identity.” The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the longer sequence and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA*, 87:2264-2268 (1990) and as discussed in Altschul, *et al.*, *J. Mol. Biol.*, 215:403-410 (1990); Karlin And Altschul,

Proc. Natl. Acad. Sci. USA, 90:5873-5877 (1993); and Altschul *et al.*, *Nucleic Acids Res.*, 25:3389-3402 (1997). The program may be used to determine percent identity over the entire length of the proteins being compared. Default parameters are provided to optimize searches with short query sequences in, for example, with the blastp program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen, *Computers and Chemistry* 17:149-163 (1993). Ranges of desired degrees of sequence identity are approximately 50% to 100% and integer values therebetween. In general, this disclosure encompasses sequences with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity with any sequence provided herein.

[0069] In general, “modification identity” refers to two polynucleotides with identical patterns of modifications on a nucleotide-to-nucleotide level. Techniques for determining modification identity may include determining the modifications of a polynucleotide and comparing these modifications to modifications of a second polynucleotide. The percent modification identity of two sequences is the number of exact modification matches between two aligned sequences divided by the length of the longer sequence and multiplied by 100. Ranges of desired degrees of modification identity are generally approximately 50% to 100%, and integer values therebetween. In general, this disclosure encompasses sequences with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% modification identity with any sequence provided herein.

[0070] As used herein, “consensus sequence”, when used in reference to a group or series of related nucleic acids, refers to a nucleotide sequence that reflects the most common choice of base at each position in the sequence where the series of related nucleic acids has been subjected to mathematical and/or sequence analysis. Unless otherwise indicated, nucleotide sequences provided herein are represented by standard nucleotide notation, as set forth by the International Union of Pure and Applied Chemistry (IUPAC). For example, the nucleotides typically found in DNA are represented by “A”, “C”, “G”, “T”; and the nucleotides typically found in RNA are represented by “A”, “C”, “G”, “U”. Nucleotide sequences provided herein may include one or more degenerate bases. A “degenerate base” generally refers to a position on a nucleotide sequence that can have more than one possible alternative. Degenerate bases are generally represented by a Roman character as set forth by the International Union of Pure and Applied

Chemistry (IUPAC). For example, the Roman character “D”, when used in relation to a nucleotide sequence, represents a degenerate base of A, G, or U.

[0071] The term “aptamer” as used herein refers to an oligonucleotide and/or nucleic acid analogues that can bind to a specific target molecule. Aptamers can include RNA, DNA, modified RNA, modified DNA, any nucleic acid analogue, and/or combinations thereof.

Aptamers can be single-stranded oligonucleotides. In some cases, aptamers may comprise more than one nucleic acid strand (*e.g.*, two or more nucleic acid strands). Aptamers may bind to a target (*e.g.*, a protein) with high affinity and specificity through non-Watson-Crick base pairing interactions. Generally, the aptamers described herein are non-naturally occurring oligonucleotides (*e.g.*, synthetically produced) that are isolated and used for the treatment of a disorder or a disease. Aptamers can bind to essentially any target molecule including, without limitation, proteins, oligonucleotides, carbohydrates, lipids, small molecules, and even bacterial cells. Aptamers may be monomeric (composed of a single unit) or multimeric (composed of multiple units). Multimeric aptamers can be homomeric (composed of multiple identical units) or heteromeric (composed of multiple non-identical units). Aptamers herein may be described by their primary structures, meaning the linear nucleotide sequence of the aptamer. Aptamer sequences herein are generally described from the 5' end to the 3' end, unless otherwise stated. Additionally or alternatively, aptamers herein may be described by their secondary structures which may refer to the combination of single-stranded regions and base-pairing interactions within the aptamer. Whereas many naturally occurring oligonucleotides, such as mRNA, encode information in their linear base sequences, aptamers generally do not encode information in their linear base sequences. Further, aptamers can be distinguished from naturally occurring oligonucleotides in that binding of aptamers to target molecules is dependent upon secondary and tertiary structures of the aptamer. Aptamers may be suitable as therapeutic agents and may be preferable to other therapeutic agents because: 1) aptamers may be fast and economical to produce because aptamers can be developed entirely by *in vitro* processes; 2) aptamers may have low toxicity and may lack an immunogenic response; 3) aptamers may have high specificity and affinity for their targets; 4) aptamers may have good solubility; 5) aptamers may have tunable pharmacokinetic properties; 6) aptamers may be amenable to site-specific conjugation of PEG and other carriers; and 7) aptamers may be stable at ambient temperatures.

[0072] An aptamer may have a secondary structure having at least two complementary regions of the same nucleic acid strand that base-pair to form a double helix (referred to herein as a “stem”). Generally, these complementary regions are complementary when read in the opposite direction. The term “stem” as used herein may refer to either of the complementary nucleotide regions individually or may encompass a base-paired region containing both complementary regions, or a portion thereof. For example, the term “stem” may refer to the 5’ side of the stem, that is, the stem sequence that is closer to the 5’ end of the aptamer; additionally or alternatively, the term “stem” may refer to the 3’ side of the stem, that is, the stem sequence that is closer to the 3’ end of the aptamer. In some cases, the term “stem” may refer to the 5’ side of the stem and the 3’ side of the stem, collectively. The term “base-paired stem” is generally used herein to refer to both complementary stem regions collectively. A base-paired stem may be perfectly complementary meaning that 100% of its base pairs are Watson-Crick base pairs. A base-paired stem may also be “partially complementary.” As used herein, the term “partially complementary stem” refers to a base-paired stem that is not entirely made up of Watson-Crick base pairs but does contain base pairs (either Watson-Crick base pairs or G-U/U-G wobble base pairs) at each terminus. In some cases, a partially complementary stem contains both Watson-Crick base-pairs and G-U/U-G wobble base pairs. In other cases, a partially complementary stem is exclusively made up of G-U/U-G wobble base pairs. A partially complementary stem may contain mis-matched base pairs and/or unpaired bases in the region between the base pairs at each terminus of the stem; but in such cases, the mis-matched base pairs and/or unpaired bases make up at most 50% of the positions between the base pairs at each terminus of the stem.

[0073] A stem as described herein may be referred to by the position, in a 5’ to 3’ direction on the aptamer, of the 5’ side of the stem (*e.g.*, the stem sequence closer to the 5’ terminus of the aptamer), relative to the 5’ side of additional stems present on the aptamer. For example, stem 1 (S1) may refer to the stem sequence that is closest to the 5’ terminus of the aptamer, its complementary stem sequence, or both stem sequences collectively. Similarly, stem 2 (S2) may refer to the next stem sequence that is positioned 3’ relative to S1, its complementary stem sequence, or both stem sequences collectively. Each additional stem may be referred to by its position, in a 5’ to 3’ direction, on the aptamer, as described above. For example, S3 may be positioned 3’ relative to S2 on the aptamer, S4 may be positioned 3’ relative to S3 on the aptamer, and so on. In some cases, the term “first stem” is used to refer to a stem in the aptamer,

irrespective of its location. For example, a first stem may be S1, S2, S3, S4 or any other stem in the aptamer. A stem may be adjacent to an unpaired region. An unpaired region may be present at a terminus of the aptamer or at an internal region of the aptamer.

[0074] As used herein, the term “loop” generally refers to an internal unpaired region of an aptamer. The term “loop” generally refers to any unpaired region of an aptamer that is flanked on both the 5' end and the 3' end by a stem region. In some cases, a loop sequence may be adjacent to a single base-paired stem, such that the loop and stem structure together resemble a hairpin. In such cases, generally the primary sequence of the aptamer contains a first stem sequence adjacent to the 5' end of the loop sequence and a second stem sequence adjacent to the 3' end of the loop sequence; and the first and second stem sequences are complementary to each other. In some cases, each terminus of a loop is adjacent to first and second stem sequences that are not complementary. In such cases, the primary sequence of the aptamer may contain an additional loop sequence that is bordered at one or both ends by stem sequences that are complementary to the first and/or second stem sequences. In cases where the two loops have different number of nucleotides, the two loops are referred to jointly herein as an “asymmetric loop” or “asymmetric loop pair,” terms that are used herein interchangeably. In cases where the two loops have the same number of nucleotides, they are referred to jointly as a “symmetric loop” or “symmetric loop pair,” terms that are used interchangeably herein.

[0075] A loop as described herein may be referred to by its position, in a 5' to 3' direction, on the aptamer. For example, loop 1 (L1) may refer to a loop sequence that is positioned most 5' on the aptamer. Similarly, loop 2 (L2) may refer to a loop sequence that is positioned 3' relative to L1, and loop 3 (L3) may refer to a loop sequence that is positioned 3' relative to L2. Each additional loop may be referred to by its position, in a 5' to 3' direction, on the aptamer, as described above. For example, L4 may be positioned 3' relative to L3 on the aptamer, L5 may be positioned 3' relative to L4 on the aptamer, and so on. In some cases, the term “first loop” is used to refer to a loop in the aptamer, irrespective of its location. For example, a first loop may be L1, L2, L3, L4 or any other loop in the aptamer.

[0076] The term “stem-loop” as used herein generally refers to the secondary structure of an aptamer of the disclosure having at least one stem and at least one loop. In some cases, a stem-loop secondary structure may include a terminal stem and a terminal loop. In some cases, a stem-loop secondary structure includes structures having more than one stem, and more than one

loop, which may include a terminal stem, at least one internal loop, at least one internal stem, and at least one terminal loop. A “terminal stem” as used herein generally refers to a stem that encompasses both the 5’ and the 3’ terminus of the aptamer. In some cases, a “terminal stem” is bordered at one or both termini by a “tail” comprising one or more unpaired nucleotides. For example, a terminal stem present in the aptamer may be bordered by a tail of one or more unpaired nucleotides (or other structures) at its 5’ end. Similarly, a terminal stem present in the aptamer may be bordered by a tail of one or more unpaired nucleotides (or other structures) at its 3’ end. In some cases, a terminal stem present in the aptamer may be bordered by a tail of one or more unpaired nucleotides (or other structures) at both its 5’ end and its 3’ end. A terminal stem may be adjacent to a loop; for example, the 5’ side of a terminal stem (*i.e.*, the terminal stem sequence closest to the 5’ end of the molecule) may be bordered at its 3’ terminus by the 5’ terminus of a loop. Similarly, the 3’ side of a terminal stem (*i.e.*, the terminal stem sequence closest to the 3’ end of the molecule) may be bordered at its 5’ terminus by the 3’ terminus of a loop. In some cases, the 5’ side of a terminal stem (*i.e.*, the terminal stem sequence closest to the 5’ end of the molecule) may be bordered at its 3’ terminus by the 5’ terminus of a loop, and the 3’ side of the terminal stem (*i.e.*, the terminal stem sequence closest to the 3’ end of the molecule) may be bordered at its 5’ terminus by the 3’ terminus of an internal stem. An “internal stem” as used herein may refer to a stem that is bordered at both termini by a loop sequence, or may refer to a stem that is bordered at one terminus by a loop sequence and bordered at the other terminus by a stem sequence. In some cases, a stem-loop secondary structure of the disclosure may include more than one internal stem. A “terminal loop” as used herein generally refers to a loop that is bordered by the same stem at both termini of the loop. For example, a terminal loop may be bordered at its 5’ end by a stem sequence, and may be bordered at its 3’ end by the complementary stem sequence. An “internal loop” as used herein generally refers to a loop that is bordered at both termini by different stems. For example, an internal loop may be bordered at its 5’ end by a first stem sequence, and may be bordered at its 3’ end by a second stem sequence that is not complementary to the first stem sequence. In some cases, a stem-loop secondary structure of the disclosure may include more than one internal loop. In some cases, a stem-loop secondary structure includes structures having more than two stems. Unless otherwise stated, when an aptamer includes more than one stem and/or more than one loop, the stems and loops

are numbered consecutively in ascending order from the 5' end to the 3' end of the primary nucleotide sequence.

[0077] The term “VEGF-A”, as used herein includes any variant or isoform of VEGF-A. Therefore, unless otherwise specified, “VEGF-A” may mean one or more of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆.

[0078] The term “pan-variant specific aptamer” as used herein refers to an aptamer that selectively binds to at least VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. A pan-variant specific aptamer may, but not necessarily, bind to one or more additional VEGF-A isoforms or variants. Generally, a pan-variant specific aptamer binds to a structural feature of VEGF-A which is common amongst VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆.

[0079] The terms “about” and “approximately” as used herein, generally refer to a range that is 15% greater than or less than a stated numerical value within the context of the particular usage. For example, “about 10” or “approximately 10” would include a range from 8.5 to 11.5.

[0080] As used herein, the term “or” is used nonexclusively to encompass “or” and “and.” For example, “A or B” includes “A but not B,” “B but not A,” and “A and B”, unless otherwise indicated.

[0081] “A”, “an”, and “the”, as used herein, can include plural referents unless expressly and unequivocally limited to one referent

Vascular endothelial growth factor-A (VEGF-A)

[0082] This disclosure generally provides compositions that bind to vascular endothelial growth factor-A (VEGF-A), and methods of using such compositions to modulate VEGF-A signaling pathways. VEGF-A is thought to be the most significant regulator of angiogenesis in the VEGF family. VEGF-A promotes growth of vascular endothelial cells which leads to the formation of capillary-like structures and may be necessary for the survival of newly formed blood vessels. Vascular endothelial cells are thought to be major effectors of VEGF signaling. Retinal pigment epithelial (RPE) cells may also express VEGF receptors and have been shown to proliferate and migrate upon exposure to VEGF. In addition, VEGF is thought to play roles beyond the vascular system. For example, VEGF may play roles in normal physiological functions, including, but not limited to, bone formation, hematopoiesis, wound healing, and development. In various aspects, the compositions provided herein include aptamers that bind to VEGF-A, thereby

inhibiting or reducing angiogenesis, *e.g.*, by inhibiting or preventing growth of vascular endothelial cells, retinal pigment epithelial cells, or both. In some cases, the anti-VEGF-A aptamers provided herein may prevent or reduce binding or association of VEGF-A with a VEGF receptor (*e.g.*, Flt-1, KDR, Nrp-1) expressed on vascular endothelial cells, retinal pigment epithelial cells, or both.

[0083] The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and placental growth factor (PlGF). The aptamers disclosed herein primarily bind to variants and isoforms of VEGF-A. In some cases, such aptamers may also bind to one or more of VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PlGF. Transcription of VEGF mRNA may be upregulated under hypoxic conditions. Furthermore, various growth factors and cytokines have been shown to upregulate VEGF mRNA expression, including, without limitation, epidermal growth factor (EGF), transforming growth factor- α (TGF- α), transforming growth factor- β (TGF- β), keratinocyte growth factor (KGF), insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), interleukin 1- α (IL-1- α), interleukin-6 (IL-6), and interleukin-8 (IL8). VEGF-A is thought to play a role in various ocular diseases and disorders such as, but not limited to, diabetic retinopathy, retinopathy of prematurity, central retinal vein occlusion, macular edema, choroidal neovascularization, neovascular (or wet) age-related macular degeneration, myopic choroidal neovascularization, punctate inner choroidopathy, presumed ocular histoplasmosis syndrome, familial exudative vitreoretinopathy, and retinoblastoma.

[0084] In some cases, the aptamers provided herein may be used to treat an ocular disease or disorder involving one or more factors that upregulate VEGF-A expression and/or activity, including, but not limited to, hypoxic conditions; a growth factor such as EGF, TGF- α , TGF- β , KGF, IGF-1, FGF, or PDGF; and a cytokine such as IL-1- α , IL6, and IL8. In some cases, the aptamers provided herein may be used to treat an ocular disease or disorder selected from the group consisting of: diabetic retinopathy, retinopathy of prematurity, central retinal vein occlusion, macular edema, choroidal neovascularization, neovascular (or wet) age-related macular degeneration, myopic choroidal neovascularization, punctate inner choroidopathy, presumed ocular histoplasmosis syndrome, familial exudative vitreoretinopathy, and retinoblastoma.

[0085] The gene for human VEGF-A contains eight exons and encodes at least 16 isoforms. The most common isoforms generated by alternative splicing mechanisms are VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. Of these, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆ each contain a C-terminal heparin binding domain (HBD). In contrast, VEGF-A₁₂₁ lacks a heparin-binding domain. Furthermore, plasmin activation may result in proteolytic cleavage of VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆, resulting in the release of the soluble VEGF-A₁₁₀ variant, which also lacks a heparin-binding domain.

[0086] In various aspects, the aptamers provided herein may bind to and inhibit a function associated with one or more VEGF-A isoforms or variants. For example, the aptamers provided herein may bind to and inhibit a function associated with one or more of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, the aptamers provided herein may be pan-variant specific aptamers. In some cases, a pan-variant specific aptamer may bind to each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, the aptamers provided herein may bind to a structural feature that is common to each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. For example, the aptamers provided herein may bind to the receptor binding face, or a portion thereof, of each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In another example, the aptamers provided herein may bind to the receptor binding domain, or a portion thereof, of each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, the aptamers provided herein may bind to a structural feature of VEGF-A other than the heparin binding domain found in VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆.

[0087] VEGF-A is known to interact with the receptor tyrosine kinases VEGFR1 (also known as Flt-1), VEGFR2 (also known as KDR or Flk-1), and Neuropilin-1 (Nrp-1). Nrp-1 is thought to be a co-receptor for KDR. VEGF receptors have been shown to be expressed by endothelial cells, macrophages, hematopoietic cells, and smooth muscle cells. KDR is a class IV receptor tyrosine kinase that binds 2:1 to VEGF-A dimers. Flt-1 is a receptor tyrosine kinase that binds to VEGF-A with a 3-10 fold higher affinity than KDR, and has also been shown to bind to VEGF-B and PlGF. Flt-1 expression may be upregulated by hypoxia, and its affinity for VEGF-A has been proposed as a negative regulator of signaling by KDR by acting as a decoy receptor. An alternative splicing variant of Flt-1 results in a soluble variant of the receptor (sFlt-1) which has been suggested to act as an anti-angiogenic sink for VEGF-A. Association of VEGF-A₁₆₅ with

KDR may be enhanced by the interaction of the heparin binding domain with co-receptor Nrp-1, which may enhance downstream signaling of KDR. Nrp-1 also has strong affinity for Flt-1, which may prevent Nrp-1 association with VEGF-A₁₆₅ and may be a secondary regulatory mechanism for VEGF-A induced angiogenesis.

[0088] In various aspects, aptamers provided herein may bind to one or more isoforms or variants of VEGF-A, and may prevent or reduce binding or association of VEGF-A with a VEGF receptor. For example, aptamers provided herein may prevent or reduce binding of one or more isoforms or variants of VEGF-A with Flt-1, KDR, Nrp-1, or any combination thereof. In some cases, aptamers provided herein may prevent or reduce binding of one or more of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆ to one or more of Flt-1, KDR, and Nrp-1. In particular cases, aptamers provided herein may prevent or reduce binding of one or more isoforms or variants of VEGF-A to KDR. In some aspects, the aptamers are pan-variant specific aptamers that bind to each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆, and reduce or prevent binding or association thereof with one or more of Flt-1, KDR, and Nrp-1.

[0089] In one instance, an amino acid sequence of human VEGF-A₂₀₆ may comprise the following sequence:

APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGG
CCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDRARQEKKSV
RGKGKGQKRKRKKSRYKSWSVYVGARCCLMPWSLPGPHPCGPCSERRKHLFVQDPQT
CKCCKNTDSRCKARQLELNERTCRCDKPRR (SEQ ID NO: 6).

[0090] In one instance, an amino acid sequence of human VEGF-A₁₈₉ may comprise the following sequence:

APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGG
CCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDRARQEKKSV
RGKGKGQKRKRKKSRYKSWSVPCGPCSERRKHLFVQDPQTCKCCKNTDSRCKARQL
ELNERTCRCDKPRR (SEQ ID NO: 7)

[0091] In one instance, an amino acid sequence of human VEGF-A₁₆₅ may comprise the following sequence:

APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGG

CCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDRARQENPCG
PCSEERRKHLFVQDPQTCKCCKNTDSRCKARQLELNERTCRCDKPRR (SEQ ID NO: 8)

[0092] In one instance, an amino acid sequence of human VEGF-A₁₂₁ may comprise the following sequence:

APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGG
CCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDRARQEKCDK
PRR (SEQ ID NO: 9)

[0093] In one instance, an amino acid sequence of human VEGF-A₁₁₀ may comprise the following sequence:

APMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGG
CCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDR (SEQ ID
NO: 10)

Aptamers

[0094] In some cases, the methods and compositions described herein include the use of one or more aptamers for the treatment of an ocular disease or disorder. In some cases, the methods and compositions described herein use one or more aptamers having a secondary structure as described herein for the treatment of an ocular disease or disorder. In some cases, the methods and compositions described herein include the use of one or more aptamers for inhibiting an activity associated with VEGF-A. In some cases, the methods and compositions described herein include the use of one or more aptamers having a secondary structure as described herein for inhibiting an activity associated with VEGF-A.

[0095] Aptamers as described herein may include any number of modifications that can affect the function or affinity of the aptamer. For example, aptamers may be unmodified or they may contain modified nucleotides to improve stability, nuclease resistance or delivery characteristics. Examples of such modifications may include chemical substitutions at the sugar and/or phosphate and/or base positions, for example, at the 2' position of ribose, the 5 position of pyrimidines, and the 8 position of purines, various 2'-modified pyrimidines and purines and modifications with 2'-amino (2'-NH₂), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe) substituents. In some cases, aptamers described herein comprise a 2'-OMe and/or a 2'-F modification to increase *in vivo* stability. In some cases, the aptamers described herein contain modified nucleotides to improve the affinity and specificity of the aptamers for a target. Examples of

modified nucleotides include those modified with guanidine, indole, amine, phenol, hydroxymethyl, or boronic acid. In other cases, pyrimidine nucleotide triphosphate analogs or CE-phosphoramidites may be modified at the 5 position to generate, for example, 5-benzylaminocarbonyl-2'-deoxyuridine (BndU); 5-[N-(phenyl-3-propyl)carboxamide]-2'-deoxyuridine (PPdU); 5-(N-thiophenylmethylcarboxamide)-2'-deoxyuridine (ThdU); 5-(N-4-fluorobenzylcarboxamide)-2'-deoxyuridine (FBndU); 5-(N-(1-naphthylmethyl)carboxamide)-2'-deoxyuridine (NapdU); 5-(N-2-naphthylmethylcarboxamide)-2'-deoxyuridine (2NapdU); 5-(N-1-naphthylethylcarboxamide)-2'-deoxyuridine (NEdU); 5-(N-2-naphthylethylcarboxamide)-2'-deoxyuridine (2NEdU); 5-(N-tryptaminocarbonyl)-2'-deoxyuridine (TrpdU); 5-isobutylaminocarbonyl-2'-deoxyuridine (IbdU); 5-(N-tyrosylcarboxamide)-2'-deoxyuridine (TyrdU); 5-(N-isobutylaminocarbonyl)-2'-deoxyuridine (iBudU); 5-(N-benzylcarboxamide)-2'-O-methyluridine, 5-(N-benzylcarboxamide)-2'-fluorouridine, 5-(N-phenethylcarboxamide)-2'-deoxyuridine (PEdU), 5-(N-3,4-methylenedioxybenzylcarboxamide)-2'-deoxyuridine (MBndU), 5-(N-imidazolylethylcarboxamide)-2'-deoxyuridine (ImdU), 5-(N-isobutylcarboxamide)-2'-O-methyluridine, 5-(N-isobutylcarboxamide)-2'-fluorouridine, 5-(N--R-threoninylcarboxamide)-2'-deoxyuridine (Thrdu), 5-(N-tryptaminocarbonyl)-2'-O-methyluridine, 5-(N-tryptaminocarbonyl)-2'-fluorouridine, 5-(N-[1-(3-trimethylammonium)propyl]carboxamide)-2'-deoxyuridine chloride, 5-(N-naphthylmethylcarboxamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxamide)-2'-fluorouridine, 5-(N-[1-(2,3-dihydroxypropyl)]carboxamide)-2'-deoxyuridine), 5-(N-2-naphthylmethylcarboxamide)-2'-O-methyluridine, 5-(N-2-naphthylmethylcarboxamide)-2'-fluorouridine, 5-(N-1-naphthylethylcarboxamide)-2'-O-methyluridine, 5-(N-1-naphthylethylcarboxamide)-2'-fluorouridine, 5-(N-2-naphthylethylcarboxamide)-2'-O-methyluridine, 5-(N-2-naphthylethylcarboxamide)-2'-fluorouridine, 5-(N-3-benzofuranylethylcarboxamide)-2'-deoxyuridine (BFdU), 5-(N-3-benzofuranylethylcarboxamide)-2'-O-methyluridine, 5-(N-3-benzofuranylethylcarboxamide)-2'-fluorouridine, 5-(N-3-benzothiophenylethylcarboxamide)-2'-deoxyuridine (BTdU), 5-(N-3-benzothiophenylethylcarboxamide)-2'-O-methyluridine, 5-(N-3-benzothiophenylethylcarboxamide)-2'-fluorouridine; 5-[N-(1-morpholino-2-ethyl)carboxamide]-2'-deoxyuridine (MOEdU); R-tetrahydrofuranylmethyl-2'-deoxyuridine (RTMdU); 3-methoxybenzyl-2'-deoxyuridine (3MBndU); 4-methoxybenzyl-2'-deoxyuridine

(4MBndU); 3,4-dimethoxybenzyl-2'-deoxyuridine (3,4DMBndU); S-tetrahydrofuranylmethyl-2'-deoxyuridine (STMdU); 3,4-methylenedioxyphenyl-2-ethyl-2'-deoxyuridine (MPedU); 4-pyridinylmethyl-2'-deoxyuridine (PyrdU); or 1-benzimidazol-2-ethyl-2'-deoxyuridine (BidU); 5-(amino-1-propenyl)-2'-deoxyuridine; 5-(indole-3-acetamido-1-propenyl)-2'-deoxyuridine; or 5-(4-pivaloylbenzamido-1-propenyl)-2'-deoxyuridine.

[0096] Modifications of the aptamers contemplated in this disclosure include, without limitation, those which provide other chemical groups that incorporate additional charge, polarizability, hydrophobicity, hydrogen bonding, electrostatic interaction, and functionality to the nucleic acid aptamer bases or to the nucleic acid aptamer as a whole. Modifications to generate oligonucleotide populations that are resistant to nucleases can also include one or more substitute internucleotide linkages, altered sugars, altered bases, or combinations thereof. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil; backbone modifications, phosphorothioate, phosphorodithioate, or alkyl phosphate modifications, methylations, and unusual base-pairing combinations such as the isobases isocytidine and isoguanosine. Modifications can also include 3' and 5' modifications such as capping, *e.g.*, addition of a 3'-3'-dT cap to increase exonuclease resistance, or conjugation of a PEG to the 5' or 3' end to increase exonuclease and endonuclease resistance.

[0097] Aptamers of the disclosure may generally comprise nucleotides having ribose in the β -D-ribofuranose configuration. In some cases, 100% of the nucleotides present in the aptamer have ribose in the β -D-ribofuranose configuration. In some cases, at least 50% of the nucleotides present in the aptamer have ribose in the β -D-ribofuranose configuration. In some cases, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the nucleotides present in the aptamer have ribose in the β -D-ribofuranose configuration.

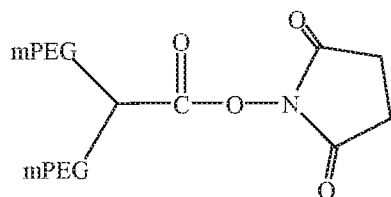
[0098] The length of the aptamer can be variable. In some cases, the length of the aptamer is less than 100 nucleotides. In some cases, the length of the aptamer is greater than 10 nucleotides. In some cases, the length of the aptamer is between 10 and 90 nucleotides. The aptamer can be, without limitation, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about

45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, or about 90 nucleotides in length.

[0099] In some instances, a polyethylene glycol (PEG) polymer chain is covalently bound to the aptamer, referred to herein as PEGylation. Without wishing to be bound by theory, PEGylation may increase the half-life and stability of the aptamer in physiological conditions. In some cases, the PEG polymer is covalently bound to the 5' end of the aptamer. In some cases, the PEG polymer is covalently bound to the 3' end of the aptamer. In some cases, the PEG polymer is covalently bound to both the 5' end and the 3' end of the aptamer. In some cases, the PEG polymer is covalently bound to a specific site on a nucleobase within the aptamer, including the 5-position of a pyrimidine or 8-position of a purine. In some cases, the PEG polymer is covalently bound to an abasic site within the aptamer.

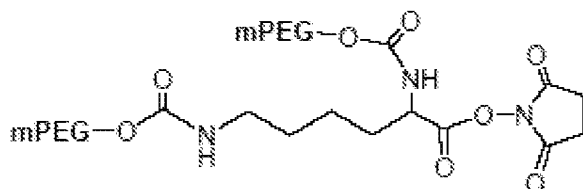
[00100] In some cases, an aptamer described herein may be conjugated to a PEG having the general formula, $H-(O-CH_2-CH_2)_n-OH$. In some cases, an aptamer described herein may be conjugated to a methoxy-PEG (mPEG) of the general formula, $CH_3O-(CH_2-CH_2-O)_n-H$. In some cases, the aptamer is conjugated to a linear chain PEG or mPEG. The linear chain PEG or mPEG may have an average molecular weight of up to about 30 kD. Multiple linear chain PEGs or mPEGs can be linked to a common reactive group to form multi-arm or branched PEGs or mPEGs. For example, more than one PEG or mPEG can be linked together through an amino acid linker (*e.g.*, lysine) or another linker, such as glycerine. In some cases, the aptamer is conjugated to a branched PEG or branched mPEG. Branched PEGs or mPEGs may be referred to by their total mass (*e.g.*, two linked 20kD mPEGs have a total molecular weight of 40kD). Branched PEGs or mPEGs may have more than two arms. Multi-arm branched PEGs or mPEGs may be referred to by their total mass (*e.g.*, four linked 10 kD mPEGs have a total molecular weight of 40 kD). In some cases, an aptamer of the present disclosure is conjugated to a PEG polymer having a total molecular weight from about 5 kD to about 200 kD, for example, about 5 kD, about 10 kD, about 20 kD, about 30 kD, about 40 kD, about 50 kD, about 60 kD, about 70 kD, about 80 kD, about 90 kD, about 100 kD, about 110 kD, about 120 kD, about 130 kD, about 140 kD, about 150 kD, about 160 kD, about 170 kD, about 180 kD, about 190 kD, or about 200 kD. In one non-limiting example, the aptamer is conjugated to a PEG having a total molecular weight of about 40 kD.

[00101] In some cases, the reagent that may be used to generate PEGylated aptamers is a branched PEG N-Hydroxysuccinimide (mPEG-NHS) having the general formula:

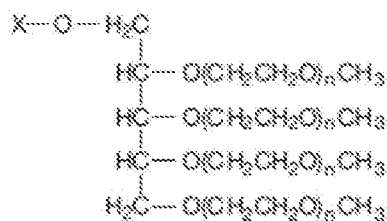


with a 20 kD, 40 kD or 60 kD total molecular weight (*e.g.*, where each mPEG is about 10kD, 20 kD or about 30 kD). As described above, the branched PEGs can be linked through any appropriate reagent, such as an amino acid (*e.g.*, lysine or glycine residues).

[00102] In one non-limiting example, the reagent used to generate PEGylated aptamers is [N²-(monomethoxy 20K polyethylene glycol carbamoyl)-N⁶-(monomethoxy 20K polyethylene glycol carbamoyl)]-lysine N-hydroxysuccinimide having the formula:

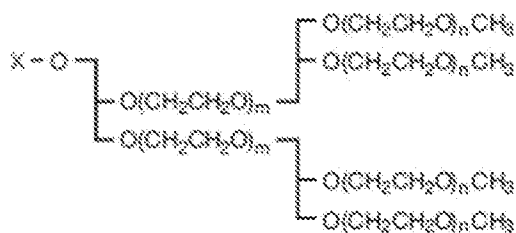


[00103] In yet another non-limiting example, the reagent used to generate PEGylated aptamers has the formula:



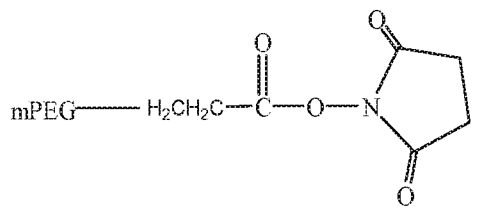
[00104] where X is N-hydroxysuccinimide and the PEG arms are of approximately equivalent molecular weight. Such PEG architecture may provide a compound with reduced viscosity compared to a similar aptamer conjugated to a two-armed or single-arm linear PEG.

[00105] In some examples, the reagent used to generate PEGylated aptamers has the formula:



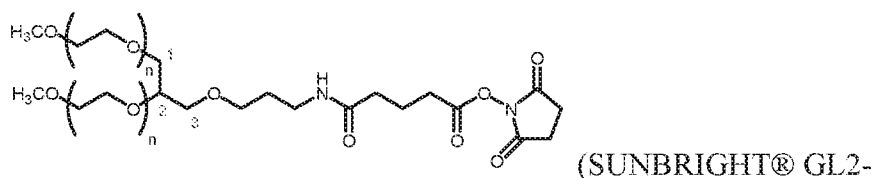
where X is N-hydroxysuccinimide and the PEG arms are of different molecular weights, for example, a 40 kD PEG of this architecture may be composed of 2 arms of 5 kD and 4 arms of 7.5 kD. Such PEG architecture may provide a compound with reduced viscosity compared to a similar aptamer conjugated to a two-armed PEG or a single-arm linear PEG.

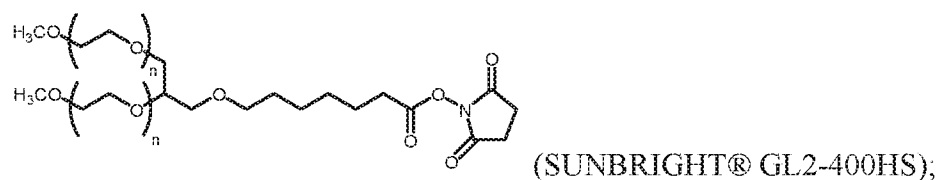
[00106] In some cases, the reagent that may be used to generate PEGylated aptamers is a non-branched mPEG-Succinimidyl Propionate (mPEG-SPA), having the general formula:



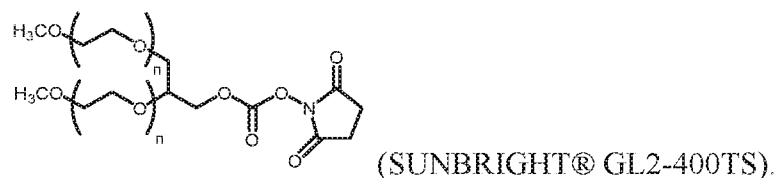
where mPEG is about 20 kD or about 30 kD. In one example, the reactive ester may be -O-CH₂-CH₂-CO₂-NHS.

[00107] In some instances, the reagent that may be used to generate PEGylated aptamers may include a branched PEG linked through glycerol, such as the SUNBRIGHT® series from NOF Corporation, Japan. Non-limiting examples of these reagents include:

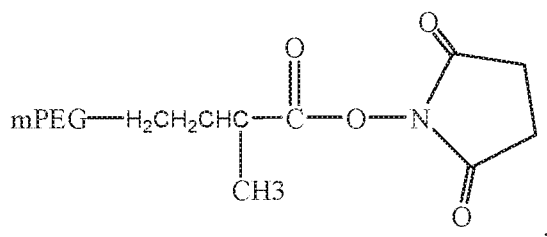




and

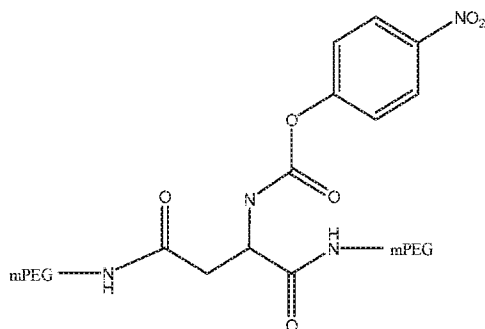


[00108] In another example, the reagents may include a non-branched mPEG Succinimidyl alpha-methylbutanoate (mPEG-SMB) having the general formula:



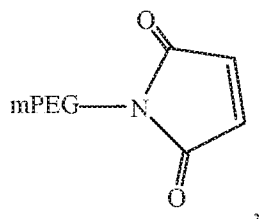
where mPEG is between 10 and 30 kD. In one example, the reactive ester may be $-O-CH_2-CH_2-CH(CH_3)-CO_2-NHS$.

[00109] In other instances, the PEG reagents may include nitrophenyl carbonate-linked PEGs, having the general formula:



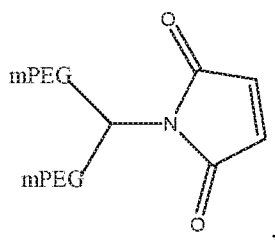
[00110] Compounds including nitrophenyl carbonate can be conjugated to primary amine containing linkers.

[00111] In some cases, the reagents used to generate PEGylated aptamers may include PEG with thiol-reactive groups that can be used with a thiol-modified linker. One non-limiting example may include reagents having the following general structure:



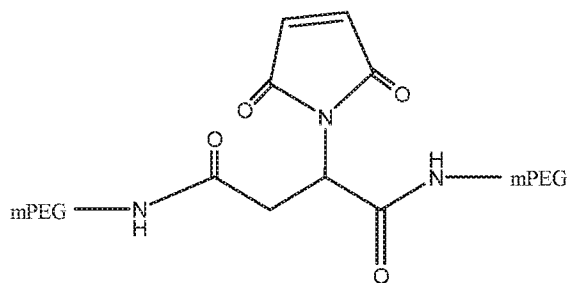
where mPEG is about 10 kD, about 20 kD or about 30 kD.

[00112] Another non-limiting example may include reagents having the following general structure:



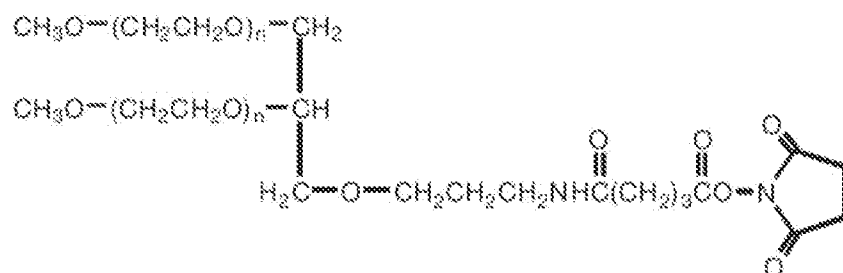
where each mPEG is about 10 kD, about 20 kD, or about 30 kD and the total molecular weight is about 20 kD, about 40 kD, or about 60 kD, respectively. Branched PEGs with thiol reactive groups that can be used with a thiol-modified linker, as described above, may include reagents in which the branched PEG has a total molecular weight of about 40 kD or about 60 kD (*e.g.*, where each mPEG is about 20 kD or about 30 kD).

[00113] In some cases, the reagents used to generated PEGylated aptamers may include reagents having the following structure:

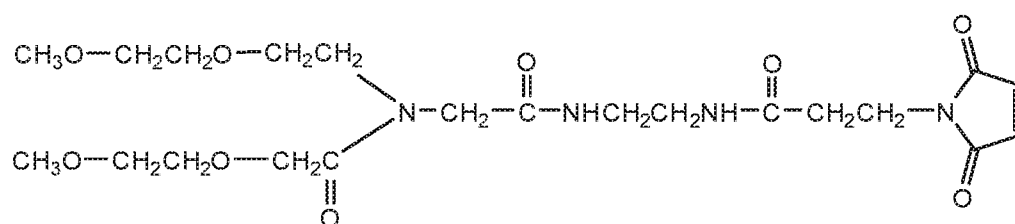


[00114] In some cases, the reaction to conjugate the PEG to the aptamer is carried out between about pH 6 and about pH 10, or between about pH 7 and pH 9 or about pH 8.

[00115] In some cases, the reagents used to generate PEGylated aptamers may include reagents having the following structure:



[00116] In some cases, the reagents used to generate PEGylated aptamers may include reagents having the following structure:



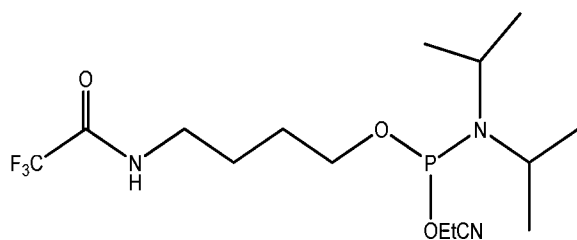
[00117] In some cases, the aptamer is associated with a single PEG molecule. In other cases, the aptamer is associated with two or more PEG molecules.

[00118] In some cases, the aptamers described herein may be bound or conjugated to one or more molecules having desired biological properties. Any number of molecules can be bound or conjugated to aptamers, non-limiting examples including antibodies, peptides, proteins, carbohydrates, enzymes, polymers, drugs, small molecules, gold nanoparticles, radiolabels, fluorescent labels, dyes, haptens (*e.g.*, biotin), other aptamers, or nucleic acids (*e.g.*, siRNA). In some cases, aptamers may be conjugated to molecules that increase the stability, the solubility or the bioavailability of the aptamer. Non-limiting examples include polyethylene glycol (PEG) polymers, carbohydrates and fatty acids. In some cases, molecules that improve the transport or delivery of the aptamer may be used, such as cell penetrating peptides. Non-limiting examples of cell penetrating peptides can include peptides derived from Tat, penetratin, polyarginine peptide Arg₈ sequence, Transportan, VP22 protein from Herpes Simplex Virus (HSV), antimicrobial peptides such as Buforin I and SynB, polyproline sweet arrow peptide molecules, Pep-1 and MPG. In some embodiments, the aptamer is conjugated to a lipophilic compound such as cholesterol, dialkyl glycerol, diacyl glycerol, or a non-immunogenic, high molecular weight compound or polymer such as polyethylene glycol (PEG) or other water-soluble

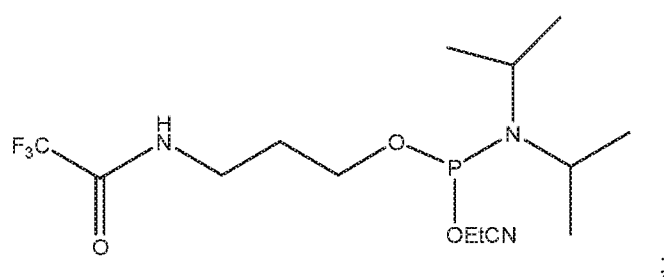
pharmaceutically acceptable polymers including, but not limited to, polyaminoamines (PAMAM) and polysaccharides such as dextran, or polyoxazolines (POZ).

[00119] The molecule to be conjugated can be covalently bonded or can be associated through non-covalent interactions with the aptamer of interest. In one example, the molecule to be conjugated is covalently attached to the aptamer. The covalent attachment may occur at a variety of positions on the aptamer, for example, to the exocyclic amino group on the base, the 5-position of a pyrimidine nucleotide, the 8-position of a purine nucleotide, the hydroxyl group of the phosphate, or a hydroxyl group or other group at the 5' or 3' terminus. In one example, the covalent attachment is to the 5' or 3' hydroxyl group of the aptamer.

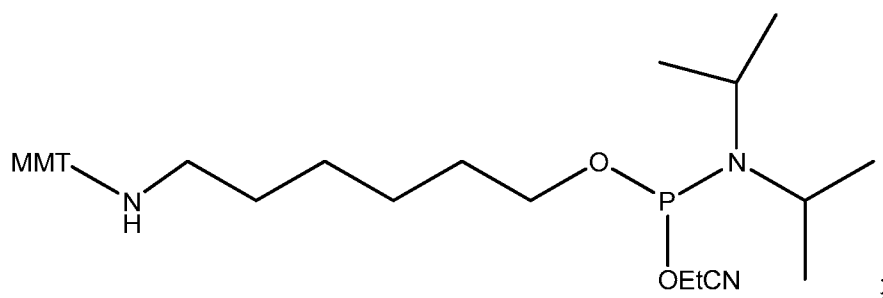
[00120] In some cases, the aptamer can be attached to another molecule directly or with the use of a spacer or linker. For example, a lipophilic compound or a non-immunogenic, high molecular weight compound can be attached to the aptamer using a linker or a spacer. Various linkers and attachment chemistries are known in the art. In a non-limiting example, 6-(trifluoroacetamido)hexanol (2-cyanoethyl-N,N-diisopropyl)phosphoramidite can be used to add a hexylamino linker to the 5' end of the synthesized aptamer. This linker, as with the other amino linkers provided herein, once the group protecting the amine has been removed, can be reacted with PEG-NHS esters to produce covalently linked PEG-aptamers. Other non-limiting examples of linker phosphoramidites may include: TFA-amino C4 CED phosphoramidite having the structure:



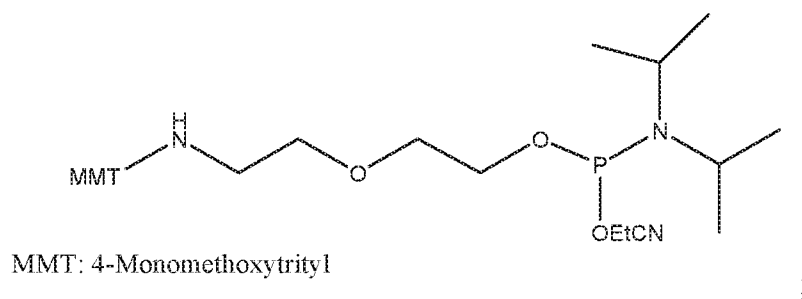
5'-amino modifier C3 TFA having the structure:



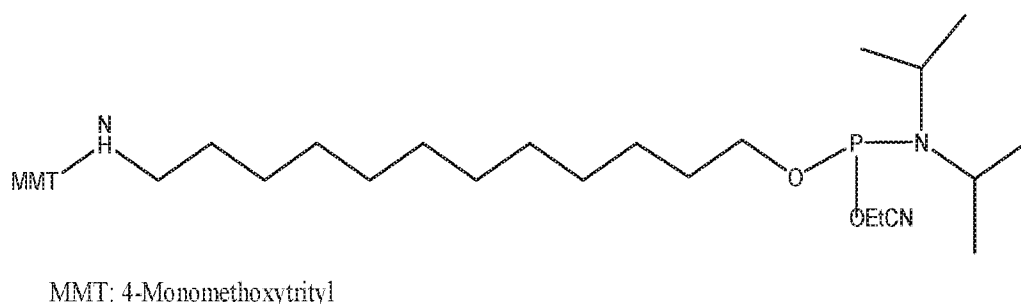
MMT amino modifier C6 CED phosphoramidite having the structure:



5'-amino modifier 5 having the structure:

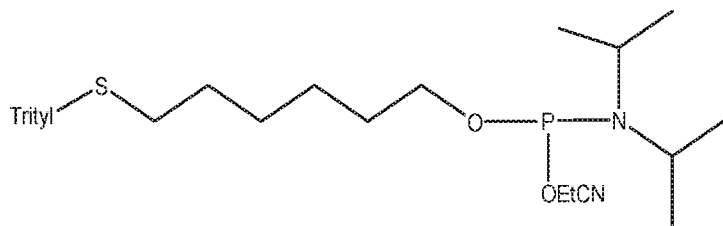


5'-amino modifier C12 having the structure:

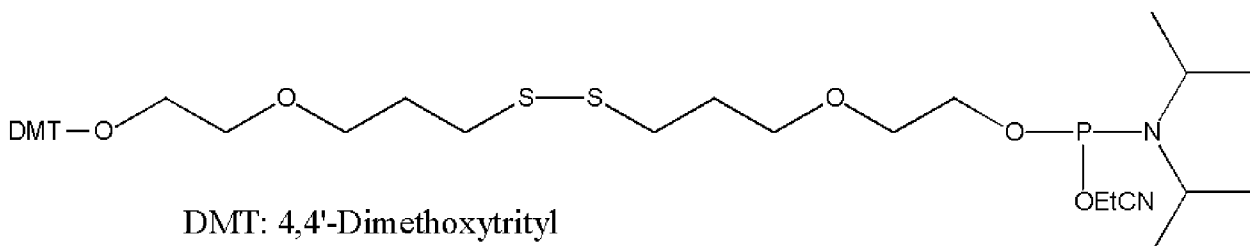


;

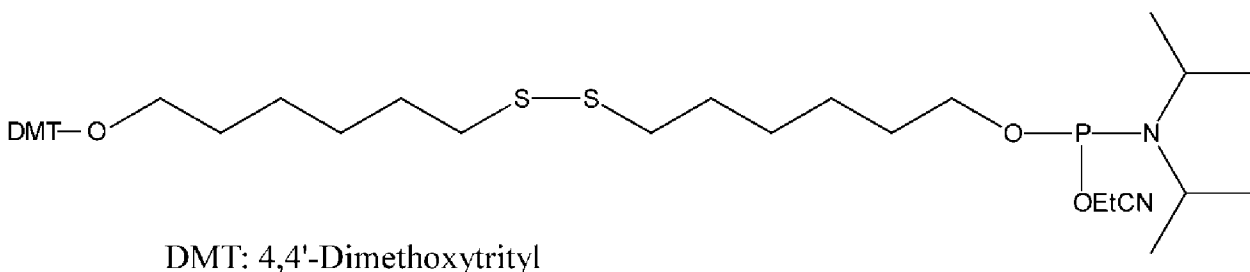
5' thiol-modifier C6 having the structure:



5' thiol-modifier C6 having the structure:



and 5' thiol-modifier C6 having the structure:



[00121] The 5'-thiol modified linker may be used, for example, with PEG-maleimides, PEG-vinylsulfone, PEG-iodoacetamide and PEG-orthopyridyl-disulfide. In one example, the aptamer may be bonded to the 5'-thiol through a maleimide or vinyl sulfone functionality.

[00122] In some cases, the aptamer formulated according to the present disclosure may also be modified by encapsulation within or displayed on the surface of a liposome. In other cases, the aptamer formulated according to the present disclosure may also be modified by encapsulation within or displayed on the surface of a micelle. Liposomes and micelles may be comprised of any lipids, and in some cases the lipids may be phospholipids, including phosphatidylcholine. Liposomes and micelles may also contain or be comprised in part or in total of other polymers and amphipathic molecules including PEG conjugates of poly lactic acid (PLA), poly DL-lactic-co-glycolic acid (PLGA), or poly caprolactone (PCL).

[00123] In some cases, the aptamers described herein may be designed to inhibit a function associated with VEGF-A. In some cases, the aptamers described herein may be designed to bind the receptor binding face of VEGF-A, or a portion thereof. In some cases, the aptamers described herein may be designed to bind the receptor binding domain of VEGF-A, or a portion thereof. The receptor binding domain of VEGF-A may include any one or more of residues 1-109 as described in **SEQ ID NOs: 6-10**. In some cases, the aptamers described herein may bind to a structural feature of VEGF-A other than the heparin binding domain of VEGF-A. The heparin binding domain of VEGF-A may include any one or more of residues 111-165 as described in **SEQ ID NOs: 6-8**. In some cases, the aptamers described herein may block or reduce binding of one or more isoforms or variants of VEGF-A to one or more of Flk-1, KDR, and Nrp-1.

[00124] In some instances, an aptamer is isolated or purified. “Isolated” (used interchangeably with “substantially pure” or “purified”) as used herein means an aptamer that is synthesized chemically, or has been separated from other aptamers.

[00125] In some cases, an aptamer of the disclosure may comprise one of the following sequences described in **Table 1**.

Table 1. VEGF-A Aptamer Sequences

Compound Name	Backbone	Sequence 5' to 3'
R6-1 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGGAUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 11), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-1 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCGCCUCCGCGC GGAGGGGAUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 12), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-2 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGAUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 13), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-2 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGAUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 14), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-3 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 15), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-3 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC

		GGAGGGGUUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUAGAAGC (SEQ ID NO: 16), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-4 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 17), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-4 full	RNA	GGGAGUGUGUACGAGGCAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCUAGAAGC (SEQ ID NO: 18), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-5 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 19), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-5 full	RNA	GGGAGUGUGUACGAGGCAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCUAGAAGC (SEQ ID NO: 20), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-6 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUAUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 21), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-6 full	RNA	GGGAGUGUGUACGAGGCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUAGAAGC (SEQ ID NO: 22), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-7 truncated	RNA	AAGCCGCCUCCGCGCGGAGGGGUUAUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 23), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-7 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCGCCUCCGCGC GGAGGGGUUAUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUAGAAGC (SEQ ID NO: 24), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-8 truncated	RNA	AAGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 25), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-8 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUAGAAGC (SEQ ID NO: 26), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-9 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUACAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 27), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-9 full	RNA	GGGAGUGUGUACGAGGCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUAGAAGC (SEQ ID NO: 28), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-12	RNA	AUGCCGCCUCCGCGCGGAGGGAUUAUCAUUAUCCCGU

truncated		UUGUCGGCUU (SEQ ID NO: 29), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-12 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCCGCCUCCGCGC GGAGGGGAUUUCAUUAUCCCGUUUGUCGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 30), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-13 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 31), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-13 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCUGCCUCCGCGC GGAGGGGUUUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 32), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-14 truncated	RNA	AAGCUGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCCGCUU (SEQ ID NO: 33), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-14 full	RNA	GGGAGUGUGUACGAGGCAUUA AAGCUGCCUCCGCGC GGAGGGGUUUUCAUUAUCCCGUUUGGCCGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 34), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-16 truncated	RNA	AAGCAUCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 35), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-16 full	RNA	GGGAGUGUGUACGAGGCAUUA AAGCAUCCUCCGCGC GGAGGGGUUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 36), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-17 truncated	RNA	AUGCAGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 37), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-17 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCAGCCUCCGCGC GGAGGGGUUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 38), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-18 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCCGGCAU (SEQ ID NO: 39), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-18 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCCGCCUCCGCGC GGAGGGGUUUUCAUUAUCCCGUUUGGCCGGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 40), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-19 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCCGGCAU (SEQ ID NO: 41), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-19 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGUCGCCUCCGCGC GGAGGGGUUUUCAUUAUCCCGUUUGGCCGGCAUUGAUA

		CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 42), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-20 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGGAUUUCAUUAUCCCGU UUGGCUGCCU (SEQ ID NO: 43), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-20 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCGCCUCCGCGC GGAGGGGAUUUCAUUAUCCCGUUUGGCUGCCUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 44), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-21 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGGAUUUCAUUAUCCCGU UUGUCGGCUU (SEQ ID NO: 45), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-21 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCGCCUCCGCGC GGAGGGGAUUUCAUUAUCCCGUUUGUCGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 46), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-22 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCAU (SEQ ID NO: 47), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-22 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 48), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-24 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUCACAAUCCCGU UUGGCUGCAU (SEQ ID NO: 49), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-24 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUUCACAAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 50), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-25 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCCGCAU (SEQ ID NO: 51), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-25 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCCGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 52), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-27 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUACAUAUCCCGU UUGGCGGCUU (SEQ ID NO: 53), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-27 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUACAUAUCCCGUUUGGCCGCGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 54), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-29 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUACAUAUCCCGU UUGGCUGCAU (SEQ ID NO: 55),

		where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-29 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCCGCCUCCGCGC GGAGGGGUUACAUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 56), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-31 truncated	RNA	AGGCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 57), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-31 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 58), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-33 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGGAUUAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 59), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-33 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCGCCUCCGCGC GGAGGGGAUUAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 60), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-34 truncated	RNA	AGGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 61), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-34 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 62), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-36 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGAUUAUUAUCCCGU UUGUCGGCAU (SEQ ID NO: 63), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-36 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCCGCCUCCGCGC GGAGGGGAUUAUUAUCCCGUUUGUCGGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 64), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-39 truncated	RNA	AGGCAUCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCCU (SEQ ID NO: 65), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-39 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCAUCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCCUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 66), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-42 truncated	RNA	AAGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 67), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-42 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 68),

		where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-43 truncated	RNA	AAGCUGCCUCCGCGCGGAGGGGUUGCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 69), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-43 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUGCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 70), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-45 truncated	RNA	AGUCAGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCUGCCU (SEQ ID NO: 71), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-45 full	RNA	GGGAGUGUGUACGAGGCAUUAAGUCAGCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGCUGCCUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 72), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-48 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGUGGCAU (SEQ ID NO: 73), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-48 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCGCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGUGGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 74), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-49 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 75), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-49 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCUGCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 76), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-50 truncated	RNA	AUGCAUCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 77), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-50 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCAUCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 78), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-53 truncated	RNA	AGGCCACCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGUGGCUU (SEQ ID NO: 79), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-53 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCACCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGUGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 80), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-54 truncated	RNA	AGUCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCGGCUA (SEQ ID NO: 81), where G is 2'F; and A, C, & U are 2'OMe modified RNA.

R6-54 full	RNA	GGGAGUGUGUACGAGGCAUUAAGUCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGCUAUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 82), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-57 truncated	RNA	AAGCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 83), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-57 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 84), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-58 truncated	RNA	AUGCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 85), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-58 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 86), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-61 truncated	RNA	AAGCAUCCUCCGCGCGGAGGGGUGUCAUCAUCCCGU UUGGCUGCUU (SEQ ID NO: 87), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-61 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCAUCCUCCGCGC GGAGGGGUGUCAUCAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 88), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-62 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUCAUUUCCCCGU UUGGCUGCAU (SEQ ID NO: 89), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-62 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCGCCUCCGCGC GGAGGGGUUUCAUUUCCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 90), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-65 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 91), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-65 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 92), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-67 truncated	RNA	AGGCCACCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGUGUCCU (SEQ ID NO: 93), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-67 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCACCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGUGUCCUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 94), where G is 2'F; and A, C, & U are 2'OMe modified RNA.

R6-68 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGAUUGCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 95), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-68 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGAUUGCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 96), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-70 truncated	RNA	UUGCCGCCUCCGCGCGGAGGGGAUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 97), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-70 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGAUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 98), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-72 truncated	RNA	ACGCAGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 99), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-72 full	RNA	GGGAGUGUGUACGAGGCAUUAACGCAGCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 100), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-75 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUCACUAUCCCGU UUGGCGGCUU (SEQ ID NO: 101), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-75 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGUUUCACUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 102), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-76 truncated	RNA	UUGCUGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCCGCAU (SEQ ID NO: 103), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-76 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCUGCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGCCGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 104), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-79 truncated	RNA	AAGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 105), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-79 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCGCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 106), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-80 truncated	RNA	AAUCAGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 107), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-80 full	RNA	GGGAGUGUGUACGAGGCAUUAUAUCAGCCUCCGCGC

		GGAGGGGUGUUAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 108), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-83 truncated	RNA	UUGCCGCUCCGCGCGGAGGGGUGUUAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 109), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-83 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGUGUUAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 110), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-85 truncated	RNA	CUGCCGCCUCCGCGCGGAGGGGUGUUAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 111), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-85 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGUGUUAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 112), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-88 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUGUUAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 113), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-88 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGUGUUAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 114), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-92 truncated	RNA	ACGCAUCCUCCGCGCGGAGGGGUGUUAUUAUCCCGU UUGGCUGCGU (SEQ ID NO: 115), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-92 full	RNA	GGGAGUGUGUACGAGGCAUUAACGCAUCCUCCGCGC GGAGGGGUGUUAUUAUCCCGUUUGGCUGCGUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 116), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-99 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUGUUAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 117), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-99 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCCGCCUCCGCGC GGAGGGGUGUUAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 118), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-101 truncated	RNA	AAGCCGCCUCCGCGCGGAGGGAUUAUUAUCCCGU UUGUCGGCUU (SEQ ID NO: 119), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-101 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCGCCUCCGCGC GGAGGGAUUAUUAUCCCGUUUGUCGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 120), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-102	RNA	UUGCUGCCUCCGCGCGGAGGGGUGUUAUUAUCCCGU

truncated		UUGGCUGCUU (SEQ ID NO: 121), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-102 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCUGCCUCCGCGC GGAGGGGUUUAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 122), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-104 truncated	RNA	UGGCCGCCUCCGCGCGGAGGGAUUUAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 123), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-104 full	RNA	GGGAGUGUGUACGAGGCAUUAUGGCCGCCUCCGCGC GGAGGGGAUUAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 124), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-110 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUUAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 125), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-110 full	RNA	GGGAGUGUGUACGAGGCAUUAUGGCCGCCUCCGCGC GGAGGGGUUUUAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 126), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-115 truncated	RNA	AGGCAUCCUCCGCGCGGAGGGAUUUAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 127), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-115 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCAUCCUCCGCGC GGAGGGGAUUAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 128), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-116 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGAUUUAUUAUCCCGU UUGUCGUCCU (SEQ ID NO: 129), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-116 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCGCCUCCGCGC GGAGGGGAUUAUUAUCCCGUUUGUCGUCCUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 130), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-117 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUGAUAAUCCCGU UUGGCUGCAU (SEQ ID NO: 131), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-117 full	RNA	GGGAGUGUGUACGAGGCAUUAUGGCCGCCUCCGCGC GGAGGGGUUUGAUAAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 132), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-119 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGAUUUCUUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 133), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-119 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCGCCUCCGCGC GGAGGGAUUUCUUUAUCCCGUUUGGCUGCUUUGAUA

		CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 134), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-123 truncated	RNA	AAUCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGAUU (SEQ ID NO: 135), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-123 full	RNA	GGGAGUGUGUACGAGGCAUUAUCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGAUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 136), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-124 truncated	RNA	AGGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCCU (SEQ ID NO: 137), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-124 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCCUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 138), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-130 truncated	RNA	AAUCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 139), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-130 full	RNA	GGGAGUGUGUACGAGGCAUUAUCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 140), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-131 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 141), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-131 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 142), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-134 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCAGCUU (SEQ ID NO: 143), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-134 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCAGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 144), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-135 truncated	RNA	AGGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 145), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-135 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 146), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-138 truncated	RNA	AUGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCAGCAU (SEQ ID NO: 147),

		where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-138 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCUGCCUCCGCGC GGAGGGGUUUC AUUAUCCCGUUUGGCAGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 148), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-139 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGAUUUC AUUAUCCCGC UUGGCUGCUU (SEQ ID NO: 149), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-139 full	RNA	GGGAGUGUGUACGAGGCAUUA AGGCCGCCUCCGCGC GGAGGGGAUUC AUUAUCCCGCUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 150), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-142 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUUGAUUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 151), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-142 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCCGCCUCCGCGC GGAGGGGUUUGAUUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 152), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-145 truncated	RNA	UUGCUGCCUCCGCGCGGAGGGGUUUC AUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 153), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-145 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCUGCCUCCGCGC GGAGGGGUUUC AUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 154), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-152 truncated	RNA	CUGCCGCCUCCGCGCGGAGGGGUUUC AUUAUCCCGU UUGGCGGCAU (SEQ ID NO: 155), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-152 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCCGCCUCCGCGC GGAGGGGUUUC AUUAUCCCGUUUGGCGGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 156), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-153 truncated	RNA	AUUCAGCCUCCGCGCGGAGGGGUUUC AUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 157), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-153 full	RNA	GGGAGUGUGUACGAGGCAUUA AUUCAGCCUCCGCGC GGAGGGGUUUC AUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 158), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-157 truncated	RNA	AAGCAGCCUCCGCGCGGAGGGGUUUC AUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 159), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-157 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCAGCCUCCGCGC GGAGGGGUUUC AUUAUCCCGUUUGGCGGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 160),

		where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-162 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGAUUUCAUUAUCCCGU UUGUCGGCCU (SEQ ID NO: 161), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-162 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCCGCCUCCGCGC GGAGGGGAUUUCAUUAUCCCGUUUGUCGGCCUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 162), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-166 truncated	RNA	AGUCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGACU (SEQ ID NO: 163), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-166 full	RNA	GGGAGUGUGUACGAGGCAUUAAGUCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGACUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 164), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-169 truncated	RNA	AGUCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGAUU (SEQ ID NO: 165), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-169 full	RNA	GGGAGUGUGUACGAGGCAUUAAGUCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGAUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 166), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-170 truncated	RNA	CUGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCAU (SEQ ID NO: 167), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-170 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 168), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-171 truncated	RNA	ACUCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCGU (SEQ ID NO: 169), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-171 full	RNA	GGGAGUGUGUACGAGGCAUUAACUCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCGUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 170), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-172 truncated	RNA	AGGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCAUCCU (SEQ ID NO: 171), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-172 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCAUCCUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 172), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-173 truncated	RNA	UUGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 173), where G is 2'F; and A, C, & U are 2'OMe modified RNA.

R6-173 full	RNA	GGGAGUGUGUACGAGGCAUUAUUGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 174), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-177 truncated	RNA	UGUCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 175), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-177 full	RNA	GGGAGUGUGUACGAGGCAUUAUGUCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 176), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-178 truncated	RNA	CUGCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 177), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-178 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 178), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-179 truncated	RNA	AGUCCGCCUCCGCGCGGAGGGGUUAUCAUUAUCCCGU UUGGCGUACU (SEQ ID NO: 179), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-179 full	RNA	GGGAGUGUGUACGAGGCAUUAAGUCCGCCUCCGCGC GGAGGGGUUAUCAUUAUCCCGUUUGGCGUACUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 180), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-181 truncated	RNA	AGCCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGUGCU (SEQ ID NO: 181), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-181 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGUGCUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 182), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-182 truncated	RNA	AGUCCACCUCCGCGCGGAGGGGUUUCAUAAUCCCGU UUGGUGGCUA (SEQ ID NO: 183), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-182 full	RNA	GGGAGUGUGUACGAGGCAUUAAGUCCACCUCCGCGC GGAGGGGUUUCAUAAUCCCGUUUGGUGGCUAUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 184), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-186 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGAUUUCGUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 185), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-186 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCCGCCUCCGCGC GGAGGGGAUUUCGUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCUGAGAAGC (SEQ ID NO: 186), where G is 2'F; and A, C, & U are 2'OMe modified RNA.

R6-197 truncated	RNA	AUGCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCCU (SEQ ID NO: 187), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-197 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCCUUGAUA CUUGAUCGCCC UAGAAGC (SEQ ID NO: 188), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-198 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGAUUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 189), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-198 full	RNA	GGGAGUGUGUACGAGGCAUUA AGGCCGCCUCCGCGC GGAGGGAUUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCC UAGAAGC (SEQ ID NO: 190), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-200 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUAUUAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 191), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-200 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCCGCCUCCGCGC GGAGGGGUAUUAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCC UAGAAGC (SEQ ID NO: 192), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-201 truncated	RNA	AUUCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 193), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-201 full	RNA	GGGAGUGUGUACGAGGCAUUA AUUCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCC UAGAAGC (SEQ ID NO: 194), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-203 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGAUUUCAUUAUCCCGU UUGGCUGCGU (SEQ ID NO: 195), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-203 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCCGCCUCCGCGC GGAGGGAUUUCAUUAUCCCGUUUGGCUGCGUUGAUA CUUGAUCGCCC UAGAAGC (SEQ ID NO: 196), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-204 truncated	RNA	AUUCGCCUCCGCGCGGAGGGGUAUCAUUAUCCCGU UUGGCGGCAU (SEQ ID NO: 197), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-204 full	RNA	GGGAGUGUGUACGAGGCAUUA AUUCCGCCUCCGCGC GGAGGGGUAUCAUUAUCCCGUUUGGCGGCAUUGAUA CUUGAUCGCCC UAGAAGC (SEQ ID NO: 198), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-205 truncated	RNA	AUGUCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU (SEQ ID NO: 199), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-205 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGUCGCCUCCGCGC

		GGAGGGGUUUCAUUAUCCCGUUUGGCGGCUUGAUA CUUGAUCGCCCAGAAGC (SEQ ID NO: 200), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-208 truncated	RNA	ACUCAGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGAUU (SEQ ID NO: 201), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-208 full	RNA	GGGAGUGUGUACGAGGCAUUAACUCAGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGAUUUGAUA CUUGAUCGCCCAGAAGC (SEQ ID NO: 202), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-210 truncated	RNA	AAGCCACCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGUGGCUU (SEQ ID NO: 203), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-210 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCACCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGUGGCUUUGAUA CUUGAUCGCCCAGAAGC (SEQ ID NO: 204), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-211 truncated	RNA	AUGCCGCCUCCGCGCGGAGGGGUUGAAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 205), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-211 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCCGCCUCCGCGC GGAGGGGUUGAAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCAGAAGC (SEQ ID NO: 206), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-214 truncated	RNA	AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGUCCU (SEQ ID NO: 207), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-214 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCCGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCGUCCUUGAUA CUUGAUCGCCCAGAAGC (SEQ ID NO: 208), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-221 truncated	RNA	AGUCCACCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGUGGCCU (SEQ ID NO: 209), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-221 full	RNA	GGGAGUGUGUACGAGGCAUUAAGUCCACCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGUGGCCUUGAUA CUUGAUCGCCCAGAAGC (SEQ ID NO: 210), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-227 truncated	RNA	CUGCUGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCUGCAU (SEQ ID NO: 211), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-227 full	RNA	GGGAGUGUGUACGAGGCAUUAUGCUGCCUCCGCGC GGAGGGGUUUCAUUAUCCCGUUUGGCUGCAUUGAUA CUUGAUCGCCCAGAAGC (SEQ ID NO: 212), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-228	RNA	AUGCCGCCUCCGCGCGGAGGGGUAGCAAUAUCCCGU

truncated		UUGGCUGCUU (SEQ ID NO: 213), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-228 full	RNA	GGGAGUGUGUACGAGGCAUUA AUGCCGCCUCCGCGC GGAGGGGUAGCAAUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 214), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-229 truncated	RNA	AAGCCGCCUCCGCGCGGAGGGGUAAUUAUCCCGU UUGGCUGCUU (SEQ ID NO: 215), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-229 full	RNA	GGGAGUGUGUACGAGGCAUUAAGCCGCCUCCGCGC GGAGGGGUAAUUAUCCCGUUUGGCUGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 216), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-241 truncated	RNA	AGGCUGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCAGCUU (SEQ ID NO: 217), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
R6-241 full	RNA	GGGAGUGUGUACGAGGCAUUAAGGCUGCCUCCGCGC GGAGGGGUUCAUUAUCCCGUUUGGCAGCUUUGAUA CUUGAUCGCCCCUAGAAGC (SEQ ID NO: 218), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 4.2	RNA	C6SS- AGGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 219), where G is 2'F; A, C, & U are 2'OMe modified RNA; C6SS is a disulfide linker; and idT is an inverted deoxythymidine residue.
Aptamer 26	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCG UUUGGCGGCUUU-idT (SEQ ID NO: 220), where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 27	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGGGGGUUCAUUAUCCCG UUUGGCGGCUUU-idT (SEQ ID NO: 221), where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 28	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGUGGGGUUCAUUAUCCCG UUUGGCGGCUUU-idT (SEQ ID NO: 222), where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 29	RNA	C6NH ₂ - UAGUCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCG

		UUUGGCGGCUUU-idT (SEQ ID NO: 223), where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 30	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCC GUUUGGCGGCUUU-idT (SEQ ID NO: 224) where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 31	RNA	C6NH ₂ - UAGACCGCCUCCGCGCGGAGGGGUUCAUUAUCCCCG UUUGGCGGCUUU-idT (SEQ ID NO: 225), where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 32	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCCG UUUGGCGGCCUU-idT (SEQ ID NO: 226), where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 47	RNA	C6NH ₂ -AGGCCGCC-Sp3- CCGCGCGGAGGGGUUCAUUAUCCCGUUUGGCGGCU U-idT (SEQ ID NO: 227), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3- propanediol).
Aptamer 48	RNA	C6NH ₂ -AGGCCGCCU-Sp3- CGCGCGGAGGGGUUCAUUAUCCCGUUUGGCGGCUU -idT (SEQ ID NO: 228), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3- propanediol).
Aptamer 49	RNA	C6NH ₂ -AGGCCGCCUC (SEQ ID NO: 229)-Sp3- GCGCGGAGGGGUUCAUUAUCCCGUUUGGCGGCUU- idT (SEQ ID NO: 230), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3- propanediol).
Aptamer 50	RNA	C6NH ₂ -AGGCCGCCUCC (SEQ ID NO: 231)-Sp3- CGCGGAGGGGUUCAUUAUCCCGUUUGGCGGCUU- idT (SEQ ID NO: 232),

		where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 51	RNA	C ₆ NH ₂ -AGGCCGCCUCCG (SEQ ID NO: 233)-Sp3-GCGGAGGGGUUCAUUAUCCCGUUUGGCGGCUU (SEQ ID NO: 234), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 52	RNA	C ₆ NH ₂ -AGGCCGCCUCCGC (SEQ ID NO: 235)-Sp3-CGGAGGGGUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 236), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 53	RNA	C ₆ NH ₂ -AGGCCGCCUCCGCG (SEQ ID NO: 237)-Sp3-GGAGGGGUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 238), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 54	RNA	C ₆ NH ₂ -AGGCCGCCUCCGCGC (SEQ ID NO: 239)-Sp3-GAGGGGUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 240), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 55	RNA	C ₆ NH ₂ -AGGCCGCCUCCGCGCG (SEQ ID NO: 241)-Sp3-AGGGGUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 242), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 56	RNA	C ₆ NH ₂ -AGGCCGCCUCCGCGCGG (SEQ ID NO: 243)-Sp3-GGGGUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 244), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).

Aptamer 57	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGA (SEQ ID NO: 245)-Sp3-GGGUUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 246), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 58	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAG (SEQ ID NO: 247)-Sp3-GGUUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 248), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 59	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGG (SEQ ID NO: 249)-Sp3-GUUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 250), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 60	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGG (SEQ ID NO: 251)-Sp3-UUUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 252), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 61	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGG (SEQ ID NO: 253)-Sp3-UUCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 254), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 62	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGU (SEQ ID NO: 255)-Sp3-UCAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 256), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 63	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUU (SEQ ID NO: 257)-Sp3-CAUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 258), where G is 2'F; and A, C, and U are 2'OMe modified RNA;

		C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 64	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUU (SEQ ID NO: 259)-Sp3-AUUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 260), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 65	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUC (SEQ ID NO: 261)-Sp3-UUAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 262), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 66	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUCA (SEQ ID NO: 263)-Sp3-UAUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 264), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 67	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUCAU (SEQ ID NO: 265)-Sp3-AUCCCGUUUGGCGGCUU-idT (SEQ ID NO: 266), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 68	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUCAU (SEQ ID NO: 267)-Sp3-UCCCGUUUGGCGGCUU-idT (SEQ ID NO: 268), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 69	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUCAUUA (SEQ ID NO: 269)-Sp3-CCCGUUUGGCGGCUU-idT (SEQ ID NO: 270), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 70	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAU

		(SEQ ID NO: 271)-Sp3-CCGUUUGGCGGCUU-idT (SEQ ID NO: 272), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 71	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUC AUUAUC (SEQ ID NO: 273)-Sp3-CGUUUGGCGGCUU-idT (SEQ ID NO: 274), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 72	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUC AUUAUCC (SEQ ID NO: 275)-Sp3-GUUUGGCGGCUU-idT (SEQ ID NO: 276), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 73	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUC AUUAUCCC (SEQ ID NO: 277)-Sp3-UUUGGCGGCUU-idT (SEQ ID NO: 278), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 74	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUC AUUAUCCCCG (SEQ ID NO: 279)-Sp3-UUGGCGGCUU-idT (SEQ ID NO: 280), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 75	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUUC AUUAUCCCCGU (SEQ ID NO: 281)-Sp3-UGGCGGCUU-idT (SEQ ID NO: 282), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).

Aptamer 76	RNA	C6NH ₂ - AGGCCGCUCCGCGCGGAGGGGUUCAUUAUCCCGU U (SEQ ID NO: 283)-Sp3-GGCGGCUU-idT, where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3- propanediol).
Aptamer 79	RNA	C6NH ₂ - AGGCCGCCCCGCGCGGAGGGGUUCAUUAUCCCGUU UGGCGGCUU-idT (SEQ ID NO: 284), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 108	RNA	C6NH ₂ - AGGCCGCUCCGCGCGGAGGGGUUCAUUAUCCCGUUU GGCGGCUU-idT (SEQ ID NO: 285), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 109	RNA	C6NH ₂ - AGGCCGCUCCGCGCGGAGGGGUUCAUUAUCCCGUUU GGCGGCUU-idT (SEQ ID NO: 286), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 112	RNA	C6NH ₂ - AGGCCGCUCCGCGCGGAAGGGGUUCAUUAUCCUGU UUGGCGGCUU-idT (SEQ ID NO: 287), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 113	RNA	C6NH ₂ - AGGCCGCUCCGCGCGGAUGGGGUUCAUUAUCCAGU UUGGCGGCUU-idT (SEQ ID NO: 288), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 114	RNA	C6NH ₂ - AGGCCGCUCCGCGCGGACGGGUUCAUUAUCCGGU UUGGCGGCUU-idT (SEQ ID NO: 289), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 115	RNA	C6NH ₂ - AGGCCGCUCCGCGCGGAGAGGUUCAUUAUCUCGU UUGGCGGCUU-idT (SEQ ID NO: 290),

		where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 116	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGUGGUUUC AUUAUCACGU UUGGCGGCUU-idT (SEQ ID NO: 291), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 117	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGCGGUUUC AUUAUCGCGU UUGGCGGCUU-idT (SEQ ID NO: 292), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 119	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGACCGGUUUC AUUACGGGU UUGGCGGCUU-idT (SEQ ID NO: 293), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 120	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGAGGGUUC AUUCCCU CGU UUGGCGGCUU-idT (SEQ ID NO: 294), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 121	RNA	C6NH ₂ - AGGCCGCGUCCGCGCGGAGGGGUUUC AUUAUCCCGU UUCGCGGCUU-idT (SEQ ID NO: 295), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 122	RNA	C6NH ₂ - AGGCCGCAUCCGCGCGGAGGGGUUUC AUUAUCCCGU UUUGCGGCUU-idT (SEQ ID NO: 296), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 123	RNA	C6NH ₂ - AGGCCGCUUCCGCGCGGAGGGGUUUC AUUAUCCCGU UUAGCGGCUU-idT (SEQ ID NO: 297), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 124	RNA	C6NH ₂ -

		AGGCCGGCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGCCGGCUU-idT (SEQ ID NO: 298), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 125	RNA	C ₆ NH ₂ - AGGCCGACUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGUCGGCUU-idT (SEQ ID NO: 299), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 126	RNA	C ₆ NH ₂ - AGGCCGUCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGACGGCUU-idT (SEQ ID NO: 300), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 127	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGCUUUCAUUAAGCCCGU UUGGCGGCUU-idT (SEQ ID NO: 301), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 128	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGACUUUCAUUAAGUCCGU UUGGCGGCUU-idT (SEQ ID NO: 302), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 129	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGUCUUUCAUUAAGACCGU UUGGCGGCUU-idT (SEQ ID NO: 303), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 130	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGUGUUUCAUUAACACCGU UUGGCGGCUU-idT (SEQ ID NO: 304), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 131	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGAGUUUCAUUAACUCCGU UUGGCGGCUU-idT (SEQ ID NO: 305), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted

		deoxythymidine residue.
Aptamer 132	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGGCGUUUCAUUAACGCCGU UUGGCGGCUU-idT (SEQ ID NO: 306) where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 133	RNA	C6NH ₂ - AGGCCGAAUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUUUCGGCUU-idT (SEQ ID NO: 307), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 134	RNA	C6NH ₂ - AGGCCGAUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUAUCGGCUU-idT (SEQ ID NO: 308), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 135	RNA	C6NH ₂ - AGGCCGUAUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUUACGGCUU-idT (SEQ ID NO: 309), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 136	RNA	C6NH ₂ - AGGCCGUUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUAACGGCUU-idT (SEQ ID NO: 310), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 137	RNA	C6NH ₂ -AGGCCGAA-Sp3- CCGCGCGGAGGGGUUUCAUUAUCCCGUUUUUCGGCU U-idT (SEQ ID NO: 311), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3- propanediol).
Aptamer 138	RNA	C6NH ₂ -AGGCCGAU-Sp3- CCGCGCGGAGGGGUUUCAUUAUCCCGUUUAUCGGCU U-idT (SEQ ID NO: 312), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3- propanediol).
Aptamer 139	RNA	C6NH ₂ -AGGCCGUA-Sp3-

		CCGCGCGGAGGGGUUCAUUAUCCCGUUUACGGCU U-idT (SEQ ID NO: 313), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 140	RNA	C6NH ₂ -AGGCCGUU-Sp3-CCGCGCGGAGGGGUUCAUUAUCCCGUUUACGGCU U-idT (SEQ ID NO: 314), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 141	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGGAGGGGUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 315), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 142	RNA	C6NH ₂ -AGGCCGUC-Sp3-CCGCGCGGAGGGGUUCAUUAUCCCGUUUGACGGCU U-idT (SEQ ID NO: 316), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 143	RNA	C6NH ₂ -AGGCCGCA-Sp3-CCGCGCGGAGGGGUUCAUUAUCCCGUUUUGCGGCU U-idT (SEQ ID NO: 317), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 144	RNA	C6NH ₂ -AGGCCGCU-Sp3-CCGCGCGGAGGGGUUCAUUAUCCCGUUUAGCGGCU U-idT (SEQ ID NO: 318), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 145	RNA	C6NH ₂ -AGGCCGCCUCCGCGCGGAGGGGUUCAUUAUCCCGUUU GGCGGCUU-idT (SEQ ID NO: 319), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted

		deoxythymidine residue.
Aptamer 146	RNA	C6NH ₂ - AGGCCGCCACCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 320), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 147	RNA	C6NH ₂ - AGGCCGCCCCCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 321), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 148	RNA	C6NH ₂ - AGGCCGCCGCGCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 322), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 149	RNA	C6NH ₂ - AGGCCGCCUCCACGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 323), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 150	RNA	C6NH ₂ - AGGCCGCCUCCCCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 324), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 151	RNA	C6NH ₂ - AGGCCGCCUCCUCGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 325), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 152	RNA	C6NH ₂ - AGGCCGCCUCCGAGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 326), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 153	RNA	C6NH ₂ - AGGCCGCCUCCGGGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 327),

		where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 154	RNA	C6NH ₂ - AGGCCGCCUCCGUGCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 328), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 155	RNA	C6NH ₂ - AGGCCGCCUCCGCACGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 329), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 156	RNA	C6NH ₂ - AGGCCGCCUCCGCCCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 330), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 157	RNA	C6NH ₂ - AGGCCGCCUCCGCUCGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 331), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 158	RNA	C6NH ₂ - AGGCCGCCUCCGCGAGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 332), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 159	RNA	C6NH ₂ - AGGCCGCCUCCGCGGGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 333), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 160	RNA	C6NH ₂ - AGGCCGCCUCCGCGUGGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 334), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 161	RNA	C6NH ₂ -

		AGGCCGCCUCCGCGCGGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 335), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 162	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCAU UUGGCGGCUU-idT (SEQ ID NO: 336), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 163	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCCU UUGGCGGCUU-idT (SEQ ID NO: 337), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 164	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCUU UUGGCGGCUU-idT (SEQ ID NO: 338), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 165	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGA UUGGCGGCUU-idT (SEQ ID NO: 339), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 166	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGC UUGGCGGCUU-idT (SEQ ID NO: 340), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 167	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGG UUGGCGGCUU-idT (SEQ ID NO: 341), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 168	RNA	C ₆ NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU AUGGCGGCUU-idT (SEQ ID NO: 342), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; and idT is an inverted

		deoxythymidine residue.
Aptamer 169	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU CUGGCGGCUU-idT (SEQ ID NO: 343), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 170	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU GUGGCGGCUU-idT (SEQ ID NO: 344), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 171	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UAGGCGGCUU-idT (SEQ ID NO: 345), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 172	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UCGGCGGCUU-idT (SEQ ID NO: 346), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 173	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUCCCGU UGGGCGGCUU-idT (SEQ ID NO: 347), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 174	RNA	C6NH ₂ - AGGCCGCCUGCGCGCGCAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 348), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 175	RNA	C6NH ₂ - AGGCCGCCUACGCGCGUAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 349), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 176	RNA	C6NH ₂ - AGGCCGCCUUCGCGCGAAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 350),

		where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 177	RNA	C6NH ₂ - AGGCCGCCUCGGCGCCGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 351), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 178	RNA	C6NH ₂ - AGGCCGCCUCAGCGCUGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 352), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 179	RNA	C6NH ₂ - AGGCCGCCUCUGCGCAGAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 353), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 180	RNA	C6NH ₂ - AGGCCGCAUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUAACCGCUU-idT (SEQ ID NO: 354), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 181	RNA	C6NH ₂ - AGGCCGCAUCCGCGCGGAGGGGUUUCAUUAUCCCGU UUAACCGAUU-idT (SEQ ID NO: 355), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 182	RNA	C6NH ₂ - AGGCCGCCUUUGCGCUUAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 356), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 183	RNA	C6NH ₂ - AGGCCGCCUCCGCGCCCAGGGGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 357), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 184	RNA	C6NH ₂ -

		AGGCCGCCUCCGCGCGGAGCCGUUUCAUUAUCCCGU UUGGCGGCUU-idT (SEQ ID NO: 358), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 185	RNA	C6NH ₂ - AGGCCGCCUCCGCGCGGAGGGGUUUCAUUAUAACGU UUGGCGGCUU-idT (SEQ ID NO: 359), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.
Aptamer 187	RNA	C6NH ₂ -AGGCCGAC-Sp3- CCGCGCGGAGGGGUUUCAUUAACCCGUUUGUCGGCU U-idT (SEQ ID NO: 360), where G is 2'F; and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3- propanediol).
Aptamer 188	RNA	C6NH ₂ -AGGCCXAC-Sp3- CCGCGCGGAGGGGUUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 361), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 189	RNA	C6NH ₂ -AGGCCGAC-Sp3- CCXCGCGGAGGGGUUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 362), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 190	RNA	C6NH ₂ -AGGCCGAC-Sp3- CCGCXCGGAGGGGUUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 363), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 191	RNA	C6NH ₂ -AGGCCGAC-Sp3- CCGCGCXGAGGGGUUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 364), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).

Aptamer 192	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGXAGGGGUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 365), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 193	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGGAXGGGUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 366), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 194	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGGAGXGGGUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 367), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 195	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGGAGGXGUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 368), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 196	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGGAGGGXUUUCAUUAUCCCGUUUGUCGGCU U-idT (SEQ ID NO: 369), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 197	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGGAGGGGUUCAUUAUCCCXUUUGUCGGCU U-idT (SEQ ID NO: 370), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 198	RNA	C6NH ₂ -AGGCCGAC-Sp3-CCGCGCGGAGGGGUUCAUUAUCCCGUUUXUCGGCU U-idT (SEQ ID NO: 371), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X

		is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 199	RNA	C ₆ NH ₂ -GCCGAC-Sp3-CCGCGCGGAGGGG ^{UUUCAUUAUCCCGUUUGUCGGC-idT (SEQ ID NO: 372)} , where G is 2'F; and A, C, and U are 2'OMe modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 201	RNA	C ₆ NH ₂ -AGGCCXAC-Sp3-CCGCGCGGAGGGG ^{XUUUCAUUAACCCCGUUUXUCGGCUU-idT (SEQ ID NO: 373)} , where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 213	RNA	C ₆ NH ₂ -XCCGAC-Sp3-CCGCGCGGAGGGG ^{UUUCAUUAUCCCGUUUGUCXXC-idT (SEQ ID NO: 374)} , where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 214	RNA	C ₆ NH ₂ -XCCXAC-Sp3-CCGCGCGGAGGGG ^{UUUCAUUAUCCCGUUUGUCXXC-idT (SEQ ID NO: 375)} , where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 215	RNA	C ₆ NH ₂ -XCCGAC-Sp3-CCGCGCGGAGGGG ^{XUUUCAUUAUCCCGUUUGUCXXC-idT (SEQ ID NO: 376)} , where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 216	RNA	C ₆ NH ₂ -XCCGAC-Sp3-CCGCGCGGAGGGG ^{UUUCAUUAUCCCGUUUXUCXXC-idT (SEQ ID NO: 377)} , where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 217	RNA	C ₆ NH ₂ -XCCXAC-Sp3-

		CCGCGCGGAGGGXUUUCAUUAUCCCGUUUGUCXXC-idT (SEQ ID NO: 378), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 218	RNA	C ₆ NH ₂ -XCCXAC-Sp3-CCGCGCGGAGGGGUUUCAUUAUCCCGUUUXUCXXC-idT (SEQ ID NO: 379), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 219	RNA	C ₆ NH ₂ -XCCGAC-Sp3-CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXUCXXC-idT (SEQ ID NO: 380), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 220	RNA	C ₆ NH ₂ -XCCXAC-Sp3-CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXUCXX-idT (SEQ ID NO: 381), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 221	RNA	C ₆ NH ₂ -CCXAC-Sp3-CCGCGCGGAGGGGUUUCAUUAUCCCGUUUGUCXX-idT (SEQ ID NO: 382), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 222	RNA	C ₆ NH ₂ -CCGAC-Sp3-CCGCGCGGAGGGXUUUCAUUAUCCCGUUUGUCXX-idT (SEQ ID NO: 383), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 223	RNA	C ₆ NH ₂ -CCGAC-Sp3-CCGCGCGGAGGGGUUUCAUUAUCCCGUUUXUCXX-idT (SEQ ID NO: 384), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT

		is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 224	RNA	C6NH ₂ -CCXAC-Sp3-CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUGUCXX-idT (SEQ ID NO: 385), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 225	RNA	C6NH ₂ -CCXAC-Sp3-CCGCGCGGAGGGGUUUCAUUAUCCCGUUUXUCXX-idT (SEQ ID NO: 386), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 226	RNA	C6NH ₂ -CCGAC-Sp3-CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUXUCXX-idT (SEQ ID NO: 387), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 227	RNA	C6NH ₂ -CCXAC-Sp3-CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUXUCXX-idT (SEQ ID NO: 388), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 228	RNA	C6NH ₂ -CXAC-Sp3-CCGCGCGGAGGGGUUUCAUUAUCCCGUUUGUCX-idT (SEQ ID NO: 389), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 229	RNA	C6NH ₂ -CGAC-Sp3-CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUGUCX-idT (SEQ ID NO: 390), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 230	RNA	C6NH ₂ -CGAC-Sp3-CCGCGCGGAGGGGUUUCAUUAUCCCGUUUXUCX-idT

		(SEQ ID NO: 391), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 231	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGXUUUCAUUAUCCCGUUUGUCX-idT (SEQ ID NO: 392), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 232	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGGUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 393), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 233	RNA	C6NH ₂ -CGAC-Sp3- CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 394), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 234	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 395), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 235	RNA	C6NH ₂ -XAC-Sp3- CCGCGCGGAGGGGUUCAUUAUCCCGUUUGUC-idT (SEQ ID NO: 396), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 236	RNA	C6NH ₂ -GAC-Sp3- CCGCGCGGAGGGXUUUCAUUAUCCCGUUUGUC-idT (SEQ ID NO: 397), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3

		spacer (1,3-propanediol).
Aptamer 237	RNA	C6NH ₂ -GAC-Sp3- CCGCGCGGAGGGGUUCAUUAUCCCGUUUXUC-idT (SEQ ID NO: 398), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 238	RNA	C6NH ₂ -XAC-Sp3- CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUGUC-idT (SEQ ID NO: 399), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 239	RNA	C6NH ₂ -XAC-Sp3- CCGCGCGGAGGGGUUCAUUAUCCCGUUUXUC-idT (SEQ ID NO: 400), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 240	RNA	C6NH ₂ -GAC-Sp3- CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUXUC-idT (SEQ ID NO: 401), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 241	RNA	C6NH ₂ -XAC-Sp3- CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUXUC-idT (SEQ ID NO: 402), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 276	RNA	C6NH ₂ -AC-Sp3- CCGCGCGGAGGGGXUUUCAUUAUCCCGUUUGU-idT (SEQ ID NO: 403), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 277	RNA	C6NH ₂ -AC-Sp3- CCGCGCGGAGGGGUUCAUUAUCCCGUUUXU-idT (SEQ ID NO: 404),

		where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 278	RNA	C ₆ NH ₂ -AC-Sp3- CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXU-idT (SEQ ID NO: 405), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 279	RNA	C ₆ NH ₂ -C-Sp3- CCGCGCGGAGGGXUUUCAUUAUCCCGUUUG-idT (SEQ ID NO: 406), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 280	RNA	C ₆ NH ₂ -C-Sp3- CCGCGCGGAGGGGUUUCAUUAUCCCGUUUX-idT (SEQ ID NO: 407), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 281	RNA	C ₆ NH ₂ -C-Sp3- CCGCGCGGAGGGXUUUCAUUAUCCCGUUUX-idT (SEQ ID NO: 408), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 282	RNA	C ₆ NH ₂ -CXAC-Sp3- CCGCGCGGAGGGGAUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 409), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 283	RNA	C ₆ NH ₂ -CXAC-Sp3- CCGCGCGGAGGGXUAUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 410), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).

Aptamer 284	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGXUUACAUAUCCCGUUUXUCX-idT (SEQ ID NO: 411), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 285	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGXUUUCAUAAUCCCGUUUXUCX-idT (SEQ ID NO: 412), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 286	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGXUUUCAUUAACCCCGUUUXUCX-idT (SEQ ID NO: 413), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 287	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGGAUUAUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 414), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 288	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGGAUUAACAUAUCCCGUUUXUCX-idT (SEQ ID NO: 415), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 289	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGGAUUAUAAUCCCGUUUXUCX-idT (SEQ ID NO: 416), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 290	RNA	C6NH ₂ -CXAC-Sp3- CCGCGCGGAGGGXUAUCAUUAACCCCGUUUXUCX-idT (SEQ ID NO: 417), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X

		is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 291	RNA	C ₆ NH ₂ -CXAC-Sp3-CCGCGCGGAGGGGXUUUCAUAACCCCGUUUXUCX-idT (SEQ ID NO: 418), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 292	RNA	C ₆ NH ₂ -CXAC-Sp3-CCGCGCGGAGGGGXUUACAUAUCCCGUUUXUCX-idT (SEQ ID NO: 419), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 293	RNA	C ₆ NH ₂ -CXAC-Sp3-CAGCGCUGAGGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 420), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 294	RNA	C ₆ NH ₂ -CXAC-Sp3-CCXCGCGGAGGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 421), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 295	RNA	C ₆ NH ₂ -CXAC-Sp3-CAXCGCUGAGGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 422), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 296	RNA	C ₆ NH ₂ -CXAC-Sp3-CCGCGCGGAGGGGAUXUCAUCAUCCCGUUUXUCX-idT (SEQ ID NO: 423), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C ₆ NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 297	RNA	C ₆ NH ₂ -CXAC-Sp3-

		CCGCGCGGAGGGGAUCUCAUXAUCCCGUUUXUCX-idT (SEQ ID NO: 424), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 298	RNA	C6NH ₂ -CXAC-Sp3-Sp3-CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 425), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp3 is the SP3 spacer (1,3-propanediol).
Aptamer 299	RNA	C6NH ₂ -CXAC-Sp6-CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 426), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp6 is the SP6 spacer (1,6-hexanediol).
Aptamer 300	RNA	C6NH ₂ -CXAC-Sp9-CCGCGCGGAGGGXUUUCAUUAUCCCGUUUXUCX-idT (SEQ ID NO: 427), where G is 2'F; and A, C, and U are 2'OMe modified RNA; X is 2'OMe G modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and Sp9 is the SP9 spacer (triethyleneglycol).
Aptamer 301	RNA	CGACUCCGCGCGGAGGGUUGGAGGUUACCCGUUUGU CG (SEQ ID NO: 439), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 302	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GUUGGAGGUUACCCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 440), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 303	RNA	CGACUCCGCGCGGAGUCCCUAAUUUGGGGCGUUUGU CG (SEQ ID NO: 441), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 304	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUAAUUUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 442), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 305	RNA	CGACUCCGCGCGGAGUCCCUUCAUUGGGGCGUUUGU CG (SEQ ID NO: 443), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 306	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUUCAUUGGGGCGUUUGUCGCUAUGUGGAAAUGG

		CGCUGU (SEQ ID NO: 444), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 307	RNA	CGACUCCGCGCGGAGGGUAAUGGCUACCCGUUUGU CG (SEQ ID NO: 445), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 308	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GUUAAUGGCUACCCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 446), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 309	RNA	CGACUCCGCGCGGAGUCCCUGUAAUGGGGCGUUUGU CG (SEQ ID NO: 447), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 310	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUGUAAUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 448), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 311	RNA	CGACUCCGCGCGGAGUCCCUAUUUUGGGGCGUUUGU CG (SEQ ID NO: 449), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 312	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUAUUUUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 450), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 313	RNA	CGACUCCGCGCGGAGAGGAGGUUACCCUCGUUUGU CG (SEQ ID NO: 451), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 314	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGA GGAGGUUACCCUCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 452), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 315	RNA	CGACUCCGCGCGGAGUCCCUGAUUUGGGGCGUUUGU CG (SEQ ID NO: 453), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 316	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUGAUUUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 454), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 317	RNA	CGACUCCGCGCGGAGGGUACUGGCUACCCGUUUGU CG (SEQ ID NO: 455), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 318	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GUUACUGGCUACCCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 456), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 319	RNA	CGACUCCGCGCGGAGUCCCUAACAUGGGGCGUUUGU CG (SEQ ID NO: 457), where G is 2'F; and A, C, & U are

		2'OMe modified RNA.
Aptamer 320	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUAACAUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 458), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 321	RNA	CGACUCCGCGCGGAGGGGAGGCAACUCCCCGUUUGU CG (SEQ ID NO: 459), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 322	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GGAGGCAACUCCCCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 460), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 323	RNA	CGACUCCGCGCGGAGUCCCUUUAUUGGGGCGUUUGU CG (SEQ ID NO: 461), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 324	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUUUAUUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 462), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 325	RNA	CGACUCCGCGCGGAGUCCCUACAAUGGGGCGUUUGU CG (SEQ ID NO: 463), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 326	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUACAAUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 464), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 327	RNA	CGACUCCGCGCGGAGUGCCGUUUGAGGUACGUUUGU CG (SEQ ID NO: 465), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 328	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU GCCGUUUGAGGUACGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 466), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 329	RNA	CGACUCCGCGCGGAGGGCUGAGGCAAUGCCCGUUUG UC (SEQ ID NO: 467), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 330	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GCUGAGGCAAUGCCCGUUUGUCCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 468), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 331	RNA	CGACUCCGCGCGGAGUCCCUACUUGGGGCGUUUGU CG (SEQ ID NO: 469), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 332	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUACUUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 470), where G is 2'F; and A, C, & U

		are 2'OMe modified RNA.
Aptamer 333	RNA	CGACUCCGCGCGGAGUCCCCUCACAUGGGGCGUUUGU CG (SEQ ID NO: 471), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 334	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUCACAUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 472), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 335	RNA	CGACUCCGCGCGGAGGGUUACAGGCUACCCGUUUGU CG (SEQ ID NO: 473), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 336	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GUUACAGGCUACCCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 474), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 337	RNA	CGACUCCGCGCGGAGUCCCCUUUGUUGGGGCGUUUGU CG (SEQ ID NO: 475), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 338	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUUUGUUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 476), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 339	RNA	CGACUCCGCGCGGAGUCCCCUAAAUGGGGCGUUUGU CG (SEQ ID NO: 477), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 340	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUAAAUGGGGCGUUUGUCGCUAUGUGGAAAUGG CGCUGU (SEQ ID NO: 478), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 341	RNA	CGACUCCGCGCGGAGGGUUUGGCUACCCGUUUGUCG (SEQ ID NO: 479), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 342	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GUUUGGCUACCCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 480), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 343	RNA	CGACUCCGCGCGGAGGCUUGAGGUAGCCGUUUGUCG (SEQ ID NO: 481), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 344	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG CUUGAGGUAGCCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 482), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 345	RNA	CGACUCCGCGCGGAGUCCCACAUGGGGCGUUUGUCG (SEQ ID NO: 483), where G is 2'F; and A, C, & U are 2'OMe modified RNA.

Aptamer 346	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCACAUGGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 484), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 347	RNA	CGACUCCGCGCGGAGGGAUGAGGUUCCCGUUUGUCG (SEQ ID NO: 485), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 348	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GAUGAGGUUCCCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 486), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 349	RNA	CGACUCCGCGCGGAGGCAUGAGGUUGCCGUUUGUCG (SEQ ID NO: 487), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 350	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG CAUGAGGUUGCCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 488), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 351	RNA	CGACUCCGCGCGGAGUGCUGAGGUGCACGUUUGUCG (SEQ ID NO: 489), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 352	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU GCUGAGGUGCACGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 490), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 353	RNA	CGACUCCGCGCGGAGUCCCUAUUGGGGCGUUUGUCG (SEQ ID NO: 491), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 354	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUAUUGGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 492), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 355	RNA	CGACUCCGCGCGGAGGGUAGGCUACCCGUUUGUCG (SEQ ID NO: 493), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 356	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GUUAGGCUACCCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 494), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 357	RNA	CGACUCCGCGCGGAGCUUCGGAUGAGGCGUUUGUCG (SEQ ID NO: 495), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 358	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGC UUCGGAUGAGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 496), where G is 2'F; and A, C, & U are 2'OMe modified RNA.

Aptamer 359	RNA	CGACUCCGCGCGGAGUCCCCUCAUGGGGCGUUUGUCG (SEQ ID NO: 497), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 360	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUCAUGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 498), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 361	RNA	CGACUCCGCGCGGAGUCCCCGUAUGGGGCGUUUGUCG (SEQ ID NO: 499), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 362	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCGUAUGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 500), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 363	RNA	CGACUCCGCGCGGAGUCCCCAAUUGGGGCGUUUGUCG (SEQ ID NO: 501), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 364	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCAAUUGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 502), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 365	RNA	CGACUCCGCGCGGAGGGUUUGGUUACCCGUUUGUCG (SEQ ID NO: 503), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 366	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGG GUUUGGUUACCCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 504), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 367	RNA	CGACUCCGCGCGGAGUCCCCAUUUGGGGCGUUUGUCG (SEQ ID NO: 505), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 368	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCAUUUGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 506), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 369	RNA	CGACUCCGCGCGGAGUCCCACAAGGGGCGUUUGUCG (SEQ ID NO: 507), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 370	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCACAAGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 508), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 371	RNA	CGACUCCGCGCGGAGCUUCGGAAGAGGCGUUUGUCG (SEQ ID NO: 509), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 372	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGC

		UUCGGAAGAGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 510), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 373	RNA	CGACUCCGCGCGGAGUCCCAUUAGGGGCGUUUGUCG (SEQ ID NO: 511), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 374	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCAUUAGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 512), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 375	RNA	CGACUCCGCGCGGAGUGCCAACUGGUACGUUUGUCG (SEQ ID NO: 513), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 376	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU GCCAACUGGUACGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 514), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 377	RNA	CGACUCCGCGCGGAGUCCCUAUAGGGGCGUUUGUCG (SEQ ID NO: 515), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 378	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUAUAGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 516), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 379	RNA	CGACUCCGCGCGGAGUCCCUAAUGGGGCGUUUGUCG (SEQ ID NO: 517), where G is 2'F; and A, C, & U are 2'OMe modified RNA.
Aptamer 380	RNA	GGGAGAGUCGGUAGCCUCAACGACUCCGCGCGGAGU CCCUAAUGGGGCGUUUGUCGCUAUGUGGAAAUGGCG CUGU (SEQ ID NO: 518), where G is 2'F; and A, C, & U are 2'OMe modified RNA.

[00126] In some aspects, an aptamer of the disclosure may have a primary nucleic acid sequence according to any one of the aptamer sequences described in **Table 1**, or may have a primary nucleic acid sequence that shares at least 50% sequence identity to any one of the aptamer sequences described in **Table 1**. In some aspects, an aptamer of the disclosure may have a primary nucleic acid sequence consisting of any one of the aptamer sequences described in **Table 1**, or may have a nucleic acid sequence that shares at least 50% sequence identity to a primary nucleic acid sequence that consists of any one of the aptamer sequences described in **Table 1**. In some cases, the nucleic acid sequence may comprise one or more modified nucleotides. In some cases, at least 50% of said nucleic acid sequence may comprise the one or

more modified nucleotides. In some cases, the one or more modified nucleotides may comprise a 2'F-modified nucleotide, a 2'OMe-modified nucleotide, or a combination thereof. In some cases, the one or more modified nucleotides may be selected from the group consisting of: 2'F-G, 2'OMe-G, 2'OMe-U, 2'OMe-A, 2'OMe-C, an inverted deoxythymidine at the 3' terminus, and any combination thereof. In some cases, the aptamer may comprise a nucleic acid sequence comprising modified nucleotides as described in **Table 1**. In some cases, the aptamer is any aptamer described in **Table 1**. For example, the aptamer may be any one of Aptamers 4.2, 26-32, 47-76, 108, 109, 112-117, 119-185, 187-199, 201, 213-241, and 276-300. In some cases, the aptamer may be conjugated to a polyethylene glycol (PEG) molecule. In some cases, the PEG molecule may have a molecular weight of 80 kDa or less (*e.g.*, 40kDa).

[00127] In some cases, an aptamer of the disclosure may have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with any aptamer described herein. For example, an anti-VEGF-A aptamer of the disclosure may have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with any aptamer described in **Table 1**.

[00128] In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 50% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 55% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 60% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 65% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 70% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 75% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 80% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 85% sequence identity with any one of the aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 90% sequence identity with any one of the

aptamer sequences described in **Table 1**. In some cases, an anti-VEGF-A aptamer of the disclosure may have at least 95% sequence identity with any one of the aptamer sequences described in **Table 1**.

[00129] In some cases, an aptamer of the disclosure may have a primary nucleotide sequence that shares at least 10, at least 11, 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 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, or at least 40 contiguous nucleotides with a nucleotide sequence described in **Table 1**.

[00130] In such cases where specific nucleotide modifications have been recited, it should be understood that any number and type of nucleotide modifications may be substituted. For example, 2'OMe-G may be substituted for 2'F-G. Non-limiting examples of nucleotide modifications have been provided herein. In some instances, all of the nucleotides of an aptamer are modified. In some instances, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the nucleotides of an aptamer of the disclosure may be modified. In some aspects, an aptamer of the disclosure has the modified nucleotide sequence of any aptamer sequence described in **Table 1**.

[00131] In some cases, an aptamer of the disclosure may have a modified nucleotide sequence. In some cases, an aptamer of the disclosure may have a modified nucleotide sequence as described in **Table 1**. In some cases, an aptamer of the disclosure may have a primary nucleotide sequence according to any aptamer described in **Table 1**, and a modified nucleotide sequence that is different than that described in **Table 1**. In such cases, an aptamer of the disclosure may have a modified nucleotide sequence that shares at least 10% modification identity with any modified nucleotide sequence described in **Table 1**. For example, an aptamer of the disclosure may have a modified nucleotide sequence that shares at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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%, or at least 99% modification identity with any modified nucleotide sequence described in **Table 1**.

[00132] In some cases, an aptamer of the disclosure may have a primary nucleotide sequence of any aptamer sequence described in **Table 1**, and a modified nucleotide sequence in which at least 10% of the C nucleotides are modified (*e.g.*, 2'OMe-C). For example, an aptamer of the disclosure may have a modified nucleotide sequence in which at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the C nucleotides are modified (*e.g.*, 2'OMe-C). In some cases, an aptamer of the disclosure may have a modified nucleotide sequence wherein at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the C nucleotides (C) are modified according to **Table 1**.

[00133] In some cases, an aptamer of the disclosure may have a primary nucleotide sequence of any aptamer sequence described in **Table 1**, and a modified nucleotide sequence in which at least 10% of the A nucleotides are modified (*e.g.*, 2'OMe-A). For example, an aptamer of the disclosure may have a modified nucleotide sequence in which at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the A nucleotides are modified (*e.g.*, 2'OMe-A). In some cases, an aptamer of the disclosure may have a modified nucleotide sequence wherein at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the A nucleotides are modified according to **Table 1**.

[00134] In some cases, an aptamer of the disclosure may have a primary nucleotide sequence of any aptamer sequence described in **Table 1**, and a modified nucleotide sequence in which at least 10% of the U nucleotides are modified (*e.g.*, 2'OMe-U). For example, an aptamer of the disclosure may have a modified nucleotide sequence in which at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the U nucleotides are modified (*e.g.*, 2'OMe-U). In

some cases, an aptamer of the disclosure may have a modified nucleotide sequence wherein at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the U nucleotides are modified according to **Table 1**.

[00135] In some cases, an aptamer of the disclosure may have a primary nucleotide sequence of any aptamer sequence described in **Table 1**, and a modified nucleotide sequence in which at least 10% of the G nucleotides are modified (*e.g.*, 2'F-G, 2'OMe-G). For example, an aptamer of the disclosure may have a modified nucleotide sequence in which at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the G nucleotides are modified (*e.g.*, 2'F-G, 2'OMe-G). In some cases, an aptamer of the disclosure may have a modified nucleotide sequence wherein at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% of the G nucleotides are modified according to **Table 1**.

[00136] In some cases, an anti-VEGF-A aptamer of the disclosure may comprise a stem-loop secondary structure. In some cases, the aptamers of the disclosure may comprise, in a 5' to 3' direction, a first side of a first base paired stem; optionally, a first loop; a first side of a second base paired stem; a second loop; a second, complementary side of the second base paired stem; a third loop; a first side of a third base paired stem; a fourth loop; a second, complementary side of the third base paired stem; a fifth loop; and a second, complementary side of the first base paired stem.

[00137] In some embodiments, each element may be adjacent to each other. For example, the anti-VEGF-A aptamers of the disclosure may comprise, in a 5' to 3' direction, a first side of a first base paired stem. The 3' terminal end of the first side of the first base paired stem may be connected to the 5' terminal end of the first loop. The first loop may be connected at its 5' terminal end to the 3' terminal end of the first side of the first base paired stem, and the first loop may be connected at its 3' terminal end to the 5' terminal end of the first side of the second base paired stem. The first side of the second base paired stem may be connected at its 5' terminal

end to the 3' terminal end of the first loop, and the first side of the second base paired stem may be connected at its 3' terminal end to the 5' terminal end of the second loop. The second loop may be connected at its 5' terminal end to the 3' terminal end of the first side of the second base paired stem, and the second loop may be connected at its 3' terminal end to the 5' terminal end of a second, complementary side of the second base paired stem. The second, complementary side of the second base paired stem may be connected at its 5' terminal end to the 3' terminal end of the second loop, and the second, complementary side of the second base paired stem may be connected at its 3' terminal end to the 5' terminal end of a third loop. The third loop may be connected at its 5' terminal end to the 3' terminal end of the second, complementary side of the second base paired stem, and the third loop may be connected at its 3' terminal end to the 5' terminal end of a first side of a third base paired stem. The first side of the third base paired stem may be connected at its 5' terminal end to the 3' terminal end of the third loop, and the first side of the third base paired stem may be connected at its 3' terminal end to the 5' terminal end of the fourth loop. The fourth loop may be connected at its 5' terminal end to the 3' terminal end of the first side of the third base paired stem, and the fourth loop may be connected at its 3' terminal end to the 5' terminal end of the second, complementary side of the third base paired stem. The second, complementary side of the third base paired stem may be connected at its 5' terminal end to the 3' terminal end of the fourth loop, and the second, complementary side of the third base paired stem may be connected at its 3' terminal end to the 5' terminal end of a fifth loop. The fifth loop may be connected at its 5' terminal end to the 3' terminal end of the second, complementary side of the third base paired stem, and the fifth loop may be connected at its 3' terminal end to the 5' terminal end of a second, complementary side of the first base paired stem. The second, complementary side of the first base paired stem may be connected at its 5' terminal end to the 3' terminal end of the fifth loop. In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a terminal stem. In some cases, the terminal stem may be the first base paired stem (*e.g.*, S1/S1'). In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a plurality of terminal loops. In some cases, the terminal loops may include the second loop (*e.g.*, L2) and/or the fourth loop (*e.g.*, L4). In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a plurality of internal stems. In some cases, the internal stems may include the second stem (*e.g.*, S2/S2'), and/or the third stem (*e.g.*, S3/S3'). In some cases, the anti-VEGF-A aptamers of the disclosure may comprise a plurality of internal loops. In some

cases, the internal loops may include the first loop (*e.g.*, L1), the third loop (*e.g.*, L3), and/or the fifth loop (*e.g.*, L5).

[00138] In a particular aspect, an anti-VEGF-A aptamer of the disclosure may have a stem-loop secondary structure comprising: (i) a first side of Stem 1 (S1); (ii) optionally, Loop 1 (L1) connected to the 3' terminal end of S1 and the 5' terminal end of a first side of Stem 2 (S2); (iii) S2 connected to the 3' terminal end of L1 (or, when L1 is absent, the 3' terminal end of S1) and the 5' terminal end of Loop 2 (L2); (iv) L2 connected to the 3' terminal end of S2 and the 5' terminal end of a second, complementary side of Stem 2 (S2'); (v) S2' connected to the 3' terminal end of L2 and the 5' terminal end of Loop 3 (L3); (vi) L3 connected to the 3' terminal end of S2' and the 5' terminal end of a first side of Stem 3 (S3); (vii) S3 connected to the 3' terminal end of L3 and the 5' terminal end of Loop 4 (L4); (viii) L4 connected to the 3' terminal end of S3 and the 5' terminal end of a second, complementary side of Stem 3 (S3'); (ix) S3' connected to the 3' terminal end of L4 and the 5' terminal end of Loop 5 (L5); (x) L5 connected to the 3' terminal end of S3' and the 5' terminal end of a second, complementary side of Stem 1 (S1'); and (xi) S1' connected to the 3' terminal end of L5. In particular aspects, an anti-VEGF-A aptamer of the disclosure may have the following stem-loop secondary structure: 5'-S1-L1-S2-L2-S2'-L3-S3-L4-S3'-L5-S1'. In other particular aspects, an anti-VEGF-A aptamer of the disclosure may have the following stem-loop secondary structure: 5'-S1-S2-L2-S2'-L3-S3-L4-S3'-L5-S1'.

[00139] In some cases, Stem 1 (S1) may comprise from two to eight contiguous base pairs. For example, S1 may comprise two, three, four, five, six, seven, or eight contiguous base pairs. In some cases, S1 may comprise one or more mismatched nucleotides. For example, S1 may comprise one, two, or three mismatched nucleotides. In some cases, the mismatched nucleotides may be adjacent to each other. In other cases, the mismatched nucleotides may be separated by one, two, or three base pairs. In some cases, the 3' terminal nucleotide of the first side of S1 (*e.g.*, nucleotide position 8), and the 5' terminal nucleotide of the second, complementary side of S1, may form a base pair (*e.g.*, nucleotide position 39). In some cases, this base pair may be C•G. In some cases, the base pair between the 3' terminal nucleotide of the first side of S1, and the 5' terminal nucleotide of the second, complementary side of S1 may be separated from other S1 base pairs by mismatched nucleotides (*e.g.*, at positions 7 and 40). In some cases, S1 may comprise a mismatch at nucleotide positions 5 and 42. In some cases, S1 may be truncated. In

some cases, the first side of S1 may comprise a consensus nucleic acid sequence of 5'-HNBYHDCC-3', and the second, complementary side of S1 may comprise a consensus nucleic acid sequence of 5'-GKYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; K is G or U; V is A, C, or G; and W is A or U. In some cases, the first side of S1 may comprise a consensus nucleic acid sequence of 5'-HNBYHDNN-3', and the second, complementary side of S1 may comprise a consensus nucleic acid sequence of 5'-NNYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; and D is A, G, or U.

[00140] In some cases, L1 may be optional. In cases in which L1 is present, L1 may comprise one nucleotide. In some cases, when L1 is present, L1 has a nucleic acid sequence of 5'-U-3'. In some cases, when L1 is present, L1 has a nucleic acid sequence of 5'-A-3'. In some cases, when L1 is present, L1 has a nucleic acid sequence of 5'-C-3'. In some cases, when L1 is present, L1 has a nucleic acid sequence of 5'-G-3'. In some cases, L1 may comprise a single non-nucleotidyl spacer 3 modification (e.g., 1,3-propanediol). In some cases, L1 may comprise two non-nucleotidyl spacer 3 modifications. In some cases, L1 may comprise a 6-carbon spacer (e.g., 1,6-hexanediol). In some cases, L1 may comprise a 9-carbon spacer (e.g., triethyleneglycol).

[00141] In some cases, S2 may comprise two base pairs. In some cases, S2 may comprise more than one base pair. In some cases, S2 may comprise less than three base pairs. In some cases, a first side of S2 may comprise a consensus nucleic acid sequence of 5'-CC-3', and a second, complementary side of S2 may comprise a consensus nucleic acid sequence of 5'-GG-3'. In some cases, the first side of S2 may comprise a consensus nucleic acid sequence of 5'-NN-3', and the second, complementary side of S2 may comprise a consensus nucleic acid sequence of 5'-NN-3', where N is A, C, G, or U.

[00142] In some cases, L2 may comprise four nucleotides. In some cases, L2 may have more than three nucleotides. In some cases, L2 may have less than five nucleotides. In some cases, L2 may comprise a consensus nucleic acid sequence of 5'-GCGC-3'. In some cases, L2 may comprise a consensus nucleic acid sequence of 5'-GYGC-3', where Y is C or U. In some cases, L2 may comprise a consensus nucleic acid sequence of 5'-KNGC-3', where K is G or U; and N is A, C, G, or U.

[00143] In some cases, S3 may comprise from four to six base pairs. For example, S3 may comprise four base pairs, five base pairs, or six base pairs. In some cases, S3 may comprise less than seven base pairs. In some cases, S3 may comprise more than three base pairs. In some cases, when S3 comprises six base pairs, a first side of S3 may be seven nucleotides in length. In such cases, the 3' terminal nucleotide of the first side of S3 may form a single mismatch. In some cases, the length of S3 may be directly related to the length of L4. In some cases, when S3 is four base pairs in length, L4 is eight nucleotides in length. In some cases, when S3 is four base pairs in length, L4 is six nucleotides in length. In some cases, when S3 is five base pairs in length, L4 is six nucleotides in length. In some cases, when S3 is five base pairs in length, L4 is four nucleotides in length. In some cases, when S3 is six base pairs in length, L4 is four nucleotides in length. In some cases, when S3 comprises six base pairs and a single mismatched nucleotide, L4 is three nucleotides in length. In some cases, a first side of S3 may comprise a consensus nucleic acid sequence of 5'-GRGRWN-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-NHYCYC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U. In some cases, a first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGGRUN3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-NWyCCC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U. In some cases, a first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGGRUN3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-NAYCCC-3', where R is A or G; N is A, C, G, or U; and Y is C or U. In some cases, a first side of S3 comprises a consensus nucleic acid sequence of 5'-GGGRUD-3', and a second, complementary side of S3 comprises a consensus nucleic acid sequence of 5'-HWYCCC-3', wherein R is A or G; D is A, G, or U; H is A, C, or U; W is A or U; and Y is C or U. In some cases, when S3 is four base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GKGN-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-NSMC-3', where K is G or U; N is A, C, G or U and M is A or C. In some cases, when S3 is four base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGGG-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-CCCC-3'. . In some cases, when S3 is four base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGGU-3', and the second, complementary

side of S3 may comprise a consensus nucleic acid sequence of 5'-ACCC-3'. In some cases, when S3 is four base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGCU-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-AGCC-3'. In some cases, when S3 is five base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GBBNY-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-RNBNC-3', where R is A or G; and Y is C or U. In some cases, when S3 is five base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGGRU-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-AYCCC-3', where R is A or G; and Y is C or U. In some cases, when S3 is five base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-SVVVK-, and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-MBBBS-3', where S is G or C; V is A, C, or G; K is G or U; M is A or C; and B is C, G, or U. In some cases, when S3 is six base pairs in length, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGGGUD-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-HAUCCC-3', where D is A, G, or U; and H is A, C, or U. In some cases, when S3 comprises six base pairs and a single mis-matched nucleotide, the first side of S3 may comprise a consensus nucleic acid sequence of 5'-GGGRUUR-3', and the second, complementary side of S3 may comprise a consensus nucleic acid sequence of 5'-UAUCCC-3', where the underlined U is the single mis-matched nucleotide, and R is A or G.

[00144] In some cases, L3 may comprise one nucleotide. In some cases, L3 may comprise less than two nucleotides. In some cases, L3 may comprise a consensus nucleic acid sequence of 5'-A-3'. In some cases, L3 may comprise a consensus nucleic acid sequence of 5'-W-3', where W is A or U.

[00145] In some cases, L4 may comprise eight, six, four, or three nucleotides. In some cases, the variation between the number of nucleotides in loop L4 may directly relate to the variation in the length of S3 (*e.g.*, as described above). In some cases, when L4 is three nucleotides in length, L4 may comprise a consensus nucleic acid sequence of 5'-MAU-3', where M is A or C. In some cases, when L4 is three nucleotides in length, L4 may comprise a consensus nucleic acid sequence of 5'-CUA-3'. In some cases, when L4 is four nucleotides in length, L4 may comprise

a consensus nucleic acid sequence of 5'-DNAH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, when L4 is four nucleotides in length, L4 may comprise a consensus nucleic acid sequence of 5'-DNDH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, when L4 is four nucleotides in length, L4 may comprise a consensus nucleic acid sequence of 5'-DNDN-3', where D is A, G, or U; N is A, C, G, or U; and N is A, C, G or U. In some cases, when L4 is six nucleotides in length, L4 may comprise a consensus nucleic acid sequence of 5'-UDNDHU-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-UDRGBU-3', where D is A, G, or U; R is A or G, N is A, C, G, or U; and B is G, C, or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-KNNNNW-3', where K is U or G, N is A, C, G, or U; and W is A, or U. In some cases, the L4 comprises a consensus nucleic acid sequence of 5'-UDUHRKYU-3', where D is A D, or U; H is A, C or U; R is A or G; K is G or U and Y is C or U. In some cases, when L4 is eight nucleotides in length, L4 may comprise a consensus nucleic acid sequence of 5'-UUUCAUUU-3'.

[00146] In some cases, L5 may comprise four nucleotides. In some cases, L5 may comprise less than five nucleotides. In some cases, L5 may comprise more than three nucleotides. In some cases, L5 may comprise a consensus nucleic acid sequence of 5'-GYUU-3', where Y is C or U. In some cases, L5 may comprise a consensus nucleic acid sequence of GNNN-3', where N is A, C, G, or U. In some cases, L5 may comprise a consensus nucleic acid sequence of 5'-GNHW-3', where N is A, C, G, or U; H is A, C, or U; and W is A or U.

[00147] In some cases, an anti-VEGF-A aptamer of the disclosure may comprise a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGGGRUDDNDHHWYCCCGYUUGKYNKVNW-3' (SEQ ID NO: 1), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G. In some cases, when S3 is five base pairs in length and L4 is six nucleotides in length, an anti-VEGF-A aptamer of the disclosure may comprise a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGGGRUUDNDHUAYCCCGYUUGKYNKVNW-3' (SEQ ID NO: 2), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; K is G or U; V is A, C, or G; and W is A or U. In some cases, when S3 is six base pairs in length and L4 is 4 nucleotides in length, an anti-VEGF-A aptamer of the disclosure may

comprise a consensus nucleic acid sequence of 5'-

WNKYHDCCUCCGCGCGGAGGGGUDDNAHHAUCCCGUUUGGYBKMHW-3' (SEQ ID NO: 3), where W is A or U; N is A, C, G, or U; K is G or U; Y is C or U; H is A, C, or U; D is A, G, or U; B is C, G, or U; and M is A or C. In some cases, when S3 is four base pairs in length and L4 is eight nucleotides in length, an anti-VEGF-A aptamer of the disclosure may comprise a consensus nucleic acid sequence of 5'-

AUGCCGCCUCCGCGCGGAGGGGUUCAUUUCCCCGUUUGGCUGCAU-3' (SEQ ID NO: 4). In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-

HNBYHDCCUCCGCGCGGAGDSBHDNNNNHNNBNCGYUUGKYNKVNW-3' (SEQ ID NO: 436), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G.

[00148] In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-

HNBYHDCCUCCGCGCGGAGKKBHYDNNNKDBVCGYUUGKYNKVNW-3' (SEQ ID NO: 437), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G. In some cases, an anti-VEGF-A aptamer of the disclosure comprises a consensus nucleic acid sequence of 5'-

HNBYHDCCUCCGCGCGGAGKSHUDRGBUDSMCGYUUGKYNKVNW-3' (SEQ ID NO: 438), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; M is A or C; and V is A, C, or G.

[00149] In some cases, an anti-VEGF-A aptamer of the disclosure may comprise a consensus nucleic acid sequence of 5'-

HNBYHDNNN*NNKNGCNNWGGGRUNDNDHNWYCCCGNNNNNNYNKVNW-3' (SEQ ID NO: 5), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; N* is A, C, G, U, can be deleted entirely, or is a non-nucleotidyl spacer 3 modification, a 6 carbon alkyl linker (1,6-hexanediol), or a spacer 9 (triethyleneglycol) modification; D is A, G, or U; K is G or U; W is A or U; R is A or G; and V is A, C, or G

Anti-VEGF-A Compositions

[00150] In some aspects, the disclosure provides anti-VEGF-A compositions that inhibit a function associated with VEGF-A. The anti-VEGF-A compositions may include one or more

anti-VEGF-A aptamers that bind to specific regions of VEGF-A with high specificity and high affinity. In some cases, the anti-VEGF-A compositions may include one or more anti-VEGF-A aptamers that bind to a region of VEGF-A that includes the receptor binding face of VEGF-A. In some cases, the anti-VEGF-A compositions may include one or more anti-VEGF-A aptamers that bind to a region of VEGF-A which includes the receptor binding domain of VEGF-A, or a portion thereof. The receptor binding domain of VEGF-A may include any one or more of residues 1-109 as described in **SEQ ID NOs: 6-10**. In some cases, the anti-VEGF-A compositions may include one or more anti-VEGF-A aptamers that prevent or reduce binding of one or more isoforms or variants of VEGF-A with Flt-1, KDR, Nrp-1, or any combination thereof.

Anti-VEGF-A Aptamers

[00151] In some aspects, anti-VEGF-A aptamers of the disclosure may block or reduce the interaction of VEGF-A with Flt-1, may block or reduce the interaction of VEGF-A with KDR, may block or reduce the interaction of VEGF-A with Nrp-1, or any combination thereof.

[00152] In some aspects, anti-VEGF-A aptamers of the disclosure bind to structural features that are common to VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, anti-VEGF-A aptamers of the disclosure bind to regions of VEGF-A other than the heparin binding domain present in VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. The heparin binding domain may include residues 111-165 as described by **SEQ ID NOs: 6-8**. Without wishing to be bound by theory, the cationic heparin binding domain of VEGF-A is thought to be the dominant epitope for aptamer recognition due to the anionic nature of the oligonucleotide sugar phosphate backbone. Therefore, selection of aptamers to regions of VEGF-A other than the heparin binding domain have proven difficult. For example, pegaptanib (brand name Macugen[®]) is an oligonucleotide inhibitor of VEGF-A which binds to the heparin binding domain. Because VEGF-A₁₂₁ and VEGF-A₁₁₀ lack the heparin binding domain, pegaptanib does not bind to or inhibit VEGF-A₁₂₁ and VEGF-A₁₁₀, thereby providing inferior VEGF-A suppression as compared to an inhibitor that binds to the receptor binding domain of VEGF-A. Similarly, additional aptamer inhibitors of VEGF-A have been described which bind to the heparin binding domain of VEGF-A. For example, a DNA aptamer specific for VEGF-A₁₆₅, but not VEGF-A₁₂₁ has been described (Hasegawa, Hijiri, Koji Sode, and Kazunori Ikebukuro. "Selection of DNA aptamers against VEGF165 using a protein competitor and the aptamer

blotting method." *Biotechnology letters* 30.5 (2008): 829-834.; Kaur, Harleen, and Lin-Yue Lanry Yung. "Probing high affinity sequences of DNA aptamer against VEGF165." *PLoS One* 7.2 (2012): e31196.). Additionally, a 2'-O-Methyl aptamer has been described that binds to VEGF-A₁₆₅, but not VEGF-A₁₂₁ (Burmeister, Paula E., et al. "Direct in vitro selection of a 2'-O-methyl aptamer to VEGF." *Chemistry & biology* 12.1 (2005): 25-33.). Similarly, DNA aptamers with an expanded 6-base nucleotide alphabet have been described that recognize VEGF-A₁₆₅, but not VEGF-A₁₂₁ (Kimoto, Michiko, et al. "Generation of high-affinity DNA aptamers using an expanded genetic alphabet." *Nature biotechnology* 31.5 (2013): 453.). Furthermore, aptamer selections against other proteins that contain heparin binding domains tend to generate aptamers to those epitopes. For example, RNA aptamers that bind to the heparin binding domain have been described for thrombin (Jeter, Martha L., et al. "RNA aptamer to thrombin binds anion-binding exosite 2 and alters protease inhibition by heparin-binding serpins." *FEBS letters* 568.1-3 (2004): 10-14.; Long, Stephen B., et al. "Crystal structure of an RNA aptamer bound to thrombin." *RNA* (2008)), basic fibroblast growth factor (Jellinek, D., et al. "High-affinity RNA ligands to basic fibroblast growth factor inhibit receptor binding." *Proceedings of the National Academy of Sciences* 90.23 (1993): 11227-11231.), interleukin-8 (Sung, Ho Jin, et al. "Inhibition of human neutrophil activity by an RNA aptamer bound to interleukin-8." *Biomaterials* 35.1 (2014): 578-589.), and *Plasmodium falciparum* erythrocyte membrane protein 1 (Barfod, Anders, Tina Persson, and Johan Lindh. "In vitro selection of RNA aptamers against a conserved region of the Plasmodium falciparum erythrocyte membrane protein 1." *Parasitology research* 105.6 (2009): 1557-1566.).

[00153] In some cases, anti-VEGF-A aptamers of the disclosure may bind to a region of VEGF-A that includes the receptor-binding face of any isoform or variant of VEGF-A, or portions thereof. The receptor-binding face of VEGF-A may include strands $\beta 2$, $\beta 5$, and $\beta 6$, and loops $\beta 1$ to $\beta 2$ of one monomer, and the N-terminal α helix and loop $\beta 3$ to $\beta 4$ of a second monomer. The receptor-binding face of VEGF-A may be as described by Muller et al. "The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 Å resolution: multiple copy flexibility and receptor binding." *Structure* 5.10 (1997): 1325-1338. In some cases, anti-VEGF-A aptamers of the disclosure may bind to one or more amino acid residues of any isoform or variant of VEGF-A, including, without limitation, Phe17, Ile43, Ile46, Glu64, Gln79, Ile83, Lys84, Pro85, Arg82, His86, Asp63, and Glu67 as described by **SEQ ID NOs: 6-10**. In some cases, anti-

VEGF-A aptamers that bind to the receptor-binding face of VEGF-A, or a portion thereof, may prevent or reduce the association of VEGF-A with one or more of Flt-1, KDR, or Nrp-1. In some cases, anti-VEGF-A aptamers that bind to the receptor-binding face of VEGF-A, or a portion thereof, may interact with recombinant bead-bound VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ as measured by flow cytometry or may interact with recombinant surface-bound VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ as measured by surface plasmon resonance (*see Examples 1 and 2*, respectively). In some cases, anti-VEGF-A aptamers that bind to the receptor-binding face of VEGF-A, or a portion thereof, may inhibit or reduce the interaction of VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ with KDR as measured by a reduction in FRET signal (*see Example 3*). In some cases, anti-VEGF-A aptamers that bind to the receptor-binding face of VEGF-A, or a portion thereof, may inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced trans autophosphorylation of the intracellular domain of KDR as measured by phospho-KDR AlphaLISA[®] (*see Example 4*). In some cases, anti-VEGF-A aptamers that bind to the receptor-binding face of VEGF-A, or a portion thereof, may inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced gene expression of tissue factor in HUVEC cells as measured by qPCR. In some cases, anti-VEGF-A aptamers that bind to the receptor-binding face of VEGF-A, or a portion thereof, may inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced tube formation of GFP-HUVECs in co-culture with human dermal fibroblasts cells as measured by change in network length or network area (*see Example 5*). In some cases, anti-VEGF-A aptamers that bind to the receptor-binding face of VEGF-A, or a portion thereof, may inhibit or reduce vascular leakage in a mouse, rat, rabbit, or primate eye following exogenous VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ challenge as measured by fluorescein angiography and Evans-blue albumin staining.

[00154] In some cases, anti-VEGF-A aptamers of the disclosure may bind to a region of VEGF-A that includes the receptor binding domain of any isoform or variant of VEGF-A, or portions thereof. The receptor binding domain of VEGF-A may include one or more of residues 1-109, as described in **SEQ ID NOs: 6-10**. In some cases, anti-VEGF-A aptamers of the disclosure may bind to one or more amino acid residues of any isoform or variant of VEGF-A, including, without limitation, Phe17, Tyr21, Tyr25, Ile43, Ile46, Ile83, Asp63, Glu64, Pro85, and His86, as described by **SEQ ID NOs: 6-10**. In some cases, anti-VEGF-A aptamers of the disclosure may bind to a region within the receptor binding domain of VEGF-A which results in global

conformational changes in VEGF-A such that it no longer binds to and activates signaling via KDR. In some cases, anti-VEGF-A aptamers that bind to the receptor binding domain of VEGF-A, or a portion thereof, may prevent or reduce the association of VEGF-A with one or more of Flt-1, KDR, and Nrp-1. In some cases, anti-VEGF-A aptamers that bind to the receptor binding domain of VEGF-A, or a portion thereof, may interact with recombinant bead-bound VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ as measured by flow cytometry or may interact with recombinant surface-bound VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ as measured by surface plasmon resonance (*see Examples 1 and 2*, respectively). In some cases, anti-VEGF-A aptamers that bind to the receptor-binding domain of VEGF-A, or a portion thereof, may inhibit or reduce the interaction of VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ with KDR as measured by a reduction in FRET signal (*see Example 3*). In some cases, anti-VEGF-A aptamers that bind to the receptor-binding domain of VEGF-A, or a portion thereof, may inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced trans autophosphorylation of the intracellular domain of KDR as measured by phospho-KDR AlphaLISA[®] (*see Example 4*). In some cases, anti-VEGF-A aptamers that bind to the receptor binding domain of VEGF-A, or a portion thereof, may inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced gene expression of tissue factor in HUVEC cells as measured by qPCR. In some cases, anti-VEGF-A aptamers that bind to the receptor binding domain of VEGF-A, or a portion thereof, may inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced tube formation of GFP-HUVECs in co-culture with human dermal fibroblasts cells as measured by change in network length or network area (*see Example 5*). In some cases, anti-VEGF-A aptamers that bind to the receptor binding domain of VEGF-A, or a portion thereof, may inhibit or reduce vascular leakage in a mouse, rat, rabbit, or primate eye following exogenous VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ challenge as measured by fluorescein angiography and Evans-blue albumin staining.

Binding Affinity

[00155] The dissociation constant (K_d) can be used to describe the affinity of an aptamer for a target (or to describe how tightly the aptamer binds to the target) or to describe the affinity of an aptamer for a specific epitope of a target. The dissociation constant may be defined as the molar concentration at which half of the binding sites of a target are occupied by the aptamer. Thus, the smaller the K_d , the tighter the binding of the aptamer to its target. In some cases, an anti-VEGF-A aptamer of the disclosure may have a K_d for one or more isoforms or variants of VEGF-A of

less than about 1000 nM, for example, less than about 500 nM, less than about 100 nM, less than about 50 nM, less than about 10 nM, less than about 5 nM, less than about 1 nM, less than about 0.5 nM, or less than about 0.1 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, an anti-VEGF-A aptamer may have a dissociation constant (K_d) for one or more isoforms or variants of VEGF-A of less than about 50 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, an anti-VEGF-A aptamer may have a dissociation constant (K_d) for one or more isoforms or variants of VEGF-A of less than about 25 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, an anti-VEGF-A aptamer may have a dissociation constant (K_d) for one or more isoforms or variants of VEGF-A of less than about 10 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, an anti-VEGF-A aptamer may have a dissociation constant (K_d) for one or more isoforms or variants of VEGF-A of less than about 5 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, an anti-VEGF-A aptamer may have a dissociation constant (K_d) for one or more isoforms or variants of VEGF-A of less than about 1 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, an anti-VEGF-A aptamer may have a dissociation constant (K_d) for one or more isoforms or variants of VEGF-A of less than about 0.5 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, an anti-VEGF-A aptamer may have a dissociation constant (K_d) for one or more isoforms or variants of VEGF-A of less than about 0.1 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, the aptamer may be a pan-variant specific aptamer that binds to each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆ with a K_d of less than about 1000 nM, for example, less than about 500 nM, less than about 100 nM, less than about 50 nM, less than about 25 nM, less than about 10 nM, less than about 5 nM, less than about 1 nM, less than about 0.5 nM, or less than about 0.1 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, the aptamer may bind to any region of VEGF-A described herein, or a portion thereof, with a K_d of less than about 1000 nM, for example, less than about 500 nM, less than about 100 nM, less than about 50 nM, less than about 25 nM, less than about 10 nM, less than about 5 nM, less than about 1 nM, less than about 0.5 nM, or less than about 0.1 nM, as measured by a surface plasmon resonance assay (*see Example 2*). In some cases, the aptamer may bind to the receptor-binding face or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 1000 nM,

for example, less than about 500 nM, less than about 100 nM, less than about 50 nM, less than about 25 nM, less than about 10 nM, less than about 5 nM, less than about 1 nM, less than about 0.5 nM, or less than about 0.1 nM, as measured by a surface plasmon assay (*see Example 2*). In some cases, the anti-VEGF-A aptamer may bind to the receptor-binding face or the receptor binding domain of VEGF-A, or portions thereof, with a K_d from about 0.5 nM to about 25 nM, as measured by a surface plasmon resonance assay (*see Example 2*).

[00156] In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor-binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 50 nM as measured by a surface plasmon assay (*see Example 2*), and may have an IC_{50} of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 50 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 10 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 50 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 50 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding

domain of VEGF-A, or portions thereof, with a K_d of less than about 50 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 0.5 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 50 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 0.1 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**).

[00157] In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 10 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 10 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 10 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see, **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 10 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 10 nM as

measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 10 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 0.5 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 10 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 0.1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*).

[00158] In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 5 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 5 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 10 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 5 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have

an IC_{50} of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 5 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 5 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 0.5 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 5 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 0.1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*).

[00159] In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 1 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 1 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 10 nM as measured by a VEGF-A:KDR competition

binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 1 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 1 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 1 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 0.5 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 1 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 0.1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*).

[00160] In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.5 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay

(see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.5 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 10 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.5 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.5 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.5 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 0.5 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.5 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 0.1 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**).

[00161] In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.1 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.1 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 10 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.1 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.1 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A, or portions thereof, with a K_d of less than about 0.1 nM as measured by a surface plasmon resonance assay (see **Example 2**), and may have an IC_{50} of less than about 0.5 nM as measured by a VEGF-A:KDR competition binding assay (see **Example 3**), a KDR phosphorylation AlphaLISA[®] assay (see **Example 4**), or an *in vitro* model of VEGF-A-induced angiogenesis (see **Example 5**). In some cases, the aptamers disclosed herein may bind to a region of VEGF-A, such as the receptor binding face of VEGF-A, or the receptor binding

domain of VEGF-A, or portions thereof, with a K_d of less than about 0.1 nM as measured by a surface plasmon resonance assay (*see Example 2*), and may have an IC_{50} of less than about 0.1 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*), a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*), or an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*).

[00162] In some aspects, the aptamers disclosed herein may have an improved half-life as compared to other therapeutics, including antibodies. In some cases, the aptamers may have an improved half-life in a biological fluid or solution as compared to an antibody. In some cases, the aptamers may have an improved half-life *in vivo* as compared to an antibody. In one example, the aptamers may have an improved half-life when injected into the eye (intraocular half-life) as compared to an antibody. In some cases, the aptamers may have an improved intraocular half-life when injected into the eye of a human. In some cases, the aptamers may demonstrate improved stability over antibodies under physiological conditions.

[00163] In some cases, the aptamers described herein may have an intraocular half-life of at least 7 days in a human. In some cases, the aptamers described herein may have an intraocular half-life of at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 20 days or greater in a human.

[00164] In some cases, the aptamers described herein may have an intraocular half-life of at least 1 day in a non-human animal (*e.g.*, rodent/rabbit/monkey/chimpanzee/pig). In some cases, the aptamers described herein may have an intraocular half-life of at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days or greater in a non-human animal such as a rodent, rabbit or monkey.

[00165] In some aspects, the aptamers described herein may have a shorter half-life as compared to other therapeutics. For example, an unmodified or unconjugated aptamer may have a lower half-life as compared to a modified or conjugated aptamer, however, the low molecular weight of the unmodified or unconjugated forms may allow for orders of magnitude greater initial concentrations, thereby achieving greater duration/efficacy. In some examples, the aptamer may have an intraocular half-life of less than about 7 days in a human. In some examples, the aptamers described herein may have an intraocular half-life of less than about 6 days, less than about 5 days or even less than about 4 days in a human.

[00166] The aptamers disclosed herein may demonstrate high specificity for VEGF-A versus other members of the VEGF family, including VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PlGF. In some cases, the aptamer may be selected such that the aptamer has high affinity for VEGF-A, but with little to no affinity for VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, or PlGF. In some cases, the aptamers of the disclosure may bind to VEGF-A with a specificity of at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 250-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, or at least 100,000-fold, or greater than 100,000-fold than the aptamers bind to VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, or PlGF at relative serum concentrations.

[00167] The activity of a therapeutic agent can be characterized by the half maximal inhibitory concentration (IC_{50}). The IC_{50} may be calculated as the concentration of therapeutic agent in nM at which half of the maximum inhibitory effect of the therapeutic agent is achieved. The IC_{50} may be dependent upon the assay utilized to calculate the value. In some examples, the IC_{50} of an aptamer described herein may be less than 100 nM, less than 50 nM, less than 25 nM, less than 10 nM, less than 5 nM, less than 1 nM, less than 0.5 nM, less than 0.1 nM or less than 0.01 nM as measured by a VEGF-A:KDR competition binding assay (*see Example 3*). In some examples, the IC_{50} of an aptamer described herein may be less than 100 nM, less than 50 nM, less than 25 nM, less than 10 nM, less than 5 nM, less than 1 nM, less than 0.5 nM, less than 0.1 nM or less than 0.01 nM as measured by a KDR phosphorylation AlphaLISA[®] assay (*see Example 4*). In some examples, the IC_{50} of an aptamer described herein may be less than 100 nM, less than 50 nM, less than 25 nM, less than 10 nM, less than 5 nM, less than 1 nM, less than 0.5 nM, less than 0.1 nM or less than 0.01 nM as measured by an *in vitro* model of VEGF-A-induced angiogenesis (*see Example 5*).

[00168] Aptamers generally have high stability at ambient temperatures for extended periods of time. The aptamers described herein may demonstrate greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, greater than 99.5%, or greater than 99.9% activity in solution under physiological conditions at 30 days or later.

[00169] In some cases, a composition of the disclosure comprises anti-VEGF-A aptamers, wherein essentially 100% of the anti-VEGF-A aptamers comprise nucleotides having ribose in

the β -D-ribofuranose configuration. In other examples, a composition of the disclosure may comprise anti-VEGF-A aptamers, wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or greater than 90% of the anti-VEGF-A aptamers have ribose in the β -D-ribofuranose configuration.

Indications

[00170] In some aspects, the methods and compositions provided herein may be suitable for the treatment of ocular diseases or disorders. In some aspects, the methods and compositions provided herein may be suitable for the prevention of ocular diseases or disorders. In some aspects, the methods and compositions provided herein may be suitable to slow or halt the progression of ocular diseases or disorders. In some cases, the ocular disease or disorder is diabetic retinopathy. In some cases, the ocular disease or disorder is retinopathy of prematurity. In some cases, the ocular disease or disorder is central retinal vein occlusion. In some cases, the ocular disease or disorder is macular edema. In some cases, the ocular disease or disorder is choroidal neovascularization. In some cases, the ocular disease or disorder is neovascular (or wet) age-related macular degeneration. In some cases, the ocular disease or disorder is myopic choroidal neovascularization. In some cases, the ocular disease or disorder is punctate inner choroidopathy. In some cases, the ocular disease or disorder is presumed ocular histoplasmosis syndrome. In some cases, the ocular disease or disorder is familial exudative vitreoretinopathy. In some cases, the ocular disease or disorder is retinoblastoma.

[00171] Additional examples of ocular diseases or disorders that may be amenable to treatment by the methods and compositions provided herein may include, without limitation, pterygium, inflammatory conjunctivitis, including allergic and giant papillary conjunctivitis, infectious conjunctivitis, vernal keratoconjunctivitis, Stevens-Johnson disease, corneal herpetic keratitis, rhegmatogenous retinal detachment, pseudo-exfoliation syndrome, endophthalmitis, scleritis, corneal ulcers, dry eye syndrome, glaucoma, ischemic retinal disease, corneal transplant rejection, complications related to intraocular surgery such as intraocular lens implantation and inflammation associated with cataract surgery, Behcet's disease, Stargardt disease, immune complex vasculitis, Fuch's disease, Vogt-Koyanagi-Harada disease, subretinal fibrosis, keratitis, vitreo-retinal inflammation, ocular parasitic infestation/migration, retinitis pigmentosa, cytomegalovirus retinitis and choroidal inflammation, ectropion, lagophthalmos, blepharochalasis, ptosis, xanthelasma of the eyelid, parasitic infestation of the eyelid, dermatitis

of the eyelid, dacryoadenitis, epiphora, dysthyroid exophthalmos, conjunctivitis, scleritis, adenovirus keratitis, corneal ulcer, corneal abrasion, snow blindness, arc eye, Thygeson's superficial punctate keratopathy, corneal neovascularization, Fuchs' dystrophy, keratoconus, keratoconjunctivitis sicca, iritis, sympathetic ophthalmia, cataracts, chorioretinal inflammation, focal chorioretinal inflammation, focal chorioretinitis, focal choroiditis, focal retinitis, focal retinochoroiditis, disseminated chorioretinal inflammation, disseminated chorioretinitis, disseminated choroiditis, disseminated retinitis, disseminated retinochoroiditis, exudative retinopathy, posterior cyclitis, pars planitis, Harada's disease, chorioretinal scars, macula scars of posterior pole, solar retinopathy, choroidal degeneration, choroidal atrophy, choroidal sclerosis, angioid streaks, hereditary choroidal dystrophy, choroideremia, choroidal dystrophy (central areolar), gyrate atrophy (choroid), ornithinaemia, choroidal haemorrhage and rupture, choroidal haemorrhage (not otherwise specified), choroidal haemorrhage (expulsive), choroidal detachment, retinoschisis, retinal artery occlusion, retinal vein occlusion, hypertensive retinopathy, diabetic retinopathy, retinopathy, retinopathy of prematurity, macular degeneration, Bull's Eye maculopathy, epiretinal membrane, peripheral retinal degeneration, hereditary retinal dystrophy, retinitis pigmentosa, retinal haemorrhage, separation of retinal layers, central serous retinopathy, retinal detachment, macular edema, glaucoma – optic neuropathy, glaucoma suspect – ocular hypertension, primary open-angle glaucoma, primary angle-closure glaucoma, floaters, Leber's hereditary optic neuropathy, optic disc drusen, strabismus, ophthalmoparesis, progressive external ophthalmoplegia, esotropia, exotropia, disorders of refraction and accommodation, hypermetropia, myopia, astigmatism, anisometropia, presbyopia, internal ophthalmoplegia, amblyopia, Leber's congenital amaurosis, scotoma, anopsia, color blindness, achromatopsia, maskun, nyctalopia, blindness, River blindness, microphthalmia, coloboma, red eye, Argyll Robertson pupil, keratomycosis, xerophthalmia, aniridia, sickle cell retinopathy, ocular neovascularization, retinal neovascularization, subretinal neovascularization; rubeosis iritis inflammatory diseases, chronic posterior and pan uveitis, neoplasms, retinoblastoma, pseudoglioma, neovascular glaucoma; neovascularization resulting following a combined vitrectomy-2 and lensectomy, vascular diseases, retinal ischemia, choroidal vascular insufficiency, choroidal thrombosis, neovascularization of the optic nerve, diabetic macular edema, cystoid macular edema, proliferative vitreoretinopathy, and neovascularization due to penetration of the eye or ocular injury.

[00172] In some aspects, the methods and compositions provided herein are suitable for the treatment of diseases that cause one or more ocular symptoms. Non-limiting examples of symptoms which may be amenable to treatment with the methods disclosed herein include, but are not limited to choroidal or vitreal neovascularization, vascular leakage, reduced reading speed, reduced color vision, macular edema, increased retinal thickening, increase in central retinal volume and/or, macular sensitivity, loss of retinal cells, increase in area of retinal atrophy, reduced best corrected visual acuity such as measured by Snellen or ETDRS scales, reduced Best Corrected Visual Acuity under low luminance conditions, impaired night vision, impaired light sensitivity, impaired dark adaptation, impaired contrast sensitivity, worsened patient reported outcomes, and any combination thereof.

[00173] In some cases, the methods and compositions provided herein may alleviate or reduce a symptom of a disease. In some cases, treatment with an aptamer provided herein may result in a reduction in the severity of any of the symptoms described herein. In some cases, treatment with an aptamer described herein may slow, halt or reverse the progression of any of the symptoms described herein. In some cases, treatment with an aptamer described herein may prevent the development of any of the symptoms described herein. In some cases, treatment with an aptamer described herein may slow, halt or reverse the progression of a disease, as measured by the number and severity of symptoms experienced. Examples of symptoms and relevant endpoints where the aptamer may have a therapeutic effect include choroidal or retinal neovascularization, vascular leakage, reduced reading speed, reduced color vision, macular edema, increased retinal thickening, increase in central retinal volume and/or, macular sensitivity, loss of retinal cells, increase in area of retinal atrophy, reduced best corrected visual acuity such as measured by Snellen or ETDRS scales, reduced Best Corrected Visual Acuity under low luminance conditions, impaired night vision, impaired light sensitivity, impaired dark adaptation, impaired contrast sensitivity, and worsening patient reported outcomes. In some instances, treatment with an aptamer described herein may have beneficial effects as measured by clinical endpoints including reading speed, choroidal or retinal neovascularization or vascular leakage as measured by fluorescein angiography, retinal thickness as measured by Optical Coherence Tomography or other techniques, central retinal volume, number and density of retinal cells, area of retinal atrophy as measured by Fundus Photography or Fundus Autofluorescence or other techniques, best corrected visual acuity such as measured by Snellen or ETDRS scales, Best Corrected

Visual Acuity under low luminance conditions, light sensitivity, dark adaptation, contrast sensitivity, and patient reported outcomes as measured by such tools as the National Eye Institute Visual Function Questionnaire and Health Related Quality of Life Questionnaires.

[00174] In some cases, the methods and compositions provided herein may alleviate or reduce a symptom of a neovascular eye disease. In some cases, treatment with an aptamer provided herein may result in a reduction in the severity of any symptoms associated with a neovascular eye disease. In some cases, treatment with an aptamer described herein may slow, halt or reverse the progression of any symptom associated with a neovascular eye disease. In some cases, treatment with an aptamer described herein may prevent the development of any symptom associated with a neovascular eye disease. In some cases, treatment with an aptamer described herein may slow, halt or reverse the progression of a neovascular eye disease, as measured by the number and severity of symptoms experienced. Non-limiting examples of symptoms associated with neovascular eye diseases where the aptamer may have a therapeutic effect include choroidal or retinal neovascularization, vascular leakage within the eye, macular edema, central retinal thickness and visual acuity.

Subjects

[00175] The terms “subject” and “patient” are used interchangeably herein to refer to a vertebrate, preferably a mammal, and more preferably a human. Mammals include, but are not limited to, rodents (*e.g.*, mice, rats, rabbits, *etc.*) simians, humans, research animals (*e.g.*, beagles, *etc.*), farm animals (*e.g.*, pigs, horses, cows, llamas, alpacas, *etc.*), sport animals, and pets. In some cases, the methods described herein may be used on tissues or cells derived from a subject and the progeny of such tissues or cells. For example, aptamers described herein may be used to affect some function in tissues or cells of a subject. The tissues or cells may be obtained from a subject *in vivo*. In some cases, the tissues or cells are cultured *in vitro* and contacted with a composition provided herein (*e.g.*, an aptamer).

[00176] In some aspects, the methods and compositions provided herein are used to treat a subject in need thereof. In some cases, the subject has, is suspected of having, or is at risk of developing, an ocular disease or disorder. In some cases, the subject is a human. In some cases, the human is a patient at a hospital or a clinic. In some cases, the subject is a non-human animal, for example, a non-human primate, a livestock animal, a domestic pet, or a laboratory animal. For example, a non-human animal can be an ape (*e.g.*, a chimpanzee, a baboon, a gorilla, or an

orangutan), an old world monkey (*e.g.*, a rhesus monkey), a new world monkey, a dog, a cat, a bison, a camel, a cow, a deer, a pig, a donkey, a horse, a mule, a lama, a sheep, a goat, a buffalo, a reindeer, a yak, a mouse, a rat, a rabbit, or any other non-human animal.

[00177] In cases where the subject is a human, the subject may be of any age. In some cases, the subject has an age-related ocular disease or disorder (*e.g.*, age-related macular degeneration). In some cases, the subject is about 50 years or older. In some cases, the subject is about 55 years or older. In some cases, the subject is about 60 years or older. In some cases, the subject is about 65 years or older. In some cases, the subject is about 70 years or older. In some cases, the subject is about 75 years or older. In some cases, the subject is about 80 years or older. In some cases, the subject is about 85 years or older. In some cases, the subject is about 90 years or older. In some cases, the subject is about 95 years or older. In some cases, the subject is about 100 years or older. In some cases, the subject is about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or greater than 100 years old. In some cases, the subject is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or greater than 20 years old.

[00178] In some aspects, the methods and compositions provided herein may be used to treat a subject having, suspected of having, or at risk of developing ocular symptoms as described herein. In some aspects, the methods and compositions provided herein may be used to treat a subject having, suspected of having, or at risk of developing an ocular disease as provided herein. In some cases, the methods and compositions provided herein may be used to treat a subject having, suspected of having, or at risk of developing an ocular disease or disorder. In some cases, the ocular disease or disorder is diabetic retinopathy. In some cases, the ocular disease or disorder is retinopathy of prematurity. In some cases, the ocular disease or disorder is central retinal vein occlusion. In some cases, the ocular disease or disorder is macular edema. In some cases, the ocular disease or disorder is choroidal neovascularization. In some cases, the ocular disease or disorder is neovascular (or wet) age-related macular degeneration. In some cases, the ocular disease or disorder is myopic choroidal neovascularization. In some cases, the ocular disease or disorder is punctate inner choroidopathy. In some cases, the ocular disease or disorder is presumed ocular histoplasmosis syndrome. In some cases, the ocular disease or disorder is familial exudative vitreoretinopathy. In some cases, the ocular disease or disorder is

retinoblastoma. In some cases, the methods and compositions provided herein may be used to treat a subject having, suspected of having, or at risk of developing an ocular disease or disorder exhibiting elevated levels of one or more isoforms or variants of VEGF-A.

Pharmaceutical compositions or medicaments

[00179] Disclosed herein are pharmaceutical compositions or medicaments, used interchangeably, for use in a method of therapy, or for use in a method of medical treatment. Such use may be for the treatment of ocular diseases or disorders. In some cases, the pharmaceutical compositions can be used for the treatment of an ocular disease or disorder. In some cases, the pharmaceutical compositions comprise one or more anti-VEGF-A aptamers for the treatment of an ocular disease or disorder. In some cases, the ocular disease or disorder is diabetic retinopathy. In some cases, the ocular disease or disorder is retinopathy of prematurity. In some cases, the ocular disease or disorder is central retinal vein occlusion. In some cases, the ocular disease or disorder is macular edema. In some cases, the ocular disease or disorder is choroidal neovascularization. In some cases, the ocular disease or disorder is neovascular (or wet) age-related macular degeneration. In some cases, the ocular disease or disorder is myopic choroidal neovascularization. In some cases, the ocular disease or disorder is punctate inner choroidopathy. In some cases, the ocular disease or disorder is presumed ocular histoplasmosis syndrome. In some cases, the ocular disease or disorder is familial exudative vitreoretinopathy. In some cases, the ocular disease or disorder is retinoblastoma. In some cases, the pharmaceutical compositions can be used for the treatment of an ocular disease or disorder that exhibits elevated levels of one or more isoforms or variants of VEGF-A.

[00180] In some cases, the one or more anti-VEGF-A aptamers may bind to one or more isoforms or variants of VEGF-A. In some cases, the one or more anti-VEGF-A aptamers are pan-variant specific aptamers that bind to each of VEGF-A₁₁₀, VEGF-A₁₂₁, VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆. In some cases, the one or more anti-VEGF-A aptamers may bind to the receptor binding face of VEGF-A, or a portion thereof. The receptor binding face of VEGF-A may include strands β 2, β 5, and β 6 and loop β 1 and β 2 of a first monomer, and the N-terminal α helix and loop β 3 to β 4 of the second monomer (see Muller, Yves A., et al. "The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 Å resolution: multiple copy flexibility and receptor binding." *Structure* 5.10 (1997): 1325-1338.). In some cases, anti-VEGF-A aptamers that bind to the receptor binding face of VEGF-A may bind to one or more of

residues Phe17, Ile43, Ile46, Glu64, Gln79, Ile83, Lys84, Pro85, Arg82, His86, Asp63, Glu67, as described in **SEQ ID NOs: 6-10**. In some cases, the one or more anti-VEGF-A aptamers may bind to the receptor binding domain of VEGF-A, or a portion thereof. The receptor binding domain of VEGF-A may include any one or more of residues 1-109, as described in **SEQ ID NOs: 6-10**. In some cases, the one or more anti-VEGF-A aptamers may prevent or reduce the binding of one or more isoforms or variants of VEGF-A with Flt-1, KDR, or Nrp-1. In some cases, the compositions may include, *e.g.*, an effective amount of the aptamer, alone or in combination, with one or more vehicles (*e.g.*, pharmaceutically acceptable compositions or *e.g.*, pharmaceutically acceptable carriers).

Formulations

[00181] Compositions as described herein may comprise a liquid formulation, a solid formulation or a combination thereof. Non-limiting examples of formulations may include a tablet, a capsule, a gel, a paste, a liquid solution and a cream. The compositions of the present disclosure may further comprise any number of excipients. Excipients may include any and all solvents, coatings, flavorings, colorings, lubricants, disintegrants, preservatives, sweeteners, binders, diluents, and vehicles (or carriers). Generally, the excipient is compatible with the therapeutic compositions of the present disclosure. The pharmaceutical composition may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and other substances such as, for example, sodium acetate, and triethanolamine oleate.

Dosage and Routes of Administration

[00182] Therapeutic doses of formulations of the disclosure can be administered to a subject in need thereof. In some cases, a formulation is administered to the eye of a subject for the treatment of an ocular disease as described herein. Administration to the eye can be; a) local ocular delivery; or b) systemic. A topical formulation can be applied directly to the eye (*e.g.*, eye drops, contact lens loaded with the formulation) or to the eyelid (*e.g.*, cream, lotion, gel). In some cases, topical administration can be to a site remote from the eye, for example, to the skin of an extremity. This form of administration may be suitable for targets that are not produced directly by the eye. In some cases, a formulation of the disclosure is administered by local ocular delivery. Non-limiting examples of local ocular delivery include intravitreal (IVT),

intracameral, subconjunctival, subtenon, retrobulbar, posterior juxtasceral, and peribulbar. In some cases, a formulation of the disclosure is delivered by intravitreal administration (IVT). Local ocular delivery may generally involve injection of a liquid formulation. In other cases, a formulation of the disclosure is administered systemically. Systemic administration can involve oral administration. In some cases, systemic administration can be intravenous administration, subcutaneous administration, infusion, implantation, and the like.

[00183] Other formulations suitable for delivery of the pharmaceutical compositions described herein may include a sustained release gel or polymer formulations by surgical implantation of a biodegradable microsize polymer system, *e.g.*, microdevice, microparticle, or sponge, or other slow release transscleral devices, implanted during the treatment of an ophthalmic disease, or by an ocular delivery device, *e.g.*, polymer contact lens sustained delivery device. In some cases, the formulation is a polymer gel, a self-assembling gel, a durable implant, an eluting implant, a biodegradable matrix or biodegradable polymers. In some cases, the formulation may be administered by iontophoresis using electric current to drive the composition from the surface to the posterior of the eye. In some cases, the formulation may be administered by a surgically implanted port with an intravitreal reservoir, an extra-vitreous reservoir or a combination thereof. Examples of implantable ocular devices can include, without limitation, the DurasertTM technology developed by Bausch & Lomb, the ODTx device developed by On Demand Therapeutics, the Port Delivery System developed by ForSight VISION4 and the Replenish MicroPumpTM System developed by Replenish, Inc. In some cases, nanotechnologies can be used to deliver the pharmaceutical compositions including nanospheres, nanoparticles, nanocapsules, liposomes, nanomicelles and dendrimers.

[00184] A composition of the disclosure can be administered once or more than once each day. In some cases, the composition is administered as a single dose (*i.e.*, one-time use). In this example, the single dose may be curative. In other cases, the composition may be administered serially (*e.g.*, taken every day without a break for the duration of the treatment regimen). In some cases, the treatment regime can be less than a week, a week, two weeks, three weeks, a month, or greater than a month. In some cases, the composition is administered over a period of at least 12 weeks, at least 16 weeks, at least 20 weeks, or at least 24 weeks. In other cases, the composition is administered for a day, at least two consecutive days, at least three consecutive days, at least four consecutive days, at least five consecutive days, at least six consecutive days, at least seven

consecutive days, at least eight consecutive days, at least nine consecutive days, at least ten consecutive days, or at least greater than ten consecutive days. In some cases, a therapeutically effective amount can be administered one time per week, two times per week, three times per week, four times per week, five times per week, six times per week, seven times per week, eight times per week, nine times per week, 10 times per week, 11 times per week, 12 times per week, 13 times per week, 14 times per week, 15 times per week, 16 times per week, 17 times per week, 18 times per week, 19 times per week, 20 times per week, 25 times per week, 30 times per week, 35 times per week, 40 times per week, or greater than 40 times per week. In some cases, a therapeutically effective amount can be administered one time per day, two times per day, three times per day, four times per day, five times per day, six times per day, seven times per day, eight times per day, nine times per day, 10 times per day, or greater than 10 times per day. In some cases, the composition is administered at least twice a day. In further cases, the composition is administered at least every hour, at least every two hours, at least every three hours, at least every four hours, at least every five hours, at least every six hours, at least every seven hours, at least every eight hours, at least every nine hours, at least every 10 hours, at least every 11 hours, at least every 12 hours, at least every 13 hours, at least every 14 hours, at least every 15 hours, at least every 16 hours, at least every 17 hours, at least every 18 hours, at least every 19 hours, at least every 20 hours, at least every 21 hours, at least every 22 hours, at least every 23 hours, or at least every day.

[00185] Aptamers as described herein may be particularly advantageous over antibodies as they may sustain therapeutic intravitreal concentrations of drug for longer periods of time, thus requiring less frequent administration. The aptamers described herein may have a longer intraocular half-life, and/or sustain therapeutic intravitreal concentrations of drug for longer periods of time than an anti-VEGF-A antibody therapy and can be dosed less frequently. In some cases, the aptamers of the disclosure are dosed at least once every 4 weeks (q4w), once every 5 weeks (q5w), once every 6 weeks (q6w), once every 7 weeks (q7w), once every 8 weeks (q8w), once every 9 weeks (q9w), once every 10 weeks (q10w), once every 11 weeks (q11w) once every 12 weeks (q12w), once every 13 weeks (q13w), once every 14 weeks (q14w), once every 15 weeks (q15w), once every 16 weeks (q16w), once every 17 weeks (q17w), once every 18 weeks (q18w), once every 19 weeks (q19w), once every 20 weeks (q20w), once every 21 weeks

(q21w), once every 22 weeks (q22w), once every 23 weeks (q23w), once every 24 weeks (q24w), or greater than once every 24 weeks.

[00186] In some aspects, a therapeutically effective amount of the aptamer may be administered. A “therapeutically effective amount” or “therapeutically effective dose” are used interchangeably herein and refer to an amount of a therapeutic agent (*e.g.*, an aptamer) that provokes a therapeutic or desired response in a subject. The therapeutically effective amount of the composition may be dependent on the route of administration. In the case of systemic administration, a therapeutically effective amount may be about 10 mg/kg to about 100 mg/kg. In some cases, a therapeutically effective amount may be about 10 µg/kg to about 1000 µg/kg for systemic administration. For intravitreal administration, a therapeutically effective amount can be about 0.01 mg to about 150 mg in about 25 µl to about 100 µl volume per eye.

Methods of Treatment

[00187] Disclosed herein are methods for the treatment of ocular diseases or disorders. In some cases, the ocular disease or disorder may be diabetic retinopathy. In some cases, the ocular disease or disorder may be retinopathy of prematurity. In some cases, the ocular disease or disorder may be central retinal vein occlusion. In some cases, the ocular disease or disorder may be macular edema. In some cases, the ocular disease or disorder may be choroidal neovascularization. In some cases, the ocular disease or disorder may be neovascular (or wet) age-related macular degeneration. In some cases, the ocular disease or disorder may be myopic choroidal neovascularization. In some cases, the ocular disease or disorder may be punctate inner choroidopathy. In some cases, the ocular disease or disorder may be presumed ocular histoplasmosis syndrome. In some cases, the ocular disease or disorder may be familial exudative vitreoretinopathy. In some cases, the ocular disease or disorder may be retinoblastoma. In some cases, the ocular disease or disorder may exhibit elevated levels of one or more isoforms or variants of VEGF-A.

[00188] In some cases, the method involves administering a therapeutically effective amount of a composition to a subject to treat an ocular disease. In some cases, the composition includes one or more aptamers as described herein. The aptamers may bind to and inhibit a function associated with one or more isoforms or variants of VEGF-A as described herein. The methods can be performed at a hospital or a clinic, for example, the pharmaceutical compositions can be

administered by a health-care professional. In other cases, the pharmaceutical compositions can be self-administered by the subject. Treatment may commence with the diagnosis of a subject with an ocular disease. In the event that further treatments are necessary, follow-up appointments may be scheduled for the administration of subsequent doses of the composition, for example, administration every 8, 12, 16, 20, or 24 weeks.

[00189] Further disclosed herein are methods of using an anti-VEGF-A composition of the disclosure to inhibit a function associated with VEGF-A. For example, the methods may involve administering a composition of the disclosure, including one or more anti-VEGF-A aptamers, to a biological system (*e.g.*, biological cells, biological tissue, a subject) to inhibit a function associated with VEGF-A. In some cases, the anti-VEGF-A aptamers may bind to the receptor binding face of VEGF-A, or portions thereof. In some cases, the anti-VEGF-A aptamers may bind to the receptor binding domain of VEGF-A. In some cases, the methods may be used to prevent or reduce binding of VEGF-A to Flt-1, KDR, Nrp-1, or any combination thereof. In some cases, the methods may be used to inhibit downstream signaling pathways associated with VEGF-A.

Methods of Generating Aptamers

The SELEXTM Method

[00190] The aptamers described herein can be generated by any method suitable for generating aptamers. In some cases, the aptamers described herein are generated by a process known as Systematic Evolution of Ligands by Exponential Enrichment ("SELEXTM"). The SELEXTM process is described in, *e.g.*, U.S. patent application Ser. No. 07/536,428, filed Jun. 11, 1990, now abandoned, U.S. Pat. No. 5,475,096 entitled "Nucleic Acid Ligands", and U.S. Pat. No. 5,270,163 (*see*, also WO 91/19813) entitled "Nucleic Acid Ligands", each of which are herein incorporated by reference. By performing iterative cycles of selection and amplification, SELEXTM may be used to obtain aptamers with any desired level of target binding affinity.

[00191] The SELEXTM method generally relies as a starting point upon a large library or pool of single stranded oligonucleotides comprising randomized sequences. The oligonucleotides can be modified or unmodified DNA, RNA, or DNA/RNA hybrids. In some examples, the pool comprises 100% random or partially random oligonucleotides. In other examples, the pool comprises random or partially random oligonucleotides containing at least one fixed sequence and/or conserved sequence incorporated within randomized sequence. In other examples, the

pool comprises random or partially random oligonucleotides containing at least one fixed sequence and/or conserved sequence at its 5' and/or 3' end which may comprise a sequence shared by all the molecules of the oligonucleotide pool. Fixed sequences are sequences common to oligonucleotides in the pool which are incorporated for a preselected purpose such as, CpG motifs, hybridization sites for PCR primers, promoter sequences for RNA polymerases (*e.g.*, T3, T4, T7, and SP6), sequences to form stems to present the randomized region of the library within a defined terminal stem structure, restriction sites, or homopolymeric sequences, such as poly A or poly T tracts, catalytic cores, sites for selective binding to affinity columns, and other sequences to facilitate cloning and/or sequencing of an oligonucleotide of interest. Conserved sequences are sequences, other than the previously described fixed sequences, shared by a number of aptamers that bind to the same target.

[00192] The oligonucleotides of the pool can include a randomized sequence portion as well as fixed sequences necessary for efficient amplification. Typically the oligonucleotides of the starting pool contain fixed 5' and 3' terminal sequences which flank an internal region of 30-50 random nucleotides. The randomized nucleotides can be produced in a number of ways including chemical synthesis and size selection from randomly cleaved cellular nucleic acids. Sequence variation in test nucleic acids can also be introduced or increased by mutagenesis before or during the selection/amplification iterations.

[00193] The random sequence portion of the oligonucleotide can be of any length and can comprise ribonucleotides and/or deoxyribonucleotides and can include modified or non-natural nucleotides or nucleotide analogs. Typical syntheses carried out on automated DNA synthesis equipment yield 10^{14} - 10^{16} individual molecules, a number sufficient for most SELEXTM experiments. Sufficiently large regions of random sequence in the sequence design increases the likelihood that each synthesized molecule is likely to represent a unique sequence.

[00194] The starting library of oligonucleotides may be generated by automated chemical synthesis on a DNA synthesizer. To synthesize randomized sequences, mixtures of all four nucleotides are added at each nucleotide addition step during the synthesis process, allowing for random incorporation of nucleotides. As stated above, in some cases, random oligonucleotides comprise entirely random sequences; however, in other cases, random oligonucleotides can comprise stretches of nonrandom or partially random sequences. Partially random sequences can be created by adding the four nucleotides in different molar ratios at each addition step.

[00195] The starting library of oligonucleotides may be RNA, DNA, substituted RNA or DNA or combinations thereof. In those instances where an RNA library is to be used as the starting library it is typically generated by synthesizing a DNA library, optionally PCR amplifying, then transcribing the DNA library *in vitro* using phage RNA polymerase or modified phage RNA polymerases, and purifying the transcribed library. The nucleic acid library is then mixed with the target under conditions favorable for binding and subjected to step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. More specifically, starting with a mixture containing the starting pool of nucleic acids, the SELEXTM method includes steps of: (a) contacting the mixture with the target under conditions favorable for binding; (b) partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules; (c) dissociating the nucleic acid-target complexes; (d) amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids; and (e) reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic acid ligands to the target molecule. In those instances where RNA aptamers are being selected, the SELEXTM method further comprises the steps of: (i) reverse transcribing the nucleic acids dissociated from the nucleic acid-target complexes before amplification in step (d); and (ii) transcribing the amplified nucleic acids from step (d) before restarting the process.

[00196] Within a nucleic acid mixture containing a large number of possible sequences and structures, there is a wide range of binding affinities for a given target. Those which have the higher affinity (lower dissociation constants) for the target are most likely to bind to the target. After partitioning, dissociation and amplification, a second nucleic acid mixture is generated, enriched for the higher binding affinity candidates. Additional rounds of selection progressively favor the best ligands until the resulting nucleic acid mixture is predominantly composed of only one or a few sequences. These can then be cloned, sequenced and individually tested as ligands or aptamers for 1) target binding affinity; and 2) ability to effect target function.

[00197] Cycles of selection and amplification are repeated until a desired goal is achieved. In the most general case, selection/amplification is continued until no significant improvement in binding strength is achieved on repetition of the cycle. The method is typically used to sample approximately 10^{14} different nucleic acid species but may be used to sample as many as about

10^{18} different nucleic acid species. Generally, nucleic acid aptamer molecules are selected in a 3 to 20 cycle procedure.

[00198] In some cases, the aptamers of the disclosure are generated using the SELEXTM method as described above. In other cases, the aptamers of the disclosure are generated using any modification or variant of the SELEXTM method.

Methods of generating pan-variant specific anti-VEGF-A aptamers

[00199] Further provided herein are methods for generating and screening pan-variant specific anti-VEGF-A aptamers. In some cases, the pan-variant specific aptamers bind to the receptor binding face of VEGF-A, or the receptor binding domain of VEGF-A. Generally, the methods provided herein bias the selection process towards aptamers that selectively bind to the receptor binding face or receptor binding domain of VEGF-A. In some cases, such aptamers do not bind to the heparin binding domain of VEGF-A. In various aspects, the methods may involve incubating an aptamer library with an isoform or variant of VEGF-A which contains a receptor binding domain but does not contain a heparin binding domain. In some cases, the isoform or variant of VEGF-A is VEGF-A₁₂₁ or VEGF-A₁₁₀. In various aspects, the methods involve immobilizing the VEGF-A variant on a solid support in a manner that does not preclude access of the library to the receptor binding face or receptor binding domain of VEGF-A. In various aspects, the methods involve performing the selection in the absence of Ca⁺⁺

[00200] In various aspects, methods for screening pan-variant specific anti-VEGF-A aptamers are provided. In some cases, the methods may involve measuring the interaction of a candidate aptamers with recombinant bead-bound VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ by flow cytometry (*see Example 1*). In such cases, interaction with each isoform or variant would indicate binding to the receptor binding domain, while only binding to VEGF-A₁₆₅ would indicate recognition of the heparin binding domain. In some cases, the methods may involve measuring the ability of the candidate aptamer to inhibit or reduce the interaction of VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ with KDR by a reduction in FRET signal (*see Example 3*). In such cases, efficacy against each variant would indicate binding to the receptor binding domain, while only inhibiting VEGF-A₁₆₅ would indicate binding to the heparin binding domain. In some cases, the methods may involve measuring the ability of the candidate aptamer to inhibit or

reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced trans autophosphorylation of the intracellular domain of KDR by phospho-KDR AlphaLISA[®] (see **Example 4**). In such cases, efficacy against each variant would indicate binding to the receptor binding domain, while only inhibiting VEGF₁₆₅ would indicate binding to the heparin binding domain. In some cases, the methods may involve measuring the ability of the candidate aptamer to inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced gene expression of tissue factor in HUVEC cells as measured by qPCR. In such cases, efficacy against each variant would indicate binding to the receptor binding domain, while only inhibiting VEGF₁₆₅ would indicate binding to the heparin binding domain. In some cases, the methods may involve measuring the ability of the candidate aptamer to inhibit or reduce VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ induced tube formation of GFP-HUVECs in co-culture with human dermal fibroblasts cells by change in network length or network area (see **Example 5**). In such cases, efficacy against each variant would indicate binding to the receptor binding domain, while only inhibiting VEGF-A₁₆₅ would indicate binding to the heparin binding domain. In some cases, the methods may involve measuring the ability of the candidate aptamer to inhibit or reduce vascular leakage in a mouse, rat, rabbit, or primate eye following exogenous VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-A₁₁₀ challenge by fluorescein angiography and Evans-blue albumin staining. In such cases, efficacy against each VEGF isoform or variant would indicate binding to the receptor binding domain, while only inhibiting VEGF-A₁₆₅ would indicate binding to the heparin binding domain.

EXAMPLES

[00201] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1A. Selection of anti-VEGF-A aptamers

[00202] Anti-VEGF-A (VEGF) aptamers targeting the receptor binding domain (RBD) were identified using an N30 library (N30S) comprised of a 30-nucleotide random region flanked by constant regions containing a built-in stem region as depicted in **FIG. 1A**. The sequence in

italics represents the forward and reverse primer binding sites. The built-in stem region is underlined and shown in bold. **FIG. 1B** depicts a representation of the N30S library with the reverse primer hybridized. For nuclease stability, the library was composed of 2'-fluoro-G (2'F GTP) and 2'-O-methyl (2'OMe) A/C/U. **FIG. 1C** depicts structures of modified nucleotides used to generate the N30S library for selection against target VEGF-A. For simplicity, the nucleosides, and not the nucleotide triphosphates are shown.

[00203] The library sequence (underlined sequences represent the built-in stem) and the sequence of oligos used to amplify the library are described in **Table 2**.

Table 2. Library sequence and sequence of oligos used to amplify the library

SEQ ID NO.		Sequence (5' to 3')
SEQ ID NO: 428	Library sequence (Total library length: 89 bases)	GGGAGTGTGTACGAGGCATT <u>AGGCCGCC</u> -N30- <u>GGCGGCCTTTGATACTTGATCGCCCTAGAAGC</u>
SEQ ID NO: 429	N30S.F	P-GGGAGTGTGTACGAGGCATTA
SEQ ID NO: 430	N30S.R	GCTTCTAGGGCGATCAAGTATCA
where G, A, T and C are deoxyribonucleotides and P-denotes a phage polymerase promoter.		

[00204] The starting library was transcribed from a pool of $\sim 10^{14}$ double-stranded DNA (dsDNA) molecules. The dsDNA library was generated by primer extension using Klenow exo(-) DNA polymerase, the pool forward primer (N30S.F; **SEQ ID NO: 429**) and synthetic single-stranded DNA (ssDNA) molecules encoding the reverse complement of the library. The dsDNA was subsequently converted to 100% backbone modified RNA via transcription using a mixture of 2'F GTP, 2'OMe ATP/CTP/UTP and a modified phage polymerase in buffer optimized to facilitate efficient transcription. Following transcription, RNAs were treated with DNase to remove the template dsDNA and purified.

[00205] In order to isolate pan-specific aptamer inhibitors of VEGF-A that bind to the RBD and inhibit VEGF-A via directly blocking its interaction with its receptor, KDR (also known as VEGFR-2), and avoid the identification of anti-VEGF-A aptamers that bound exclusively to the heparin binding domain (HBD) of the protein, aptamer selections were performed using a combination of the 121 and 110 variants of VEGF-A (VEGF-A₁₂₁ or VEGF-A₁₁₀). Both of these variants lack the HBD but possess the RBD and therefore maintain high affinity for the target receptor, KDR.

[00206] Aptamer selection was facilitated by the use of biotin-tagged recombinant human VEGF-A₁₂₁ or VEGF-A₁₁₀. To ensure the biotin tag did not interfere with the availability of desired epitopes within the RBD during the selection, biotin was chemically conjugated to the terminal sugars on the glycan. These VEGF-A monomers bear a single N-linked glycosylation site on Asn74, which is located in the center of the RBD, distal to the receptor binding face, making this an ideal site for target immobilization. In brief, VEGF-A glycans were oxidized with 100 μ M sodium periodate for 30 minutes, and excess periodate was quenched with 1 mM glycerol. 1 mM biotin-PEG₁₂-alkoxyamine and 10 mM anthranilic acid final were added to the reaction and allowed to incubate for another 2 hours. Reactions were cleaned up by membrane filtration using a 10 kDa cutoff filter.

[00207] Biotinylated VEGF-A variants were immobilized on magnetic streptavidin capture beads (Dynal, Streptavidin MyOne™) at a constant ratio of 1 μ L beads per 1 μ g protein. Briefly, beads were washed three times with binding buffer SB1T(-) (40 mM HEPES, pH 7.5, 125 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 0.05% Tween-20) and were resuspended in 50 μ L of recombinant VEGF-A in SB1T(-) and incubated at room temperature for 30 minutes. The beads were subsequently blocked with excess biotin for 10 minutes. The amount of target protein varied with each round (**Table 3**). The beads were washed three times with SB1T(-) buffer to remove any unbound protein.

[00208] For the first round of selection, ~1 nanomole of the Round 0 RNA pool, ~3x coverage for 2×10^{14} sequences, was used. Prior to each round, the library was thermally equilibrated by heating at 90°C for 3 minutes and cooled at room temperature for 15 minutes in the presence of a 1.5-fold molar excess of reverse primer (N30S.R; **SEQ ID NO: 430**) to allow the library to refold. Following renaturation, the final volume of the reaction was adjusted to 50 μ L in SB1T(-) supplemented with 1 μ g/ml ssDNA and 0.1% BSA.

[00209] For the first round, the library was added to VEGF-A₁₁₀ immobilized on beads and incubated at 37°C for 30 minutes on a tube rotary. After 30 minutes, the beads were washed three times using 0.5 mL SB1T(-) buffer to remove unbound aptamers. After washing, VEGF-A₁₁₀-bound aptamers were eluted using 200 μ L elution buffer (2M Guanidine-HCl in SB1T(-) buffer) two times (total volume 400 μ L). The eluted aptamers, in 400 μ L of elution buffer, were ethanol precipitated. The recovered library was converted to DNA by reverse transcription using Super Script IV reverse transcriptase, and the ssDNA was subsequently amplified by PCR. The

resulting dsDNA library was subsequently converted back into modified RNA via transcription as described above. DNaseI, purified RNA was used for subsequent rounds.

[00210] Following the first round, a negative selection step was included in all the subsequent rounds. For the negative selection, the pool was prepared as described before and first incubated with biotin saturated beads (in the absence of target protein) for 30 minutes at 37°C in SB1T(-) buffer. The beads were pelleted and the supernatant, containing molecules that did not bind to the beads, was incubated with VEGF-A-labeled beads for an additional 30 minutes at 37°C. For rounds 2-4, the input RNA was kept fixed at 25 picomoles, and the protein target was fixed at 0.5 µg.

[00211] For rounds 5-7, the target was varied between VEGF-A₁₂₁ and VEGF-A₁₁₀ and a solution capture method was implemented. Negative selections were implemented as described above, and positive selection involved incubation with biotinylated target without the presence of beads for 30 minutes at 37°C. For capture, 2 µL of streptavidin beads were washed with 0.5 mL of SB1T(-) and the positive selection was used to re-suspend the bead pellet. After a 5 minute incubation, the beads were washed according to **Table 3** prior to elution and precipitation as described above.

Table 3. Selection Details

Round	Input library (moles/conc.)	Target protein	Target dimer (pMoles/conc.)	1 µg/ml ssDNA & 0.1% BSA	Solution capture	Washes	# cycles
1	1 nm/19.5 µM	VEGF-A ₁₁₀	80 pm/1.5 µM	NO	NO	3x0 min.	24
2	25 pm/0.5 nM	VEGF-A ₁₁₀	20 pm/400 nM	YES	NO	3x0 min.	33
3	25 pm/0.5 nM	VEGF-A ₁₁₀	20 pm/400 nM	YES	NO	3x0 min.	22
4	25 pm/0.5 nM	VEGF-A ₁₁₀	20 pm/400 nM	YES	NO	3x5 min.	22
5	25 pm/0.5 nM	VEGF-A ₁₂₁	5 pm/100 nM	YES	YES	3x5 min.	27
6	25 pm/0.5 nM	VEGF-A ₁₂₁	5 pm/100 nM	YES	YES	0, 5, 15 min.	25
7	25 pm/0.5 nM	VEGF-A ₁₁₀	1 pm/20 nM	YES	YES	0, 5, 15 min.	28

Example 1B. Assessing the progress of selection

[00212] Flow cytometry was used to assess the progress of the selection. For these assays, RNA from each round was first hybridized with reverse complement oligonucleotide composed of 2'OMe RNA labeled with Dylight[®] 650 (Dy650-N30S.R.OMe, sequence identical to N30S.R). Briefly, the library was combined with a 1.5-fold molar excess of Dy650-N30S.R.OMe, heated at 90°C for 3 minutes and allowed to cool at room temperature for 15 minutes, after which it was incubated with unlabeled “Negative” beads or beads labelled with VEGF-A₁₂₁ in SB1T(-) buffer supplemented with 0.1% BSA and 1 mg/mL ssDNA final. Following incubation for 30 minutes at 37°C, the beads were washed 3 times with SB1T(-), re-suspended in SB1T(-) buffer, and analyzed by flow cytometry. As shown in **FIG. 2A** and **FIG. 2B**, an improvement in fluorescent signal was observed by Round 4. After Round 5, there was little change in the binding signal through Round 7. “Negative” and “VEGF 121” refers to the signal of unlabeled and VEGF-A₁₂₁-labeled beads, respectively, in the absence of fluorescently labeled RNA. Titrations of Rounds 5-7 prepared as described above also showed dose-dependent responses in median fluorescence for the bead populations functionalized with VEGF-A₁₂₁ and VEGF-A₁₁₀, which indicated that the selection strategy was successful in enriching for aptamers that bind to the RBD of VEGF-A (**FIG. 2C** and **FIG. 2D**). Importantly, similar binding experiments performed in the presence of 1 μ M non-biotinylated VEGF-A₁₂₁ demonstrated a significant loss in binding signal (**FIG. 2E**; “Round 6 decoy”). Thus, the iterative rounds of selection resulted in the enrichment of an aptamer population capable of specifically binding the RBD of VEGF-A when both immobilized on beads and, more importantly, in solution.

Example 1C. Selection, purification and characterization of clones

[00213] The enriched aptamer population from Round 7 was cloned into a TOPO TA vector, transformed into competent cells, and sequenced by Sanger sequencing. All *in silico* analyses were performed using Geneious software (Biomatters Inc., Newark NJ, USA). Of the 45 sequences identified, 10 were unique and all fell into a single family with one sequence representing 32 identical reads. The dominant sequence, Aptamer 4.2 (r7-01) was chemically synthesized with 2'-fluoro-G and 2'-O-methyl (2'OMe) A/C/U modified phosphoramidites along with a 3' inverted deoxythymidine and a 5' C6 disulfide linker, which was conjugated to Dylight[®] 650 maleimide (**Table 4**). The aptamer was purified by reversed phase high-performance liquid chromatography (HPLC) and assayed for activity in the flow cytometry assay

described above. A dose response against beads bearing VEGF-A₁₂₁ or VEGF-A₁₆₅ demonstrated that Aptamer 4.2 was capable of binding to both isoforms (**FIG. 3**). Therefore, Aptamer 4.2 bound to a common epitope on multiple isoforms of VEGF-A, with VEGF-A recognition independent of the HBD. In combination with the bead binding results above for VEGF-A₁₁₀, the surface plasmon resonance binding data shown below in **Example 2**, and the receptor competition shown below in **Example 3**, these data support the notion that aptamers in this family of molecules bind to the VEGF-A RBD.

Table 4. Chemical synthesis and testing of Round 7 sequence

SEQ ID NO	Aptamer Number	Backbone	Sequence 5' to 3'	Binding 165	Binding 121
SEQ ID NO: 219	Aptamer 4.2	RNA	C6SS- AGGCCGCCUCCGCGC GGAGGGGUUUCAUUA UCCCGUUUGGCGGCU U-idT	+	+
where G is 2'F, A, C, & U are 2'OMe modified RNA; C6SS is a disulfide linker; and idT is an inverted deoxythymidine residue.					
+ indicates dose-dependent binding of the target					

Example 1D. Deep sequencing, chemical synthesis, and assessment of VEGF-A inhibition

[00214] In order to gain deeper insight to the progression of the selection, the enriched libraries from Rounds 3-6 were sequenced using next-generation sequencing (NGS) to identify individual functional clones. Data from greater than 100,000 individual sequences per round were processed by trimming the flanking constant regions from the library. Aptamers with identical sequences were de-duplicated into stacks and annotated with frequency. Stacks were ranked by frequency within the libraries and organized into families by clustering aptamers with similar sequence relatedness using the MAFFT alignment algorithm. To a first approximation, the number of times a sequence occurs in a stack directly correlates with its molecular function; more functional molecules typically occur more times. Thus, the rank order of each stack can be thought of as a proxy for fitness. From an analysis of Rounds 5 and 6, clones related to r7-01 were selected for testing, chemically synthesized with 2'-fluoro-G and 2'-O-methyl (2'OMe)

A/C/U modified phosphoramidites, purified by reversed phase HPLC, and desalted into nuclease-free H₂O via buffer exchange before further analysis (**Table 5**).

[00215] Aptamer function was determined by assessing the ability to inhibit VEGF-A₁₆₅ and VEGF-A₁₂₁ induced KDR phosphorylation using a cell-based assay described in detail in **Example 3**. Of the 7 compounds assayed, 5 were able to inhibit KDR phosphorylation by both VEGF-A₁₆₅ and VEGF-A₁₂₁ (**Table 5**).

Table 5. A summary of the clones tested capable of inhibiting VEGF-A induced receptor phosphorylation

SEQ ID NO	Aptamer Number	Backbone	Sequence (5' to 3')	pKDR 165	pKDR 121
SEQ ID NO: 220	Aptamer 26	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGAGGGGUUUCAUUA UCCCGUUUGGCGGCCUUU-idT	++	+
SEQ ID NO: 221	Aptamer 27	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGGGGGUUUCAUUA UCCCGUUUGGCGGCCUUU-idT	-	-
SEQ ID NO: 222	Aptamer 28	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGUGGGGUUUCAUUA UCCCGUUUGGCGGCCUUU-idT	++	-
SEQ ID NO: 223	Aptamer 29	RNA	C6NH ₂ - UAGUCCGCCUCCGCGCGGAGGGGUUUCAUUA UCCCGUUUGGCGGCCUUU-idT	++	+
SEQ ID NO: 224	Aptamer 30	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGAGGGGUUUCAUUU AUCCCGUUUGGCGGCCUUU-idT	++	+
SEQ ID NO: 225	Aptamer 31	RNA	C6NH ₂ - UAGACCGCCUCCGCGCGGAGGGGUUUCAUUA UCCCGUUUGGCGGCCUUU-idT	++	+
SEQ ID NO: 226	Aptamer 32	RNA	C6NH ₂ - UAGGCCGCCUCCGCGCGGAGGGGUUUCAUUA UCCCGUUUGGCGGCCUUU-idT	++	+
where G is 2'F; A, C, & U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.					
++ indicates >50% inhibition at 10 nM; + indicates >50% inhibition at 100 nM; - indicates no inhibition at 100 nM					

Example 2. Binding affinity and confirmation of pan-specificity with surface plasmon resonance

[00216] To measure binding affinity of the identified aptamer and confirm its pan-specific binding to VEGF-A variants described in **Example 1**, SPR experiments were conducted against VEGF-A₁₆₅, VEGF-A₁₂₁, and VEGF-A₁₁₀ with Aptamer 26 (**Table 5**) as compared to a previously described HBD binding aptamer, Aptamer 7 (**Table 6**) (Ruckman, 1998). Aptamer 26 was identical in sequence to Aptamer 4.2 but contained a hexylamine linker in place of the C6 disulfide linker at the 5' terminus. Aptamer 7 has been demonstrated to bind directly to the HBD of VEGF-A at an epitope overlapping the binding site of the VEGF-A₁₆₅ co-receptor neuropilin-1 (Ruckman et al. 1998). Briefly, in the SPR studies, 1.0-2.5 µg/mL of glycan biotinylated VEGF-A₁₆₅, VEGF-A₁₂₁, and VEGF-A₁₁₀ were diluted in HEPES running buffer (10 mM HEPES, 137.5 mM NaCl, 5.7 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 0.05% Tween20; pH 7.4) and immobilized to a streptavidin dextran chip (GE Healthcare Life Sciences) for 1-2 minutes. The chip was then blocked with 0.2 mg/mL biotin in running buffer, followed by 3 cycles of buffer blanks and regenerated with 50 mM NaOH to equilibrate the chip. This resulted in 200-3000 RIU immobilized. After coating and blocking, samples were screened at 100 nM in duplicate injections at a flow rate of 25 µL/min, with an association time of 3 minutes 20 seconds and a dissociation time of 10 minutes. Regeneration conditions of chips were optimized based on the protein immobilized and effected with either 50 mM NaOH for 30 seconds or 2M guanidine HCl for 2 x 40 seconds. To determine binding affinities, aptamers were subsequently run in an 11-point, 2-fold dose response with Aptamer 26 starting at a top concentration of 1.0 µM, and Aptamer 7 starting at 500 nM.

Table 6. An anti-VEGF-A HBD aptamer

SEQ ID NO	Aptamer Number	Backbone	Sequence (5' to 3')
SEQ ID NO: 431	Aptamer 7	RNA	C6NH ₂ - fCmGmGAAfUfCmAmGfUmGmAmAfUmGfCfUfUmAfUmAfC mAfUfCfCmG-idT
where mG and mA are 2'OMe modified RNA; fC and fU are 2'F modified RNA; C6NH ₂ is a hexylamine linker; and idT is an inverted deoxythymidine residue.			

[00217] Data was fit to a 1:1 kinetic binding model. Binding affinities were calculated based on these fits and are shown in **Table 7**. Results of the assay demonstrate that Aptamer 26 bound to all three variants of VEGF-A tested, whereas, consistent with the literature, Aptamer 7 only bound to VEGF-A₁₆₅. The affinity for Aptamer 7 to VEGF-A₁₆₅ as determined here was consistent with the literature reported value (Ruckman et al. 1998). This result provided further evidence that the selection effectively identified pan-specific anti-VEGF-A aptamers, which recognized an epitope of VEGF-A contained within the RBD. This differentiated the aptamers identified in the selection described herein from previously described aptamers to VEGF-A, such as Aptamer 7, which recognize an epitope of VEGF-A contained within the HBD, and thus do not bind the non-HBD bearing variants VEGF-A₁₁₀ and VEGF-A₁₂₁.

Table 7. Binding affinity (nM) of aptamers to VEGF-A variants as determined by SPR

Aptamer	VEGF-A ₁₆₅	VEGF-A ₁₂₁	VEGF-A ₁₁₀
Aptamer 26	0.5 ± 0.2	4.6 ± 1.7	9.9 ± 3.5
Aptamer 7	0.6	no binding	no binding
"No binding" means no change in signal observed at the highest concentration tested			

Example 3. Characterization of mechanisms of interaction by VEGF-A:KDR competition binding

[00218] The SPR data in **Example 2** demonstrated that Aptamer 26 bound to the RBD of VEGF-A. To further define the epitope within the RBD of VEGF-A recognized by Aptamer 26, the mechanism of action of Aptamer 26 was interrogated by testing the ability of Aptamer 26 to directly inhibit KDR binding to either VEGF-A₁₆₅ or VEGF-A₁₂₁ as compared to a clone of an anti-VEGF-A antibody (Ferrara 2004 b) known to bind an epitope contained in the RBD that defines the receptor binding face of VEGF-A (**SEQ ID NO: 432** and **SEQ ID NO: 433**, shown in **Table 8**), and as compared to Aptamer 7.

[00219] Briefly, the aptamers were heated at 90°C for 3 minutes and allowed to cool to room temperature for a minimum of 10 minutes. Aptamers and anti-VEGF-A antibody were serially diluted in a polypropylene plate and 5 µL was transferred to a white low volume 384 well Optiplate (Perkin Elmer). A solution of VEGF-A₁₆₅ (Acro BioSystems) or VEGF-A₁₂₁ (Acro

BioSystems) that was glycan biotinylated was prepared and added to the assay plate containing aptamers or anti-VEGF-A antibody to yield a final assay concentration of 2 nM. A mixture of human recombinant his-tagged KDR, (Sino Biological), AlphaLISA[®] nickel chelate acceptor beads (Perkin Elmer), and AlphaScreen[®] streptavidin donor beads (Perkin Elmer) was then prepared and 10 μ L was added to the assay plate. The final concentrations of KDR and beads were 5 nM and 5 μ g/mL, respectively. The assay plate was sealed and incubated in the dark for approximately 2 hours, after which it was read on a Biotek CYTATION[™] 5 plate reader using the Alpha 384 well optical cube. The low signal control was determined using excess anti-VEGF-A inhibitor and the high signal control was determined by buffer only.

[00220] Percent inhibition for each sample was calculated by the following formula:

$$\% \text{ inhibition} = 1 - (\text{sample-low control}) / (\text{high control-low control}) * 100$$

The values were fit by using a four-parameter non-linear fit in GraphPad Prism Version 7.0.

[00221] The results (**FIG. 4A** and **FIG. 4B**) demonstrate that Aptamer 26 and the anti-VEGF-A mAb directly block the interaction of VEGF-A (VEGF-A₁₆₅ or VEGF-A₁₂₁) with KDR. In contrast, despite its high affinity for VEGF-A₁₆₅ as provided in **Example 2**, Aptamer 7 shows no activity in this assay against VEGF-A₁₆₅, demonstrating that it does not directly inhibit the interaction between VEGF-A and its receptor. As expected, given the lack of interaction between Aptamer 7 and VEGF-A₁₂₁, Aptamer 7 also showed no inhibition of the interaction between this variant of VEGF-A and KDR. Aptamer 26 blocked the interaction of VEGF-A₁₆₅ with KDR with an IC₅₀ of 3.7 ± 2.4 nM and the interaction of VEGF-A₁₂₁ with KDR with an IC₅₀ of 19 ± 19 nM, consistent with the affinity of Aptamer 26 for the respective VEGF-A variants. Similarly, the anti-VEGF mAb also inhibited the interaction of VEGF-A₁₆₅ and VEGF-A₁₂₁ with an IC₅₀ of 0.71 ± 0.48 nM and 0.63 ± 0.22 nM, respectively, consistent with its affinity for these VEGF-A variants.

[00222] The data presented in **FIG. 4A** and **FIG. 4B** demonstrated that Aptamer 26 bound to an epitope consisting of, or overlapping with, the receptor binding face contained within the RBD of VEGF-A, and thus directly blocked the interaction of VEGF-A with its cognate receptor. The absence of inhibition of the interaction of VEGF-A₁₆₅ with KDR by Aptamer 7 is consistent with the literature, which demonstrates that this aptamer engages VEGF-A by binding to an epitope

within the HBD and does not directly block binding of VEGF-A to its receptor, KDR (Ruckman, 1998; Lee 2005; Ng, 2006). The lack of inhibition of VEGF-A₁₂₁ activity by Aptamer 7 is consistent with the SPR binding data described in **Example 2** and the published literature (Ruckman, 1998).

Table 8. Anti-VEGF-A mAb

SEQ ID NO	Name	Type	Sequence (N to C terminus)
SEQ ID NO: 432	Light Chain	Protein	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQYSTVPWTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 433	Heavy Chain	Protein	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAP GKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQ MNSLRAEDTAVYYCAKYPHYGSSHWYFDVWGQGLTVTS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Example 4. Characterization of inhibition of VEGF-A signal transduction by KDR phosphorylation AlphaLisa[®]

[00223] When the RBD of VEGF-A binds to KDR, the receptor dimerizes leading to trans-autophosphorylation and activation of VEGF-A signaling. To determine if Aptamer 26 binding to the RBD of VEGF-A resulted in inhibition of VEGF-A activity, Aptamer 26 was tested for its ability to inhibit KDR phosphorylation induced by either VEGF-A₁₆₅ or VEGF-A₁₂₁ as compared to a clone of an anti-VEGF-A antibody (Ferrara 2004 b) (**SEQ ID NO: 432** and **SEQ ID NO: 433**, shown in **Table 8**), and as compared to Aptamer 7 for inhibition of VEGF-A₁₆₅-induced KDR phosphorylation.

[00224] Briefly, HEK293 cells engineered to stably overexpress KDR were plated overnight in collagen coated 96 well plates at 50k cells/well. Aptamers in SB1+ (40 mM HEPES, pH 7.5, 125 mM NaCl, 5 mM KCl, 1 mM MgCl₂) were heated to 90°C for 3 minutes and allowed to cool to room temperature for a minimum of 10 minutes. VEGF-A₁₂₁ (Biolegend) and VEGF-A₁₆₅ (R&D Systems) were prepared at 12.5 nM in DMEM + 0.8% FBS, a 20X stock for the reaction. 15 µL of VEGF-A was added to 15 µL titrated aptamer in a polypropylene plate and diluted to 300 µL with TS buffer (10 mM Tris pH 7.5; 100 mM NaCl; 5.7 mM KCl; 1 mM MgCl₂; 1 mM CaCl₂). The aptamer/VEGF-A mixture was incubated at 37°C for 30 minutes, after which 100 µL was added to the cells for 5 minutes at 37°C in 5% CO₂. Treatments were aspirated from cells, and cells were lysed with 100 µL cold lysis buffer [20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5 mM sodium orthovanadate (freshly prepared), 1 mM PMSF (freshly prepared), 1x protease inhibitor cocktail (freshly prepared)] on ice for 10 minutes. Plates were centrifuged at 4000 x g for 10 minutes before transferring the cell lysis to the AlphaLISA[®] assay plate.

[00225] To perform the AlphaLISA[®] assay, 10 µL of cell lysis was transferred to a white low volume 384 well Optiplate (Perkin Elmer). A mixture of the following components was made in order of which they are listed: 1.25 nM anti-hVEGFR2 polyclonal goat IgG antibody (R&D Systems), 10 µg/ml AlphaLISA[®] anti-goat IgG acceptor beads (Perkin Elmer), 1.25 nM P-tyrosine biotinylated mouse mAb (Cell Signaling Technology), and 10 µg/ml AlphaScreen[®] streptavidin donor beads (Perkin Elmer). 10 µL of this reagent mixture was added to the assay plate that contained 10 µL of cell lysate. The assay plate was sealed and incubated in the dark for approximately 2 hours, then was read on a Biotek CYTATION[™] 5 plate reader using the Alpha 384 well optical cube. Percent inhibition was calculated by subtracting TS buffer background from each value and normalizing to VEGF-A only controls. The values were fit by using a four-parameter non-linear fit in GraphPad Prism Version 7.0.

[00226] The results demonstrated that Aptamer 26 and the anti-VEGF-A mAb achieved full inhibition of the phosphorylation of KDR by inhibiting its interaction with VEGF-A₁₆₅ or VEGF-A₁₂₁ (**FIG. 5A** and **FIG. 5B**). Consistent with their measured affinities for VEGF-A₁₆₅ and VEGF-A₁₂₁, calculated IC₅₀ values for Aptamer 26 were 1.5 ± 0.6 nM and 20 ± 5.2 nM, and for the anti-VEGF-A mAb 1.1 ± 0.20 nM and 2.8 ± 1.7 nM, respectively, for inhibition of phosphorylation of KDR by VEGF-A₁₆₅ or VEGF-A₁₂₁. Conversely, Aptamer 7 only partially

inhibited phosphorylation of KDR by VEGF-A₁₆₅ (maximum inhibition of ~80%), with an IC₅₀ of 2.2 nM (**FIG. 5A** and **FIG. 5B**).

[00227] The potency and complete inhibition of VEGF-A₁₆₅ or VEGF-A₁₂₁ induced phosphorylation of KDR by Aptamer 26, and its comparable activity profile to an anti-VEGF-A mAb, was consistent with the ability of Aptamer 26 to bind to the receptor binding face present within the RBD of VEGF-A and directly block the interaction between VEGF-A and KDR. The partial inhibition of VEGF-A₁₆₅-induced phosphorylation of KDR by Aptamer 7 was consistent with indirect inhibition of KDR phosphorylation via blocking the interaction of neuropilin-1 with VEGF-A₁₆₅ (Soker, 1998). This result supported the conclusion that binding of Aptamer 26 to the receptor binding face within the RBD of VEGF-A variants confers inhibition of VEGF-A signaling. Further, these results differentiated the mechanism of action of Aptamer 26 from previously described HBD binding aptamers such as Aptamer 7.

Example 5. Secondary selection of a VEGF-A inhibiting aptamer

[00228] To further define the secondary structure of the active aptamer, as well as to potentially identify VEGF-A aptamers with increased potency, secondary selections were performed utilizing a partially randomized library based on Aptamer 26 (85% of the parental sequence + 5% of the other three nucleotides at each position within the aptamer). The library sequence and the sequence of oligos used to amplify the library are described in **Table 9**.

Table 9. Library sequence and sequence of oligos used to amplify the degenerate library

SEQ ID NO.		Sequence (5' to 3')
SEQ ID NO: 434	Doped library sequence (Total library length: 89 bases)	GGGAGAGTCGGTAGCATACT- AGGCCGCCTCCGCGCGGAGGGGTTTCATTATCCC GTTTGGCGGCTT -TGATACTTGATCGCCCTAGAAGC
SEQ ID NO: 435	Dope.F24	P-GGGAGAGTCGGTAGCATACT
SEQ ID NO: 430	N30S.R	GCTTCTAGGGCGATCAAGTATCA

where A, C, G and T are deoxyribonucleotides and P- denotes a phage polymerase promoter. Bold denotes doped positions where the indicated parent nucleotide comprises 85% of the phosphoramidite mix and each of the other 3 nucleotides account for 5%.

[00229] Six rounds of selection were conducted against VEGF-A₁₁₀. The progress of the selection was monitored by flow cytometry to ensure the enrichment for function (data not shown).

[00230] Library DNA from Round 1 through Round 6 were barcoded, pooled, and sequenced on a MiniSeq high throughput sequencer (Illumina), which yielded approximately 400,000 sequences per round. Sequences were trimmed to remove constant regions from the 5' and 3' ends, leaving the core 30 nucleotide region from the library and the terminal 8 base pair stem. Identical sequences were de-duplicated to form stacks of identical sequences. The resultant stacks were then rank ordered based on the total number of sequences within each stack. To a first approximation, the number of times a sequence occurs in a stack directly correlates with molecular function; more functional molecules typically occur more times. Thus, the rank order of each stack can be thought of as a proxy for fitness.

[00231] Alignment of the top 250 stacks for Aptamer 26, which represented ~185,000 de-duplicated sequences and corresponded to the top performing ~40% of the selected population from Round 6 of the secondary selection, revealed a significant level of conservation in the identity of each nucleotide within the aptamer family. Most positions displayed conservation levels > 90% (**FIG. 6**), with half of the positions proving to be invariant (conservation = 100%). Close examination of these stacks strongly supported the predicted stem loop secondary structure (**FIG. 7**).

Example 6. Sequence Analysis for degenerate selection of Aptamer 26

[00232] Comparison of the top 250 stacks revealed that the enriched sequences readily adopted a stem loop structure consistent with the secondary structure shown in **FIG. 7**. Such a structure may comprise a terminal stem S1 that may be connected to the 5' terminal end of loop L1. Loop L1 may be connected to the 3' terminal end of stem S1 and the 5' terminal end of stem S2. Stem S2 may be connected to the 3' terminal end of loop L1 and the 5' terminal end of loop L2. Loop L2 may be connected to the 3' terminal end of stem S2 and the 5' terminal end of the complementary region of stem S2. The complementary region of stem S2 may be connected to the 3' terminal end of loop L2 and the 5' terminal end of loop L3. Loop L3 may be connected to the 3' terminal end of the complementary region of stem S2 and the 5' end of stem S3. Stem S3 may be connected to the 3' terminal end of loop L3 and the 5' terminal end of loop L4. Loop L4

may be connected to the 3' terminal end of stem S3 and the 5' terminal end of the complementary region of stem S3. The complementary region of stem S3 may be connected to the 3' end of loop L4 and the 5' end of loop L5. Loop L5 may be connected to the 3' terminal end of the complementary region of stem S3 and the 5' terminal end of the complementary region of stem S1. The complementary region of stem S1 may be connected to the 3' terminal end of loop L5.

[00233] A comparison of sequences observed in stem S1 are shown in **Table 10**. These data provide additional support of the formation of Stem 1, as indicated by the sequence covariation in this region. In some cases, stem S1 contained one, two or three mismatched nucleotides. In some cases, the mismatched nucleotides were adjacent to each other. In some cases, the mismatched nucleotides were separated by one, two, or three base pairs. In some cases, stem S1 was composed of two to eight contiguous base pairs that ended in a base pair at positions 8 and 39. As depicted in **FIG. 6**, positions 8 and 39 were 100% conserved and formed a C:G pair that closed stem S1 adjacent to loop L1 and loop L5. In some cases, the base pair between positions 8 and 39 (8/39) was separated from other contiguous base pairs in stem S1 by mismatched nucleotides at positions 7 and 40 (7/40). In some cases, the preferred length of the contiguous run within stem S1 that ends in a base pair at positions 8 and 39 was three base pairs.

[00234] In some cases, stem S1 contained a mismatch at positions 5 and 42, demonstrated by a preference for conversion from G in the parent sequence to a U in approximately 69% of the sequences represented in the top 250 stacks (**FIG. 6**). The introduction of the mispairing in the middle of stem S1 suggested the possibility that truncation may be favorable. Together, these data suggested that the consensus sequence was 5'-HNB^YHDCC-3' for the 5' side of the stem. The consensus sequence for the 3' complementary side of stem S1 was 5'-GKY^NKNVNW-3', where Y is C or U; W is A or U; K is G or U; B is C, G or U; D is A, G or U; H is A, C, or U; V is A, C, or G; and N is A, C, G, or U.

Table 10. Doped selection sequence variation observed in stem S1 (S1) (Full sequences and truncated sequences are described in **Table 1**).

Sequence (5' to 3')		Sequence (5' to 3')	
Aptamer 26	AGGCCGCC/GGCGGCUU	R6-92	ACGCA <u>U</u> CC/GGC <u>U</u> GCGU
R6-1	AGGCCGCC/GGC <u>U</u> GCUU	R6-101	AAGCCGCC/G <u>U</u> CGGCUU

R6-2	A <u>U</u> GCCGCC/GGC <u>U</u> GCUU	R6-102	<u>U</u> UGCUGCC/GGC <u>U</u> GCUU
R6-3	AUGCCGCC/GGC <u>U</u> GCAU	R6-104	<u>U</u> GGCCGCC/GGC <u>U</u> GCUU
R6-4	A <u>U</u> GCCGCC/GGCGGC <u>U</u> U	R6-115	AGGCA <u>U</u> CC/GGC <u>U</u> GCUU
R6-7	AAGCCGCC/GGC <u>U</u> GCUU	R6-116	AGGCCGCC/G <u>U</u> CG <u>U</u> CCU
R6-8	AAGC <u>U</u> GCC/GGC <u>U</u> GCUU	R6-123	AAUCCGCC/GGCGGAU
R6-12	A <u>U</u> GCCGCC/G <u>U</u> CGGC <u>U</u> U	R6-124	AGGC <u>U</u> GCC/GGC <u>U</u> GCCU
R6-13	AUGC <u>U</u> GCC/GGC <u>U</u> GCAU	R6-130	AA <u>U</u> CCGCC/GGCGGC <u>U</u> U
R6-14	AAGC <u>U</u> GCC/GGCC <u>U</u> GCUU	R6-134	A <u>U</u> GCUGCC/GGCAGC <u>U</u> U
R6-16	AAGCA <u>U</u> CC/GGC <u>U</u> GCUU	R6-135	AGGCUGCC/GGCGGC <u>U</u> U
R6-17	A <u>U</u> GCA GCC/GGCUGC <u>U</u> U	R6-138	AUGCUGCC/GGCAGCAU
R6-18	AUGCCGCC/GGCGGCAU	R6-145	<u>U</u> UGCUGCC/GGCGGC <u>U</u> U
R6-19	AUGUCGCC/GGCGGCAU	R6-152	<u>C</u> UGCCGCC/GGCGGCAU
R6-20	AGGCCGCC/GGC <u>U</u> GCCU	R6-153	AU <u>U</u> CAGCC/GGCUGC <u>U</u> A
R6-21	AGGCCGCC/G <u>U</u> CGGC <u>U</u> U	R6-157	AAGCAGCC/GGCGGC <u>U</u> U
R6-22	AUGCUGCC/GGCGGCAU	R6-162	AUGCCGCC/G <u>U</u> CGGC <u>U</u> U
R6-25	AUGC <u>U</u> GCC/GGCC <u>U</u> GCAU	R6-166	AGUCCGCC/GGC <u>U</u> GACU
R6-31	AGGCAGCC/GGCUGCUU	R6-169	AGUCCGCC/GGCGGAU
R6-34	AGGC <u>U</u> GCC/GGC <u>U</u> GCUU	R6-170	<u>C</u> UGCUGCC/GGCGGCAU
R6-36	AUGCCGCC/G <u>U</u> CGGCAU	R6-171	AC <u>U</u> CAGCC/GGCUGC <u>U</u> G
R6-39	AGGCA <u>U</u> CC/GGC <u>U</u> GCCU	R6-172	AGGC <u>U</u> GCC/GGCA <u>U</u> CCU
R6-42	AAGCUGCC/GGCGGC <u>U</u> U	R6-173	<u>U</u> UGC <u>U</u> GCC/GGC <u>U</u> GCAU
R6-45	AG <u>U</u> CAGCC/GGCUGC <u>U</u> U	R6-177	<u>U</u> GUCCGCC/GGCGGC <u>U</u> U
R6-48	AUGCCGCC/GGUGGCAU	R6-178	<u>C</u> UGCAGCC/GGCUGC <u>U</u> U
R6-49	A <u>U</u> GCUGCC/GGCGGC <u>U</u> U	R6-179	AGUCCGCC/GGCG <u>U</u> ACU
R6-50	AUGCA <u>U</u> CC/GGC <u>U</u> GCAU	R6-181	AGCCGCC/GGCG <u>U</u> GCU
R6-53	AGGCCACC/GGUGGCUU	R6-182	AG <u>U</u> CCACC/GGUGGC <u>U</u> A
R6-54	AG <u>U</u> CCGCC/GGCGGC <u>U</u> A	R6-197	AUGCAGCC/GGCUGC <u>U</u> U
R6-57	AAGCAGCC/GGCUGCUU	R6-198	AGGCCGCC/GGC <u>U</u> GCAU
R6-58	AUGCAGCC/GGCUGCAU	R6-201	A <u>U</u> UCAGCC/GGCUGC <u>U</u> U
R6-67	AGG <u>C</u> CACC/GGUG <u>U</u> CCU	R6-203	AUGCCGCC/GGC <u>U</u> GCGU
R6-70	<u>U</u> UGCCGCC/GGC <u>U</u> GCUU	R6-204	AU <u>U</u> CCGCC/GGCGGC <u>U</u> A
R6-72	A <u>C</u> GCA GCC/GGCUGC <u>U</u> U	R6-205	A <u>U</u> GUCGCC/GGCGGC <u>U</u> U
R6-76	<u>U</u> UGCUGCC/GGCC <u>U</u> GCAU	R6-208	AC <u>U</u> CAGCC/GGCUGA <u>U</u> U
R6-79	AAGCCGCC/GGCGGC <u>U</u> U	R6-210	AAGCCACC/GGUGGCUU

R6-80	AA <u>U</u> CAGCC/GGCUG <u>C</u> UU	R6-214	AGG <u>C</u> CGCC/GGCG <u>U</u> CCU
R6-83	<u>U</u> UGCCGCC/GGCGGC <u>U</u> U	R6-221	AG <u>U</u> CCACC/GGUGG <u>C</u> CU
R6-85	<u>C</u> UGCCGCC/GGCGGC <u>U</u> U	R6-227	<u>C</u> UGC <u>U</u> GCC/GGCUGCA <u>U</u>
R6-88	A <u>U</u> GC <u>U</u> GCC/GGC <u>U</u> GC <u>U</u> U	R6-241	AGGCUGCC/GGCAGCUU
Differences from the parent (Aptamer 26) stem S1 are denoted in bold . Unpaired residues are underlined.			

[00235] The identity of loop L1 was found to be 100% conserved across the top 250 stacks of sequences analyzed in the doped selection (**FIG. 6**). Thus, the nucleotide sequence of loop L1 was 5'-U-3'.

[00236] The identity of stem S2 was found to be 100% conserved across the top 250 stacks of sequences analyzed in the doped selection (**FIG. 6**). Thus, the nucleotide sequence of stem S2 was 5'-CC/GG-3'.

[00237] The identity of loop L2 was found to be 100% conserved across the top 250 stacks of sequences analyzed in the doped selection (**FIG. 6**). Thus, the nucleotide sequence of loop L2 was 5'-GCGC-3'.

[00238] The identity of loop L3 was found to be 100% conserved across the top 250 stacks of sequences analyzed in the doped selection (**FIG. 6**). Thus, the nucleotide sequence of loop L3 was 5'-A-3'.

[00239] A comparison of sequences observed in stem S3 suggested that the preferred length of stem S3 was four, five, or six base pairs. When stem S3 was six base pairs in length, the 5' side of the stem may be seven nucleotides in length. As such, the six base pair stem S3 may contain a single mis-match at the penultimate nucleotide in the 5' side of the stem. These data provided additional support of the formation of stem S3, as indicated by the sequence covariation in this region (**Table 11**). The variation between four, five, and six base pairs in stem S3 directly related to the variation of loop L4 between eight, six, four and three nucleotides. When stem S3 was four base pairs in length, loop L4 was eight nucleotides in length. When stem S3 was five base pairs in length, loop L4 was six nucleotides in length. When stem S3 was six base pairs in length, loop L4 was four nucleotides in length. When stem S3 was six base pairs in length and contained a mismatch, loop L4 was three nucleotides in length.

[00240] Together, these data suggested that the consensus sequence of stem S3 for all variants observed with the top 250 stacks of Aptamer 26 was 5'-GGGRUD-3' for the 5' side of stem S3 and 5'-HWYCCC-3' for the 3' side of stem S3, where R is A or U; D is A, G, or U; H is A, C, or

U; W is A or U; and Y is C or U. In some cases, when stem S3 was four base pairs, the consensus sequence was 5'-GGGG-3' for the 5' side of stem S3 and 5'-CCCC-3' for the 3' side of stem S3. In some cases, when stem S3 was 5 base pairs, the consensus sequence was 5'-GGGRU-3' for the 5' side of stem S3 and 5'-AYCCC-3' for the 3' side, where R is A or G; and Y is C or U. In some cases, when stem S3 was six base pairs, the consensus sequence was 5'-GGGGUD-3' for the 5' side of stem S3 and 5'-HAUCCC-3' for the 3' side, where D is A, G or U; and H is A, C, or U. In some cases, when stem S3 was six base pairs long and contained a single mis-matched nucleotide, the consensus was 5'-GGGRUUR-3' for the 5' side of stem S3 and 5'-UAUCCC-3' for the 3' side, where the underlined U is the single mis-matched nucleotide; and R is A or G.

Table 11. Doped selection sequence variation observed in stem S3 (Full sequences and truncated sequences are described in **Table 1**).

Sequence (5' to 3')	
Aptamer 26	GGGGU / AUCCC
R6-9	GGGGU <u>U</u> A/UAUCCC
R6-33	GGGAU <u>U</u> A/UAUCCC
R6-43	GGGGU <u>U</u> G/UAUCCC
R6-68	GGGAU <u>U</u> G/UAUCCC
R6-5	GGGGUU/AAUCCC
R6-6	GGGGUA/UAUCCC
R6-61	GGGGUG/CAUCCC
R6-1	GGGAU / AUCCC
R6-65	GGGGU / ACCCC
R6-62	GGGG / CCCC
Differences from the parent (Aptamer 26) stem S3 are denoted in bold .	

[00241] A comparison of sequences observed in loop L4 suggested that the preferred length of loop L4 was eight, six, four, or three nucleotides (**Table 12**). The variation between eight, six, four and three nucleotides in loop L4 directly related to the variation in the length of stem S3 between four, five, six base pairs, or six base pairs and a single mismatch. Together, these data

suggested that when loop L4 was three nucleotides in length, the consensus sequence for loop L4 was 5'-MAU-3', where M is A or C. When loop L4 was four nucleotides in length, the consensus sequence for loop L4 was 5'-DNAH-3', where D is A, G or U; H is A, C, or U; and N is A, C, G, or U. In some cases, when loop L4 was six nucleotides in length, the consensus sequence for loop L4 was 5'-UDNDHU-3', where D is A, G or U; H is A, C, or U; and N is A, C, G, or U. In some cases, when loop L4 was eight nucleotides in length, the consensus sequence for loop L4 was 5'-UUUCAUUU-3'.

Table 12. Doped selection sequence variation observed in loop L4 (Full sequences and truncated sequences are described in **Table 1**).

Sequence (5' to 3')		Sequence (5' to 3')	
Aptamer 26	UUCAUU	R6-186	UUCGUU
R6-62	UUUCAUUU	R6-5	UCAU
R6-27	U A CAUU	R6-24	UC A C
R6-43	U G CAUU	R6-29	A CAU
R6-75	UUC A CU	R6-117	UGAU
R6-99	UU A AUU	R6-200	UU A U
R6-110	UUU A UU	R6-228	G CAA
R6-119	UUC U UU	R6-229	UU A U
R6-131	UUC A AU	R6-9	CAU
R6-142	UUG A UU	R6-211	A AU
Differences from the parent (Aptamer 26) loop L4 are denoted in bold .			

[00242] A comparison of sequences observed in loop L5 suggested that the preferred length of loop L5 was four bases (**Table 13**). Together, these data suggest the consensus sequence for loop L5 was 5'-GYUU-3', where Y is C or U.

Table 13. Doped selection sequence variation observed in loop L5 (Full sequences and truncated sequences are described in **Table 1**).

Sequence (5' to 3')	
Aptamer 26	GUUU
R6-139	G C UU
Differences from the parent (Aptamer 26) loop L5 are denoted in bold .	

[00243] Using the data from the degenerate selection, the consensus sequence for all sequence variants within the top 250 stacks of the Aptamer 26 family of sequence members was 5'-HNBYHDCC-U-CC-GCGC-GG-A-GGGRUD-DNDH-HWYCCC-GYUU-GKYNKVNW-3' (SEQ ID NO: 1), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G; and is represented with a six base pair stem S3 and a four nucleotide loop L4 in FIG. 7. The alternate pairings of stem S3 and loop L4 are highlighted by dashed boxes. When stem S3 was five base pairs and loop L4 was six nucleotides in length, the consensus sequence for the Aptamer 26 family of sequence members was 5'-HNBYHDCC-U-CC-GCGC-GG-A-GGGRU-UDNDHU-AYCCC-GYUU-GKYNKVNW-3' (SEQ ID NO: 2), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; K is G or U; V is A, C, or G; and W is A or U. When stem S3 was six base pairs and loop L4 was four nucleotides in length, the consensus sequence for the Aptamer 26 family of sequence members was 5'-WNKYHDCC-U-CC-GCGC-GG-A-GGGGUD-DNAH-HAUCCC-GUUU-GGYBKMHW-3' (SEQ ID NO: 3), where W is A or U; N is A, C, G, or U; K is G or U; Y is C or U; H is A, C, or U; D is A, G, or U; B is C, G, or U; and M is A or C. When stem S3 was four base pairs and loop L4 was eight nucleotides in length, the consensus sequence for the Aptamer 26 family of sequence members was 5'-AUGCCGCC-U-CC-GCGC-GG-A-GGGG-UUCAUUU-CCCC-GUUU-GGCUGCAU-3' (SEQ ID NO: 4).

[00244] FIG. 7 also depicts the motif variations for each structural element (*e.g.*, stem S1, loop L1, stem S2, loop L2, loop L3, stem S3, loop L4, loop L5) observed within the top 250 sequence stacks from the selection. By combining the provided motifs in the proper order for the respective structural elements of this aptamer family, one can assemble extant or novel Aptamer 26-like variants with anti-VEGF-A activity.

Example 7. Linker scanning Aptamer 26

[00245] To better understand the nucleotide dependence and secondary structure of Aptamer 26, variants of the parent aptamer were synthesized in which every position from U9 to U38 was replaced, individually, with a non-nucleotide 3-carbon spacer (SP3; 1,3-propanediol) and assayed for activity using a receptor phosphorylation AlphaLISA[®] and competition TR-FRET (Table 14 and FIG. 8).

[00246] Briefly, for competition TR-FRET, 2-fold dilutions of thermally equilibrated aptamers were made in TR-FRET Buffer (50 mM MOPS, pH 7.4, 125 mM NaCl, 5 mM KCl, 50 μ M CHAPS, 0.1 mg/mL BSA, 1 mM CaCl₂, and 1 mM MgCl₂). 5 μ L of aptamer or control solution was added to a 15 μ L mix of glycan biotinylated VEGF-A₁₂₁ (5 nM final), ALEXA FLUOR[®] 647-labeled Aptamer (30 nM final), and Streptavidin-Eu (Perkin Elmer; 2.5 nM final) in a black wall low volume 384 well plate (Greiner). For control, 5 μ L of 1000-fold excess of unlabeled parent aptamer or 5 μ L TR-FRET buffer alone was added to the mix of ALEXA FLUOR[®] 647-labeled aptamer, biotin-VEGF-A, and Streptavidin-Eu. The plate was covered with a plate seal and subsequently incubated in the dark for 1 hour at room temperature. The plate was read on a Biotek CYTATION[™] 5 plate reader. Samples were excited at 330 nm and fluorescent values were collected at 665 nm. Data analysis was performed by subtracting the background value and plotting as percent inhibition, normalized to baseline in the absence of competitor. The values were fit by [Inhibitor] vs. response - Variable slope (four parameters) using GraphPad Prism Version 7.0 and then normalized to aptamer control to obtain an IC₅₀ relative to parent aptamer. Data is presented as log values of relative IC₅₀.

[00247] Consistent with the high level of conservation observed during the degenerate selection (FIG. 6), the majority of positions could not be replaced with a linker without a substantial loss in activity (>10-fold worse). Interestingly, positions 24 through 29 (see Table 14; Aptamers 62, 63, 64, 65, 66, and 67), tolerated replacement by SP3 with relatively equal activity to the parent. This further supported the secondary structure proposed in Example 5 and shown in FIG. 7, demonstrating that these positions were in a loop or at the end of a stem and not essential for structure. More surprisingly, replacement of loop L1 (position 9) with an SP3 linker led to a >10-fold enhancement of activity against VEGF-A₁₂₁, even though it was found to be 100% conserved in the degenerate selection (Table 14 and FIG. 8; Aptamer 47).

Table 14. Linker Scan Analysis

SEQ ID NO with modifications:	Aptamer Number	Sequence (5' to 3')	pKDR	TR- FRET
		S1 L1 S2 L2 S2 L3 S3 L4 S3 L5 S1		
220	Aptamer 26	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	parent	parent
227	Aptamer 47	C6NH ₂ -AGGCCGCC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	++	++

228	Aptamer 48	C6NH ₂ -AGGCCGCC-U-3C-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	----	nd
229/230	Aptamer 49	C6NH ₂ -AGGCCGCC-U-C3-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	----	nd
231/232	Aptamer 50	C6NH ₂ -AGGCCGCC-U-CC-3CGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	----	nd
233/234	Aptamer 51	C6NH ₂ -AGGCCGCC-U-CC-G3GC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	----	nd
235/236	Aptamer 52	C6NH ₂ -AGGCCGCC-U-CC-GC3C-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	---	nd
237/238	Aptamer 53	C6NH ₂ -AGGCCGCC-U-CC-GCG3-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	---	nd
239/240	Aptamer 54	C6NH ₂ -AGGCCGCC-U-CC-GCGC-3G-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	---	nd
241/242	Aptamer 55	C6NH ₂ -AGGCCGCC-U-CC-GCGC-G3-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	---	nd
243/244	Aptamer 56	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-3-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	---	nd
245/246	Aptamer 57	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-3GGGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	---	nd
247/248	Aptamer 58	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-G3GGU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	---	nd
249/250	Aptamer 59	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GG3GU-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	----	nd
251/252	Aptamer 60	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGG3U-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	----	nd
253/254	Aptamer 61	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGG3-UUCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	----	---
255/256	Aptamer 62	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-3UCAUU-AUCCC-GUUU-GGCGGCCUU-IdT	~	--
257/258	Aptamer 63	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-U3CAUU-AUCCC-GUUU-GGCGGCCUU-IdT	~	~
259/260	Aptamer 64	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UU3AUU-AUCCC-GUUU-GGCGGCCUU-IdT	~	--
261/262	Aptamer 65	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUC3UU-AUCCC-GUUU-GGCGGCCUU-IdT	~	~
263/264	Aptamer 66	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCA3U-AUCCC-GUUU-GGCGGCCUU-IdT	~	~
265/266	Aptamer 67	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAU3-AUCCC-GUUU-GGCGGCCUU-IdT	~	~
267/268	Aptamer 68	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-3UCCC-GUUU-GGCGGCCUU-IdT	--	---
269/270	Aptamer 69	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-A3CCC-GUUU-GGCGGCCUU-IdT	---	nd
271/272	Aptamer 70	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AU3CC-GUUU-GGCGGCCUU-IdT	---	nd
273/274	Aptamer 71	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUC3C-GUUU-GGCGGCCUU-IdT	---	nd
275/276	Aptamer	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCC3-GUUU-GGCGGCCUU-IdT	----	nd

	72			
277/278	Aptamer 73	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- 3 UUU-GGCGGCCUU-idT	---	nd
279/280	Aptamer 74	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-G 3 UU-GGCGGCCUU-idT	---	nd
281/282	Aptamer 75	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GU 3 U-GGCGGCCUU-idT	---	nd
283	Aptamer 76	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUU 3 -GGCGGCCUU-idT	----	nd
where G is 2'F, and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; 3 is the SP3 spacer (1,3-propanediol); and bold letters denote differences from the parent Aptamer 26. Dashes (-) delineate the structural features of the aptamers (<i>e.g.</i> , stems, loops).				
Key: ~ = 2 fold worse to 2 fold better, + = 2-10 fold better, ++ = 10-100 fold better, +++ more than 100 fold better, - = 2-10 fold worse, 10-100 fold worse, --- = more than 100 fold worse. nd = not determined.				

Example 8. Structure validation and analysis by selective mutagenesis

[00248] To better understand the sequence requirements and to confirm the stem structures of the Aptamer 26 family as determined from sequence covariation analysis from the secondary (degenerate) selection, a series of variants that included mutations and deletions to the predicted stems were synthesized and screened. Activity of each of these variants were tested using a time resolved competition TR-FRET assay, as described in **Example 7**, in which a labeled parent was competed with increasing concentrations of unlabeled variants for binding to VEGF-A₁₂₁.

[00249] Replacing the sequence of stem S1 found in Aptamer 26, 5'-AGGCCGCC/GGCGGCCUU-3', with single base pair A/U or U/A covariations in positions 7/40 and 8/39 (**Table 15** and **FIG. 9**; Aptamers 122, 123, 125, and 126) showed activity within approximately 2-fold of the parent molecule, confirming the presence of a stem. Other modifications in these positions resulted in only a modest loss in activity (approximately 2-10 fold; Aptamers 124, 135 and 136) further supporting stem formation. Importantly, mutations leading to intentional mispairing of the terminal base pairs in stem S1 led to a complete loss of activity (>100-fold; **Table 15** and **FIG. 9**; Aptamers 180 and 181). Together with the data from the doped selection (**Table 10**), these data further support the formation of stem S1, and the requirement for the formation of a base pair between positions 8 and 39 of the stem. Therefore, stem S1 may be composed of two to eight contiguous base pairs that end in a base pair at positions 8 and 39. The identity of the nucleotides can be varied with little effect on activity provided pairing is maintained. When combined with consensus sequence from the degenerate

selection, the consensus sequence was 5'-HNBYHDNN-3' for the 5' side of the stem and 5'-NNYNKVNW-3' for the 3' complementary side of stem S1, where N is A, C, G, or U; B is C, G or U; Y is C or U; H is A, C, or U; D is A, G or U; K is G or U; V is A, C, or G; and W is A or U.

[00250] The identity of stem S2 was found to be 100% conserved during the degenerate selection (FIG. 6). To confirm the identity of this stem, we replaced nucleotides to assess the effect of alternate base pairings on aptamer function. Replacing nucleotides at positions 10 and 17, or 11 and 16, resulted in only a modest loss of aptamer activity (approximately 2 -10 fold; Table 15 and FIG. 9; Aptamers 174-179) provided that base pairing was maintained. Mutations leading to mispairing of the stem S2 led to a complete loss of activity (>100-fold; Table 15 and FIG. 9; Aptamers 182 and 183), thus confirming the requirement of a stem. When combined with consensus sequence from the degenerate selection, this expanded the consensus for stem S2. Thus, Stem S2 was composed of 2 base pairs with a consensus sequence of 5'-NN-3' for both the 5' and 3' sides of the stem, where N is A, C, G, or U.

[00251] A comparison of sequences observed in stem S3 suggested that the preferred length of stem S3 was four, five, or six base pairs and that the variation between four, five, and six base pairs in stem S3 was directly related to the length of loop L4, which varied between eight, six, and four nucleotides, respectively. To confirm the identity of this stem, the effect of alternate base pairings on aptamer function was assessed. For simplicity, replacements were done in the context of Aptamer 26, which had a five base pair stem S3 and six nucleotides in loop L4.

[00252] Replacing the G:C base pair formed between positions 19 and 34 within this stem with any other canonical pairing resulted in a complete loss of activity (>100-fold; Table 15 and FIG. 9, Aptamers 112, 113 and 114). Similarly, replacing the G:C base pair formed between positions 20 and 33 with either a U:A or a C:G pair resulted in a complete loss of activity (>100 fold; Table 15 and FIG. 9, Aptamers 116 and 117). When replaced with an A:U, however, the resulting molecule (Aptamer 115) demonstrated only a modest loss in activity (approximately 7 -fold) supporting the formation of a stem, albeit one with a preference for a purine at position 20 and a pyrimidine at position 33. Interestingly, other modifications to these within the center of the stem (paired positions 21:32 and 22:31) proved refractory to change leading to significant (>10-fold; Aptamer 128) or complete (>100-fold; Aptamers 127, 129, 130, 131 and 132) loss of activity. It was unclear if this was the result of a preference for a specific base pairing within the

stem (as with position 19 and 34) or due to misfolding. Indeed, it was possible that modification of the primary sequence elsewhere in the molecule or within the stem itself could compensate for this observation and overcome these negative effects. For example, when the deleterious C:G pairing at positions 19 and 34, and 20 and 33 were combined with a U to C mutation at position 32, the resultant molecule only demonstrated a modest loss in activity (Aptamer 119; approximately 9-fold). Similar results were observed from the combination of an A:U pair at positions 20 and 33 with a G:C at positions 23 and 30 and a U to C mutation at position 31 (Aptamer 120; approximately 9-fold). These results, together with the high degree of conservation of pairing within this stem observed during the primary and degenerate selections and the observation that intentional mispairings in stem S3 resulted in molecules with no activity (>100-fold; Aptamer 184 and 185), strongly supported the formation of a stem. Finally, because shortened versions of stem S3 had been observed during the degenerate selection (**Table 11**), the effects of truncations in stem S3 were tested. Removal of the terminal A:U at positions 23 and 30 (Aptamer 108) resulted in the complete loss of activity (>100-fold), whereas removal of the penultimate pair, positions 22 and 31 (Aptamer 109), only resulted in a modest loss in activity. These data, when combined with sequence data observed in the primary and degenerate selections, supported the observation that stem S3 can be four, five or six base pairs in length, but expanded the observation that a four base pair stem S3 can be combined with a six or eight nucleotide loop L4. These data also further expanded the consensus for stem S3. When stem S3 was five base pairs long and loop L4 was six nucleotides in length, the consensus sequence was 5'-SVVVK-3' for the 5' side of the stem and 5'-MBBBS-3' for the complementary region of stem S3, where S is G or C; V is A, C, or G; K is G or U; M is A or C; and B is C, G, or U.

Table 15. Analysis of Stems 1, 2, and 3

SEQ ID NO with modific ations:	Aptamer Number	Sequence (5' to 3')	TR-FRET
		S1 L1 S2 L2 S2 L3 S3 L4 S3 L5 S1	
220	Aptamer 26	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	parent
295	Aptamer 121	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-CCCGGCUU-idT	~
296	Aptamer 122	C6NH ₂ -AGGCCGCA-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-UGCGGCUU-idT	~
297	Aptamer 123	C6NH ₂ -AGGCCGCU-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-AGCGGCUU-idT	~

298	Aptamer 124	C6NH ₂ -AGGCCGGC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	-
299	Aptamer 125	C6NH ₂ -AGGCCGAC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGCCUU-idT	~
300	Aptamer 126	C6NH ₂ -AGGCCGUC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GACGCCUU-idT	~
307	Aptamer 133	C6NH ₂ -AGGCCGAA-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-UUCGCCUU-idT	---
308	Aptamer 134	C6NH ₂ -AGGCCGAU-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-AUCGCCUU-idT	--
309	Aptamer 135	C6NH ₂ -AGGCCGUA-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-UACGCCUU-idT	-
310	Aptamer 136	C6NH ₂ -AGGCCGUU-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-AACGCCUU-idT	-
354	Aptamer 180	C6NH ₂ -AGGCCGCA-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-AACGCCUU-idT	---
355	Aptamer 181	C6NH ₂ -AGGCCGCA-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCC-CGUUU-AACCGAUU-idT	---
348	Aptamer 174	C6NH ₂ -AGGCCGCC-U-GC-GCGC-GC-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	-
349	Aptamer 175	C6NH ₂ -AGGCCGCC-U-AC-GCGC-GU-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	-
350	Aptamer 176	C6NH ₂ -AGGCCGCC-U-UC-GCGC-CA-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	-
351	Aptamer 177	C6NH ₂ -AGGCCGCC-U-CG-GCGC-CG-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	--
352	Aptamer 178	C6NH ₂ -AGGCCGCC-U-CA-GCGC-UG-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	~
353	Aptamer 179	C6NH ₂ -AGGCCGCC-U-CU-GCGC-AG-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	-
356	Aptamer 182	C6NH ₂ -AGGCCGCC-U-UU-GCGC-UU-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	----
357	Aptamer 183	C6NH ₂ -AGGCCGCC-U-CC-GCGC-CC-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCCUU-idT	---
287	Aptamer 112	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-AGGGU-UUCAUU-AUCCU-GUUU-GCGGCCUU-idT	----
288	Aptamer 113	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-UGGGU-UUCAUU-AUCCA-GUUU-GCGGCCUU-idT	---
289	Aptamer 114	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-CGGGU-UUCAUU-AUCCG-GUUU-GCGGCCUU-idT	---
290	Aptamer 115	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GAGGU-UUCAUU-AUCUC-GUUU-GCGGCCUU-idT	-
291	Aptamer 116	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GUGGU-UUCAUU-AUCAC-GUUU-GCGGCCUU-idT	---
292	Aptamer 117	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-CGGU-UUCAUU-AUCGC-GUUU-GCGGCCUU-idT	---
293	Aptamer 119	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-CCGGU-UUCAUU-ACC GG-GUUU-GCGGCCUU-idT	-
294	Aptamer	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GAGGG-UUCAUU-CCCUC-GUUU-GCGGCCUU-idT	-

	120		
301	Aptamer 127	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGCU-UUCAUU-AGCCC-GUUU-GGCGGCUU-idT	----
302	Aptamer 128	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GG ACU -UCAUU-AG UCC -GUUU-GGCGGCUU-idT	---
303	Aptamer 129	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GG UCU -UCAUU-AG ACC -GUUU-GGCGGCUU-idT	----
304	Aptamer 130	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GG UGU -UCAUU-AC ACC -GUUU-GGCGGCUU-idT	----
305	Aptamer 131	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GG AGU -UCAUU-AC UCC -GUUU-GGCGGCUU-idT	----
306	Aptamer 132	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GG CGU -UCAUU-AC GCC -GUUU-GGCGGCUU-idT	----
358	Aptamer 184	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-G CCGU -UCAUU-AU CCC -GUUU-GGCGGCUU-idT	---
359	Aptamer 185	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AU AAC -GUUU-GGCGGCUU-idT	---
285	Aptamer 108	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGG_ <u> </u> -UCAUU-_ <u> </u> UCCC-GUUU-GGCGGCUU-idT	---
286	Aptamer 109	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGU_ <u> </u> -UCAUU-_ <u> </u> ACCC-GUUU-GGCGGCUU-idT	-
where G is 2'F and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; and bold letters denote differences from the parent Aptamer 26. Dashes (-) delineate the structural features of the aptamers (<i>e.g.</i> , stems, loops) and underline (<u> </u>) denotes a deletion.			
Key: ~ = 2 fold worse to 2 fold better, + = 2-10 fold better, ++ = 10-100 fold better, +++ more than 100 fold better, - = 2-10 fold worse, 10-100 fold worse, --- = more than 100 fold worse.			

[00253] To better understand the role of loops in the Aptamer 26 sequence family, residues in loop L1, loop L2, loop L3, loop L4, and loop L5 were replaced with alternate base identities and the effects of substitutions on activity were determined.

[00254] During the degenerate selection, the identity of loop L1 (position 9) was a highly conserved U (100%; **FIG. 6**). Interestingly, this position could be replaced with any other base (C, G, or A) or deleted entirely with only modest effects on activity (**Table 16** and **FIG. 10**; Aptamers 146, 147, 148 and 79). For example, replacement with an A lead to a modest (~3-fold) increase in activity. Together, this data indicated that the identity or even the presence of a nucleotide at this position was not necessary for activity, and suggested that loop L1 could be a flexible linker joining stems S1 and S2. Thus, the consensus for loop L1 can be expanded to 5'-N*-3', where N* is A, C, G, U, a non-nucleotidyl spacer 3 modification (Sp3), or can be deleted entirely.

[00255] The identity of loop L2 was found to be 100% conserved during the degenerate selection (FIG. 6). Consistent with this, base modifications to positions within this loop were poorly tolerated. For example, replacement of position 12 within the loop with an A or C resulted in >100-fold loss in activity (Table 16 and FIG. 10; Aptamers 149 and 150). Replacement with a U was slightly less detrimental, but still resulted in >10-fold loss in activity (Aptamer 151). Position 13 proved to be the most tolerant but still resulted in a significant (>10-fold) loss in activity for substitution with A and G (Table 16 and FIG. 10; Aptamers 152 and 153). Replacement with U was the least detrimental to activity, resulting in only a 4-fold loss in activity (Aptamer 154). For both positions 14 and 15, replacement with any nucleotide other than that found in the parent resulted in >100-fold loss in activity (Table 16 and FIG. 10; Aptamers 155 – 160). Thus, the nucleotide sequence of loop L2 may be expanded to 5'-KNGC-3', where K is G or U; and N is A, C, G, or U. In a preferred embodiment, loop L2 is 5'-GYGC-3', where Y is C or U. In another preferred embodiment, loop L2 is 5'-GCGC-3'.

[00256] The identity of loop L3 was found to be 100% conserved during the degenerate selection (FIG. 6). Consistent with this observation, when the conserved A was mutated to a C or a G, the resultant molecules (Aptamers 27 and 161) lost >100-fold activity. Additionally, when mutated to a U, the resultant molecule (Aptamer 28) only demonstrated a 10-fold loss of activity. Thus, the nucleotide sequence of L3 may be 5'-W-3', where W is A or U.

[00257] A comparison of sequences observed in loop L4 suggested that positions 24, 25, and 29 within the loop were not highly conserved and remained equal to the composition of the starting library, ~85%. Positions 26, 27, and 28, on the other hand, were more conserved (97%, 99%, and 96%, respectively). The identity of loop L4 was explored by deletion analysis to assess if all nucleotides in the position were required for function. To this end, when the nucleotides at positions 24 and 29 were removed, only a modest loss in activity was observed (Aptamer 145; approximately 8-fold). Together with data from the primary and degenerate selections, these data further expand the relationship between stem S3 and loop L4. Stem S3 can be four, five or six base pairs in length. When stem S3 is four base pairs in length, loop L4 may be six or eight nucleotides in length. When stem S3 is five base pairs in length, loop L4 may be four or six nucleotides in length and when stem S3 is six base pairs, loop L4 may be four nucleotides in length. When loop L4 is four nucleotides in length, the consensus sequence for loop L4 may be 5'-DNAH-3', where D is A, G or U; H is A, C, or U; and N is A, C, G, or U. In some cases,

when loop L4 is six nucleotides in length, the consensus sequence for loop L4 may be 5'-UDNDHU-3', where D is A, G or U; H is A, C, or U; and N is A, C, G, or U. In some cases, when loop L4 is eight nucleotides in length, the consensus sequence for loop L4 may be 5'-UUUCAUUU-3'.

[00258] A comparison of sequences observed in loop L5 from the degenerate selection suggested a high degree of conservation for all positions in this domain (**FIG. 6**). Consistent with this, replacement of the parental G at position 35 with any other nucleotide resulted in a complete loss in activity (>100-fold; Aptamers 162, 163, and 164). Surprisingly, similar replacements in other positions of the loop were tolerated much better (2-10 fold and 10-100 fold; Aptamers 165-173). Consistent with observations from the degenerate selection, the most tolerant substitutions were allowed at position 36, where replacement of the parental U with C had almost no effect on activity (Aptamer 166). Together, these data expanded the consensus sequence of loop L5 to 5'-GNNN-3', where N is A, C, G, or U. In a preferred embodiment, the consensus sequence of loop L5 may be 5'-GNHW-3', where N is A, C, G, or U; H is A, C, or U; and W is A or U. In another preferred embodiment, the consensus sequence of loop L5 may be 5'-GYUU-3', where Y is C or U.

Table 16. Analysis and optimization of Loops 1, 2, 3, 4, and 5

SEQ ID NO with modific ations:	Aptamer Number	Sequence (5' to 3')	TR-FRET
		S1 L1 S2 L2 S2 L3 S3 L4 S3 L5 S1	
220	Aptamer 26	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	parent
320	Aptamer 146	C6NH ₂ -AGGCCGCC- A -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	+
321	Aptamer 147	C6NH ₂ -AGGCCGCC- C -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	-
322	Aptamer 148	C6NH ₂ -AGGCCGCC- G -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	~
284	Aptamer 79	C6NH ₂ -AGGCCGCC- _ -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	-
323	Aptamer 149	C6NH ₂ -AGGCCGCC-U-CC- AC GC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	----
324	Aptamer 150	C6NH ₂ -AGGCCGCC-U-CC- CC GC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	----
325	Aptamer 151	C6NH ₂ -AGGCCGCC-U-CC- UC GC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	---
326	Aptamer	C6NH ₂ -AGGCCGCC-U-CC- GAGC -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	--

	152		
327	Aptamer 153	C6NH ₂ -AGGCCGCC-U-CC-G GGC -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	---
328	Aptamer 154	C6NH ₂ -AGGCCGCC-U-CC-G UGC -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	-
329	Aptamer 155	C6NH ₂ -AGGCCGCC-U-CC-GC AC -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	----
330	Aptamer 156	C6NH ₂ -AGGCCGCC-U-CC-GC CC -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	----
331	Aptamer 157	C6NH ₂ -AGGCCGCC-U-CC-GC UC -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	----
332	Aptamer 158	C6NH ₂ -AGGCCGCC-U-CC-GC GA -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	----
333	Aptamer 159	C6NH ₂ -AGGCCGCC-U-CC-GC GG -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	----
334	Aptamer 160	C6NH ₂ -AGGCCGCC-U-CC-GC GU -GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	---
335	Aptamer 161	C6NH ₂ -AGGCCGCC-U-CC-GC GC -GG- C -GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU-idT	---
221	Aptamer 27	C6NH ₂ - U AGGCCGCC-U-CC-GC-GCGG- G -GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU U -idT	---
222	Aptamer 28	C6NH ₂ - U AGGCCGCC-U-CC-GC-GCGG- U -GGGGU-UUCAUU-AUCCC-GUUU-GGCGGCUU U -idT	--
319	Aptamer 145	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU- _UCAU_ -AUCCC-GUUU-GGCGGCUU-idT	-
336	Aptamer 162	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- AUUU -GGCGGCUU-idT	---
337	Aptamer 163	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- CUUU -GGCGGCUU-idT	---
338	Aptamer 164	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- UUUU -GGCGGCUU-idT	----
339	Aptamer 165	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- CAUU -GGCGGCUU-idT	-
340	Aptamer 166	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GCUU -GGCGGCUU-idT	~
341	Aptamer 167	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GGUU -GGCGGCUU-idT	-
342	Aptamer 168	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GUUU -GGCGGCUU-idT	--
343	Aptamer 169	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GUCU -GGCGGCUU-idT	-
344	Aptamer 170	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GUGU -GGCGGCUU-idT	--
345	Aptamer 171	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GUUA -GGCGGCUU-idT	-
346	Aptamer 172	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GUUC -GGCGGCUU-idT	--
347	Aptamer 173	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- GUUG -GGCGGCUU-idT	--

where G is 2'F and A, C, and U are 2'OMe modified RNA; C6NH₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; an underscore (_) denotes an internal deletion; and bold letters denote differences from the parent Aptamer 26. Dashes (-) delineate the structural features of the aptamers (*e.g.*, stems, loops).

Key: ~ = 2 fold worse to 2 fold better, + = 2-10 fold better, ++ = 10-100 fold better, +++ more than 100 fold better, - = 2-10 fold worse, 10-100 fold worse, --- = more than 100 fold worse.

Example 9A. Lead optimization and characterization

[00259] Linker scanning analysis (**Table 14** and **FIG. 8**) revealed that replacement of the parental U in loop L1 (position 9) with a non-nucleotidyl spacer 3 modification, lead to a significant improvement in aptamer activity (10-100 fold) as determined by competition TR-FRET. To better characterize this substitution, we assessed the effect of this modification on stem S3 modifications that were previously tested for function (**Table 15**). As shown in **Table 17** and **FIG. 11**, replacement of position 9 with a non-nucleotidyl spacer 3 modification lead to an improvement in the activity of all molecules tested (compare the activities of Aptamer 137, 138, 139, 141, 142, 143, and 144 with Aptamers 133, 134, 135, 125, 126, 122, and 123 in **FIG. 9**). Only Aptamer 140 failed to show any improvement in activity. These data support the assertion that a non-nucleotidyl spacer 3 modification is a beneficial mutation and that the consensus for loop L1 is 5'-N*-3', where N* is A, C, G, U, a non-nucleotidyl spacer 3 modification or deleted entirely. In a preferred embodiment, loop L1 is 5'-A-3'. Loop L1 can also be a non-nucleotide spacer. In another preferred embodiment, loop L1 is 5'-U-3'. In another preferred embodiment, loop L1 is 5'-Sp3-3', where Sp3 is non-nucleotidyl spacer 3 modification.

Table 17. Optimization of lead consensus mutations

SEQ ID NO with modific ations:	Aptamer Number	Sequence (5' to 3')	TR-FRET
		S1 L1 S2 L2 S2 L3 S3 L4 S3 L5 S1	
220	Aptamer 26	C6NH ₂ -AGGCCGCC-U-CC-GCGC-GC-A-GGGGU-UUCAUU-AUCCC-GUUU-GCGGCUU-idT	parent
311	Aptamer 137	C6NH ₂ -AGGCCG AA -3-CC-GCGC-GC-A-GGGGU-UUCAUU-AUCCC-GUUU- UUC GCGCUU-idT	++
312	Aptamer 138	C6NH ₂ -AGGCCG AU -3-CC-GCGC-GC-A-GGGGU-UUCAUU-AUCCC-GUUU- AUC GCGCUU-idT	~
313	Aptamer 139	C6NH ₂ -AGGCCG UA -3-CC-GCGC-GC-A-GGGGU-UUCAUU-AUCCC-GUUU- UAC GCGCUU-idT	+
314	Aptamer	C6NH ₂ -AGGCCG UU -3-CC-GCGC-GC-A-GGGGU-UUCAU C -AUCCC-GUUU- AAC GCGCUU-idT	--

	140		
315	Aptamer 141	C6NH ₂ -AGGCCG AC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-G U CGGCUU-idT	++
360	Aptamer 187	C6NH ₂ -AGGCCG AC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-A CCCC -GUUU-G U CGGCUU-idT	++
316	Aptamer 142	C6NH ₂ -AGGCCG UC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-C AC GGCUU-idT	++
317	Aptamer 143	C6NH ₂ -AGGCCG CA -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU- U GCGGCUU-idT	+
318	Aptamer 144	C6NH ₂ -AGGCCG CU -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU- AG CGGCUU-idT	+
where G is 2'F and A, C, and U are 2'OMe modified RNA; C6NH ₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; 3 is the SP3 spacer (1,3-propanediol); and bold letters denote differences from the parent Aptamer 26. Dashes (-) delineate the structural features of the aptamers (<i>e.g.</i> , stems, loops).			
Key: ~ = 2 fold worse to 2 fold better, + = 2-10 fold better, ++ = 10-100 fold better, +++ more than 100 fold better, - = 2-10 fold worse, 10-100 fold worse, --- = more than 100 fold worse.			

Example 9B. Lead characterization by TR-FRET

[00260] To characterize the binding affinities of lead aptamers and an anti-VEGF-A mAb to VEGF-A₁₆₅ and VEGF-A₁₂₁, a time resolved FRET (TR-FRET) assay was performed. Briefly, ALEXA FLUOR[®] 647-labeled aptamers were heated to 90°C for 3 minutes and cooled to room temperature. Titrations of ALEXA FLUOR[®] 647-labeled aptamers and ALEXA FLUOR[®] 647-labeled anti-VEGF-A antibody were made in TR-FRET buffer (50 mM MOPS, pH 7.4, 125 mM NaCl, 5 mM KCl, 50 μM CHAPS, 0.1 mg/mL BSA, 1 mM CaCl₂, and 1 mM MgCl₂) in a polypropylene plate. 5 μL of aptamers or antibody were transferred to the black wall low volume 384 well assay plate (Greiner), followed by 5 μL glycan biotinylated VEGF-A₁₂₁ (2.5 nM final), and 5 μL Streptavidin-Eu (Perkin Elmer; 2.5 nM final). The plate was covered with a plate seal and subsequently incubated in the dark for 2 hours at room temperature. The plate was read on a Biotek CYTATION[™] 5 plate reader. Samples were excited at 330 nm and fluorescent values were collected at 665 nm.

[00261] No protein background fluorescent values were averaged and subtracted from test compounds, with data normalized as a fold change to highest concentrations of aptamers and antibody. These values were fit using a four-parameter non-linear fit in GraphPad Prism Version 7.0. Representative binding affinity curves of Aptamers 26, 47, and 141, compared to the anti-VEGF-A mAb described in Table 8, is shown in FIG. 12A and FIG. 12B. Results indicated that Aptamers 26, 47, and 141 have a binding affinity to VEGF-A₁₆₅ with calculated K_d values of

4.0 ± 0.64 nM, 2.1 ± 0.21 nM, and 1.2 ± 0.21 nM, respectively, which is comparable to the anti-VEGF-A mAb tested, with a K_d of 1.2 ± 0.07 nM. For VEGF-A₁₂₁, Aptamers 26, 47, and 141 demonstrated calculated K_d values of $46 \pm$ nM, 11 ± 2.1 nM, and 4.3 nM, respectively, with the anti-VEGF-A mAb with a K_d of 1.3 nM. Importantly, the calculated K_d values for Aptamer 141 (for VEGF-A₁₆₅) and the anti-VEGF-A mAb (for both VEGF-A₁₆₅ and VEGF-A₁₂₁) were limited by the input VEGF-A concentration. These results were consistent with binding affinity SPR data from **Example 2** and supported the pan-isoform specificity of the lead aptamers.

Example 9C. Lead characterization by receptor inhibition AlphaLISA®

[00262] The mechanism of action of lead aptamers was characterized by interrogating their ability to inhibit the VEGF-A:KDR interaction with both VEGF-A₁₆₅ and VEGF-A₁₂₁. To do this, Aptamers 26, 47, 141 and a comparator anti-VEGF-A mAb were tested and analyzed by an AlphaLISA® assay, as described in **Example 3**.

[00263] Representative curves of Aptamers 26, 47, 141 and comparator anti-VEGF-A mAb is shown in **FIG. 13A** and **FIG. 13B**. Results indicated that Aptamers 26, 47, 141 and the anti-VEGF-A mAb directly blocked the interaction of VEGF-A (VEGF-A₁₆₅ or VEGF-A₁₂₁) with KDR. Aptamers 26, 47, and 141 blocked the interaction of VEGF-A₁₆₅ with KDR with IC₅₀ values of 3.7 ± 2.4 nM, 0.84 ± 0.50 nM, and 0.81 ± 0.22 nM, respectively, which is in line with the anti-VEGF-A mAb comparator that demonstrated an IC₅₀ of 0.71 ± 0.48 nM. Similarly, Aptamers 26, 47, and 141 blocked the interaction of VEGF-A₁₂₁ with KDR with IC₅₀ values of 19 ± 19 nM, 3.6 ± 2.3 nM, and 3.2 ± 1.6 nM, respectively, which is in line with the anti-VEGF-A mAb comparator that demonstrated an IC₅₀ of 0.63 ± 0.22 nM. Importantly, the calculated IC₅₀ values for all aptamers (for VEGF-A₁₆₅) and the anti-VEGF-A mAb (for both VEGF-A₁₆₅ and VEGF-A₁₂₁) were limited by the input VEGF-A concentration.

[00264] The data presented in **FIG. 13A** and **FIG. 13B** demonstrates that these aptamers bound to an epitope consisting of or overlapping with the receptor binding face contained within the RBD of VEGF-A, and thus directly blocked the interaction of VEGF-A with its cognate receptor.

Example 9D. Lead characterization by receptor phosphorylation

[00265] When the RBD of VEGF-A binds to KDR, the receptor dimerizes leading to trans-autophosphorylation and activation of VEGF-A signaling. To determine if lead aptamers bound to the RBD of VEGF-A and resulted in inhibition of VEGF-A activity, Aptamers 26, 47, and 141 were tested for their ability to inhibit KDR phosphorylation induced by VEGF-A₁₆₅ as compared to an anti-VEGF-A antibody. This characterization was performed by a KDR phosphorylation AlphaLISA[®], as described in **Example 4**.

[00266] Representative curves of Aptamers 26, 47, 141 and comparator anti-VEGF-A mAb is shown in **FIG. 14**. Results indicated that Aptamers 26, 47, 141 and the anti-VEGF-A mAb achieved full inhibition of the phosphorylation of KDR by inhibiting its interaction with VEGF-A₁₆₅. Calculated IC₅₀ values for Aptamers 26, 47, and 141 and the comparator anti-VEGF-A mAb were 1.5 ± 0.60 nM, 2.6 ± 0.26 nM, 1.7 ± 0.46 nM, and 1.1 ± 0.02 nM, respectively. The potency and complete inhibition of VEGF-A₁₆₅-induced phosphorylation of KDR by Aptamers 26, 47, and 141 and their comparable activity profile to an anti-VEGF-A mAb, is consistent with the ability of Aptamers 26, 47, and 141 to bind to the receptor binding face present within the RBD of VEGF-A and directly block the interaction between VEGF-A and KDR.

Example 9E. Lead characterization by inhibition of angiogenesis

[00267] VEGF-A plays an important role in inducing angiogenesis in both normal tissues and diseased pathologies (Ferrara, 2004). It is therefore of interest to evaluate potential VEGF-A antagonists in an angiogenesis assay. *In vitro* models have been established to study VEGF-A-stimulated angiogenesis. These models can therefore be used with the selected aptamers to determine their ability to inhibit angiogenesis via binding to either VEGF-A₁₆₅ or VEGF-A₁₂₁ and inhibiting VEGF-A induced signal transduction via KDR activation.

[00268] Co-culture of fibroblasts and endothelial cells leads to the formation of tubes after stimulation with either VEGF-A₁₆₅ or VEGF-A₁₂₁. Briefly, at Day 0, primary human dermal fibroblasts (ATCC) were plated at 20k cells/well in 100 μ L fibroblast growth media (ATCC) and incubated at room temperature for 1 hour. GFP-infected HUVEC cells (Lonza, infected in-house) were then plated at 10k cells/well with 100 μ L HUVEC growth media (without VEGF-A supplement, PromoCell) and incubated at room temperature for 1 hour. The plate was then placed in the IncuCyte[®] Zoom (Essen Bioscience) set to the default angiogenesis analysis definition with imaging programmed every 4 hours. After 24 hours of incubation (Day 1), old

media was removed and replaced with 150 μ L/well of IncuCyte[®] Angiogenesis Prime Kit Optimized Assay Medium (Essen Bioscience). After 24 hours (Day 2), media was changed with fresh media with or without treatment. Treatment included 400 pM VEGF-A₁₆₅ or 800 pM VEGF-A₁₂₁ premixed with or without a dilution of aptamer or the anti-VEGF-A mAb. Assays were carried out for 6 days with media changes and subsequent treatments performed on Days 4 and 6.

[00269] Network length (mm/mm³) of the tubes formed from the GFP-HUVEC cells was measured as a primary indication of angiogenesis. Percent inhibition was calculated by subtracting assay media background from each value and normalizing to VEGF-A only controls. The values were fit using a four-parameter non-linear fit in GraphPad Prism Version 7.0. IC₅₀ values for VEGF-A₁₆₅ and VEGF-A₁₂₁ were calculated at a time point that reflected an EC₉₀-EC₁₀₀ of the VEGF-A alone induction of tube formation. Representative dose response curves of Aptamers 26, 47, and 141, compared to the anti-VEGF-A mAb described in **Table 8**, are shown in **FIG. 15A** and **FIG. 15B**. Data indicated that all aptamers had an inhibitory effect against VEGF-A₁₆₅ with IC₅₀ values of 1.9 ± 0.85 nM, 0.64 ± 0.17 nM, and 0.50 ± 0.21 nM, respectively which was comparable to the anti-VEGF-A mAb tested, with an IC₅₀ of 0.51 ± 0.05 nM. When angiogenesis was stimulated by VEGF-A₁₂₁, Aptamers 26, 47, and 141 likewise had an inhibitory effect with IC₅₀ values of 18 ± 5.6 nM, 5.5 ± 2.4 nM, and 3.1 ± 1.8 nM, respectively, with the anti-VEGF-A mAb inhibiting at 0.91 ± 0.06 nM. This is also represented qualitatively in **FIG. 16A** and **FIG. 16B**, in which Aptamers 26, 47, and 141 as well as the anti-VEGF-A mAb were shown to inhibit tube formation when GFP-HUVEC cells (shown) were treated with either VEGF-A₁₆₅ or VEGF-A₁₂₁. These data supported the notion that lead aptamers could inhibit both VEGF-A₁₆₅ and VEGF-A₁₂₁-induced angiogenesis.

Example 10. Lead stabilization and truncation

[00270] 2'OMe modifications are known to impart higher duplex stability and have greater coupling efficiency during synthesis compared to 2'F-containing nucleotides. The use of these nucleotides also avoids the potential loss of the 2'F group, which can happen in production during the deprotection step and during exposure to heat. The effect of 2'F-G to 2'OMe-G substitution on target binding was probed, using Aptamer 141, an improved variant of Aptamer

26 that contained a non-nucleotidyl spacer 3 modification, and an A:U base pair at positions 7 and 40 within stem S1.

[00271] Starting at position 6, 2'F-G residues were systematically replaced, one at a time, and the activity of the resultant molecule was assessed by competition TR-FRET, as described in **Example 7**, against the parent molecule, Aptamer 141. As shown in **Table 18** and **FIG. 17**, the 2'OMe-G substitution was tolerated at numerous positions throughout the molecule, including positions 6, 12, 14, 16, 17, 22, 39 and 40 (Aptamers 188-198), with modification at some of these positions (positions 22 and 39) leading to a modest improvement in activity (~2-fold; Aptamer 196 and Aptamer 198).

[00272] In addition, an initial attempt at truncating stem S1 of Aptamer 141 to six base pairs resulted in a ~2-fold increase in activity (Aptamer 199). In the context of Aptamer 199, the replacement of the terminal 2'F-G residues outside of the region systematically examined by Aptamers 188 – 198 was tested. As shown in **Table 18** and **FIG. 17**, replacement of the terminal stem S1 positions (Aptamer 213) had no effect on aptamer activity relative to Aptamer 199 (~2-fold improvement in activity over Aptamer 141).

[00273] In summary, 2'OMe-G substitution was tolerated at numerous positions throughout the molecule and was particularly well tolerated in all of the positions within stem S1 as well as positions 12, 22, and 39 (Aptamers 189, 196, and 198).

Table 18. O-Methyl G substitution analysis

SEQ ID NO with modifications:	Aptamer Number	Sequence (5' to 3')											TR-FRET
		S1	L1	S2	L2	S2	L3	S3	L4	S3	L5	S1	
315	Aptamer 141	C6NH ₂ -AGGCCG AC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT											parent
361	Aptamer 188	C6NH ₂ -AGGCC XAC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT											~
362	Aptamer 189	C6NH ₂ -AGGCCCG AC -3-CC- X C GC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT											~
363	Aptamer 190	C6NH ₂ -AGGCCCG AC -3-CC-GC X C-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT											--
364	Aptamer 191	C6NH ₂ -AGGCCCG AC -3-CC-GCGC- XG -A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT											-
365	Aptamer 192	C6NH ₂ -AGGCCCG AC -3-CC-GCGC- GX -A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT											--
366	Aptamer 193	C6NH ₂ -AGGCCCG AC -3-CC-GCGC-GG-A- X GGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT											---
367	Aptamer	C6NH ₂ -AGGCCCG AC -3-CC-GCGC-GG-A- G XGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-											---

	194	idT	
368	Aptamer 195	C6NH ₂ -AGGCCG AC -3-CC-GCGC-GG-A-GG X GU-UUCAUU-AUCCC-GUUU-GUCGGCUU- idT	----
369	Aptamer 196	C6NH ₂ -AGGCCG AC -3-CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU-GUCGGCUU- idT	~
370	Aptamer 197	C6NH ₂ -AGGCCG AC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC- XUUU -GUCGGCUU- idT	---
371	Aptamer 198	C6NH ₂ -AGGCCG AC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU- XU CGGCUU- idT	+
373	Aptamer 201	C6NH ₂ -AGGCC XAC -3-CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU- XU CGGCUU- idT	~
372	Aptamer 199	C6NH ₂ -___GCCG AC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGC___- idT	+
374	Aptamer 213	C6NH ₂ -___ XCCGAC -3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUC XXC ___- idT	~
where G is 2'F and A, C, and U are 2'OMe modified RNA; X is 2'OMe-G modified RNA; C6NH₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; 3 is the SP3 spacer (1,3-propanediol); and bold letters denote differences from the parent Aptamer 26. Dashes (-) delineate the structural features of the aptamers (e.g., stems, loops). Key: ~ = 2 fold worse to 2 fold better, + = 2-10 fold better, ++ = 10-100 fold better, +++ more than 100 fold better, - = 2-10 fold worse, 10-100 fold worse, --- = more than 100 fold worse.			

[00274] To further probe the relationship between 2'F-G to 2'OMe-G replacement and aptamer length, a series of constructs bearing stem S1 lengths of six, five, four, three, two, and one nucleotide were synthesized and the combinatorial effect of 2'F-G to 2'OMe-G replacements that were previously observed to be permissible were explored (Table 19 and FIG. 18). The length of stem S1 could be reduced to three base pairs in length without any adverse effect on activity (Aptamers 233-241). Moreover, the 2'F-G to 2'OMe-G substitutions could be readily accommodated into all these molecules. Shortening the length of stem S1 to less than three base pairs in length (Aptamers 276-281) resulted in a significant loss in activity (>100-fold).

[00275] Together, these data support the finding that the 2'F-G residues at positions 6, 22, 39, and 40 could be replaced with a 2'OMe-G without negatively affecting the activity of Aptamer 141. Moreover, 2'OMe-G replacements at positions 6, 22, and 40 could be combined without negatively affecting the activity of Aptamer 141. Finally, stem S1 of aptamer 141, or variants bearing 2'F-G to 2'OMe-G replacements could be eight, six, five, four, or three base pairs in length.

Table 19. O-Methyl G consensus and truncation analysis

SEQ ID NO with modifications:	Aptamer Number	Sequence (5' to 3')	TR-FRET
		S1 L1 S2 L2 S2 L3 S3 L4 S3 L5 S1	
315	Aptamer 141	C6NH ₂ -ACGCCGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT	parent
375	Aptamer 214	C6NH ₂ -XCCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCXXC-idT	+
376	Aptamer 215	C6NH ₂ -XCCGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCXXC-idT	+
377	Aptamer 216	C6NH ₂ -XCCGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	+
378	Aptamer 217	C6NH ₂ -XCCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCXXC-idT	+
379	Aptamer 218	C6NH ₂ -XCCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	~
380	Aptamer 219	C6NH ₂ -XCCGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	+
381	Aptamer 220	C6NH ₂ -XCCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	+
382	Aptamer 221	C6NH ₂ -CCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCXXC-idT	~
383	Aptamer 222	C6NH ₂ -CCGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCXXC-idT	+
384	Aptamer 223	C6NH ₂ -CCGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	~
385	Aptamer 224	C6NH ₂ -CCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCXXC-idT	+
386	Aptamer 225	C6NH ₂ -CCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	~
387	Aptamer 226	C6NH ₂ -CCGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	~
388	Aptamer 227	C6NH ₂ -CCXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCXXC-idT	+
389	Aptamer 228	C6NH ₂ -CXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCX-idT	+
390	Aptamer 229	C6NH ₂ -CGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCX-idT	+
391	Aptamer 230	C6NH ₂ -CGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCX-idT	+
392	Aptamer 231	C6NH ₂ -CXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCX-idT	+
393	Aptamer 232	C6NH ₂ -CXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCX-idT	+
394	Aptamer 233	C6NH ₂ -CGAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCX-idT	+
395	Aptamer 234	C6NH ₂ -CXAC-3-CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-XUCX-idT	+

396	Aptamer 235	C6NH ₂ - XAC-3 -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUC-idT	+
397	Aptamer 236	C6NH ₂ - GAC-3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU-GUC-idT	+
398	Aptamer 237	C6NH ₂ - GAC-3 -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU- XUC -idT	+
399	Aptamer 238	C6NH ₂ - XAC-3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU-GUC-idT	+
400	Aptamer 239	C6NH ₂ - XAC-3 -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU- XUC -idT	+
401	Aptamer 240	C6NH ₂ - GAC-3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU- XUC -idT	+
402	Aptamer 241	C6NH ₂ - XAC-3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU- XUC -idT	+
403	Aptamer 276	C6NH ₂ - AC-3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU-GU-idT	---
404	Aptamer 277	C6NH ₂ - AC-3 -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU- XU -idT	---
405	Aptamer 278	C6NH ₂ - AC-3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU- XU -idT	---
406	Aptamer 279	C6NH ₂ -C- 3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU-G-idT	---
407	Aptamer 280	C6NH ₂ -C- 3 -CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU- X -idT	----
408	Aptamer 281	C6NH ₂ -C- 3 -CC-GCGC-GG-A-GGG XU -UCAUU-AUCCC-GUUU- X -idT	----
where G is 2'F and A, C, and U are 2'OMe modified RNA; X is 2'OMe-G modified RNA; C6NH₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; 3 is the SP3 spacer (1,3-propanediol); and bold letters denote differences from the parent Aptamer 26. Dashes (-) delineate the structural features of the aptamers (<i>e.g.</i>, stems, loops). Key: ~ = 2 fold worse to 2 fold better, + = 2-10 fold better, ++ = 10-100 fold better, +++ more than 100 fold better, - = 2-10 fold worse, 10-100 fold worse, --- = more than 100 fold worse.			

[00276] Aptamer 234, a 2'OMe-G bearing variant of Aptamer 141 with a four base pair stem S1, was used to further explore the effects of various stem S3/loop L4 mutations identified in the degenerate selection on aptamer function. The proposed effect of these substitutions on the stem/loop structure are illustrated in **FIG. 19**. As shown in **Table 20** and **FIG. 20**, almost all of these substitutions were well tolerated, providing molecules with activity levels comparable to Aptamer 234; a 2-10 fold improvement over Aptamer 141. Of particular note, the replacement of the U at position 29 (numbering based on Aptamer 141) with an A, generating a predicted six base pair stem S3 and a four nucleotide loop L4, resulted in an approximately 10-fold improvement in activity over Aptamer 141 (Aptamer 285). Additionally, these data demonstrated

that a six base pair stem S3 could contain a single mis-matched nucleotide (*e.g.*, Aptamer 284, and 288). Thus, when stem S3 is six base pairs long and contains a single mis-matched nucleotide, the length of loop L4 may be three nucleotides in length.

[00277] These data further highlighted the relationship between stem S3 and loop L4. When combined with sequence data observed in the primary and degenerate selections, these data further supported the observation that stem S3 could be four, five, or six base pairs in length. When stem S3 is six base pairs in length, it may contain a single mis-match at the penultimate nucleotide in the 5' side of the stem. When stem S3 is four base pairs in length, loop L4 may be six or eight nucleotides in length. When stem S3 is five base pairs in length, loop L4 may be four or six nucleotides in length and when stem S3 is six base pairs, loop L4 may be four nucleotides in length. When stem S3 is six base pairs long and contains a single mis-matched nucleotide, the length of loop L4 may be three nucleotides in length.

[00278] Together these data expand the consensus sequence of stem S3 for all of the variants observed or tested for Aptamer 26. Stem S3 may be expanded to 5'-GGGRUN3' for the 5' side of stem S3 and 5'-NWYCCC-3' for the 3' side of stem S3, where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U. When stem S3 is four base pairs, the consensus sequence may be 5'-GGGG-3' for the 5' side of stem S3 and 5'-CCCC-3' for the 3' side of stem S3. In some cases, when stem S3 is five base pairs, the consensus sequence may be 5'-GGGRU-3' for the 5' side of stem S3 and 5'-AYCCC-3' for the 3' side, where R is A or G; and Y is C or U. In some cases, when stem S3 is six base pairs, the consensus sequence may be 5'-GGGRUN-3' for the 5' side of stem S3 and 5'-NAYCCC-3' for the 3' side, where R is A or G; N is A, C, G, or U; D is A, G, or U; and H is A, C, or U. When stem S3 is six base pairs long and contains a single mis-matched nucleotide, the consensus sequence may be 5'-GGGRUUR-3' for the 5' side of stem S3 and 5'-UAUCCC-3' for the 3' side, where U is the single mis-matched nucleotide; and R is A or G. When stem S3 is six base pairs long and contains a single mis-matched nucleotide, loop L4 may be three nucleotides long. When loop L4 is three nucleotides long, the sequence may be 5'-CUA-3'.

[00279] Aptamer 234 was also used to examine the effects of an alternate pairing at positions 10 and 17 in stem S2 (numbering based on Aptamer 141) and the effect of an additional 2'F-G to 2'OMe substitution at position 12 in loop L2 (numbering based on Aptamer 141) on aptamer function (Aptamers 293, 294, and 295). Consistent with the secondary structure prediction, the

C:G pair at positions 10 and 17 could be replaced with an A:U pair with only a modest loss in activity relative to Aptamer 141 (Aptamers 293; approximately 6-fold loss). Interestingly, while the effect of the 2'F-G to 2'OMe substitution at position 12 was well tolerated (Aptamer 294; 1-2 fold loss), the combination of both the base pair substitution and the 2'OMe substitution resulted in a more significant loss of activity (Aptamer 295; 12-fold loss). The data further supported the consensus sequence for stem S2. Stem S2 may be composed of two base pairs with a consensus sequence of 5'-NN-3' for both the 5' and 3' sides of the stem.

[00280] Finally, using Aptamer 234, the effects of linker length on loop L1 (position 7; numbering based on Aptamer 141; Aptamers 298-300) was explored. Increasing the length of the non-nucleotidyl linker in loop L1 resulted in a loss in activity relative to Aptamer 141. The longer the linker, the less potent the molecule (compare Aptamers 298, 299, and 300; **FIG. 20**). Together, these data further expanded the range of moieties that could be substituted into loop L1. Thus, the expanded consensus for loop L1 is 5'-N*-3', where N* is A, C, G, U, can be deleted entirely, or is a non-nucleotidyl spacer 3 (1,3-propanediol) modification (**Table 20; 3**), two spacer 3 modifications (**Table 20; 33**), a 6 carbon alkyl linker (1,6-hexanediol) (**Table 20; 6**), or a spacer 9 (triethyleneglycol) modification (**Table 20; 9**).

Table 20. Lead optimization by loop L4 / stem S3 mutations discovered in degenerate selection

SEQ ID NO with modifications:	Aptamer Number	Sequence (5' to 3')	TR-FRET
		S1 L1 S2 L2 S2 L3 S3 L4 S3 L5 S1	
315	Aptamer 141	C6NH ₂ -AGGCCGAC-3---CC-GCGC-GG-A-GGGGU-UUCAUU-AUCCC-GUUU-GUCGGCUU-idT	parent
395	Aptamer 234	C6NH ₂ -CXAC-3---CC-GCGC-GG-A-GGGXU-UUCAUU-AUCCC-GUUU-XUCX-idT	+
409	Aptamer 282	C6NH ₂ -CXAC-3---CC-GCGC-GG-A-GGGAU-UUCAUU-AUCCC-GUUU-XUCX-idT	~
410	Aptamer 283	C6NH ₂ -CXAC-3---CC-GCGC-GG-A-GGGXU-AUCAUU-AUCCC-GUUU-XUCX-idT	+
411	Aptamer 284	C6NH ₂ -CXAC-3---CC-GCGC-GG-A-GGGXU-UACAUU-AUCCC-GUUU-XUCX-idT	+
412	Aptamer 285	C6NH ₂ -CXAC-3---CC-GCGC-GG-A-GGGXU-UUCAUA-AUCCC-GUUU-XUCX-idT	++
413	Aptamer 286	C6NH ₂ -CXAC-3---CC-GCGC-GG-A-GGGXU-UUCAUU-ACCCC-GUUU-XUCX-idT	~
414	Aptamer 287	C6NH ₂ -CXAC-3---CC-GCGC-GG-A-GGGAU-AUCAUU-AUCCC-GUUU-XUCX-idT	~

415	Aptamer 288	C6NH ₂ -C XAC -3---CC-GCGC-GG-A-GGG AU -U ACA UU-AUCCC-GUUU- XUCX -idT	+
416	Aptamer 289	C6NH ₂ -C XAC -3---CC-GCGC-GG-A-GGG AU -U UCA U A -AUCCC-GUUU- XUCX -idT	~
417	Aptamer 290	C6NH ₂ -C XAC -3---CC-GCGC-GG-A-GGG XU - AUCA UU-AUCCC-GUUU- XUCX -idT	+
418	Aptamer 291	C6NH ₂ -C XAC -3---CC-GCGC-GG-A-GGG XU -U UCA U A -AUCCC-GUUU- XUCX -idT	+
419	Aptamer 292	C6NH ₂ -C XAC -3---CC-GCGC-GG-A-GGG XU -U ACA U A -AUCCC-GUUU- XUCX -idT	+
423	Aptamer 296	C6NH ₂ -C XAC -3---CC-GCGC-GG-A-GGG AU - XUCA UC-AUCCC-GUUU- XUCX -idT	~
424	Aptamer 297	C6NH ₂ -C XAC -3---CC-GCGC-GG-A-GGG AU - CUCA U X -AUCCC-GUUU- XUCX -idT	~
420	Aptamer 293	C6NH ₂ -C XAC -3---CA-GCGC- UG -A-GGG XU -U UCA UU-AUCCC-GUUU- XUCX -idT	-
421	Aptamer 294	C6NH ₂ -C XAC -3---CC- XCGC -GG-A-GGG XU -U UCA UU-AUCCC-GUUU- XUCX -idT	~
422	Aptamer 295	C6NH ₂ -C XAC -3---CA- XCGC - UG -A-GGG XU -U UCA UU-AUCCC-GUUU- XUCX -idT	--
425	Aptamer 298	C6NH ₂ -C XAC - 33 -CC-GCGC-GG-A-GGG XU -U UCA UU-AUCCC-GUUU- XUCX -idT	-
426	Aptamer 299	C6NH ₂ -C XAC - 6 ---CC-GCGC-GG-A-GGG XU -U UCA UU-AUCCC-GUUU- XUCX -idT	--
427	Aptamer 300	C6NH ₂ -C XAC - 9 ---CC-GCGC-GG-A-GGG XU -U UCA UU-AUCCC-GUUU- XUCX -idT	--
where G is 2'F and A, C, and U are 2'OMe modified RNA; X is 2'OMe-G modified RNA; C6NH₂ is a hexylamine linker; idT is an inverted deoxythymidine residue; 3 is the SP3 spacer (1,3-propanediol); 6 is the SP6 spacer (1,6-hexanediol); 9 is the SP9 spacer (triethyleneglycol); and bold letters denote differences from the parent Aptamer 26. Dashes (-) delineate the structural features of the aptamers (e.g., stems, loops). Key: ~ = 2 fold worse to 2 fold better, + = 2-10 fold better, ++ = 10-100 fold better, +++ more than 100 fold better, - = 2-10 fold worse, 10-100 fold worse, --- = more than 100 fold worse.			

[00281] In total, when the data from the degenerate selection (Example 6) is combined with the data from our structure activity relationship studies (Examples 7, 8, 9 and 10), the consensus sequences for the Aptamer 26 family of molecules is 5'-

HNBYHDNNN*NNKNGC>NNWGGGRUNDNDHNWYCCCGNNNNNNYNKVNW-3' (SEQ ID NO: 5) where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; N* is A, C, G, U, can be deleted entirely, or is a non-nucleotidyl spacer 3 modification, a 6 carbon alkyl linker (1,6-hexanediol), or a spacer 9 (triethyleneglycol) modification; D is A, G, or U; K is G or U; W is A or U; R is A or G; and V is A, C, or G; and is represented with a six base pair stem S3 and a four nucleotide loop L4 in FIG. 7.

Example 11 Optimization of S3/L4 by SELEX

[00282] To better understand the roles of S3 and L4 in aptamer function we generated two different randomized libraries based on the sequence and structure of Aptamer 285, in which the non-nucleotidyl linker was replaced with a random nucleotide, N, where N is A, G, U or C, and where the entirety of Stem S3 and Loop L4 (S3-L4) was replaced with a library composed of 16 or 14 random positions. The sequence of the libraries used were:

GGGAGAGTCGGTAGCCTCAACGACNCCGCGCGGAN16GTTTGTCGCTATGTGGAAAT
GGCGCTGT,

GGGAGAGTCGGTAGCCTCAACGACNCCGCGCGGAN14GTTTGTCGCTATGTGGAAAT
GGCGCTGT (N16, N14; where N16 is 16 N positions, N14 is 14 N positions and N is A, G, U or C).

[00283] Using these libraries, we performed two separate selections targeting human VEGF 121 in a manner similar to that described for the original selection (**Example 1**). Following 3 rounds of selection, each library was found to demonstrate significant VEGF binding activity (~10-fold signal over background) as determined by flow cytometry. The libraries were subsequently adapted for sequencing and sequenced on an Illumina MiSeq sequencer. Sequence analysis was performed as described previously.

[00284] For the S3-L4 N16 library, we analyzed the sequence data from 174,583 reads. An alignment of the sequences corresponding to the top 20 stacks from this library are shown in **Table 21**. These 20 stacks correspond to the top 44% of the enriched library sequences.

[00285] As shown in **Table 21**, the sequences from these top 20 stacks from the S3-L4 N16 library strongly support the structural and sequence requirements summarized in **FIG. 7** in which the preferred identity of nucleotide in loop L1 is U, and stem S3 is composed of 4, 5 or 6 base pairs with a corresponding loop L4 of 8, 6 or 4 nucleotides, respectively. The results also demonstrate that S3 contains a significant degree of covariation, is not highly conserved in sequence identity except for the first base pair in the stem which prefers to be G:C pair. The consensus sequence for this randomized region (S3-L4) within the N16 library from the top 20 stacks is GDSBHDNNNNHNNBNC. When S3 is composed of 4 base pairs, the consensus sequence for the S3-L4 region is GGGT-TRVWGGYT-ACCC. When S3 is composed of 5 base pairs, consensus sequence for the S3-L4 region is GKSCY-KNNNNW-RKKVC, and when S3 is

composed of 6 base pairs consensus sequence for the S3-L4 region is GRGGAG-GYWA-CYYCYC. When stem S3 is 4 based pairs long, loop L4 is 8 nucleotides long and the consensus is 5'-URVWGGY-3'. When stem S3 is 5 base pairs long, loop L4 is 6 nucleotides long and the consensus is 5'-KNNNNW-3'. When stem S3 is 6 base pairs long loop L4 is 4 nucleotides long and the consensus is 5'GYWA-3'.

Table 21. Top 20 sequence stacks identified in from the S3-L4 N16 library.

SEQ ID NO:	S1	L1	S2	L2	S2	L3	S3	L4	S3	L6	S1
439:	CGAC	U	CC	GCGC	GG	A	GGGU	UGGAGGUU	ACCC	GUUU	GUCG
441:	CGAC	U	CC	GCGC	GG	A	GUCCC	UAAUUU	GGGGC	GUUU	GUCG
443:	CGAC	U	CC	GCGC	GG	A	GUCCC	UUCAUU	GGGGC	GUUU	GUCG
445:	CGAC	U	CC	GCGC	GG	A	GGGU	UAAUGGCU	ACCC	GUUU	GUCG
447:	CGAC	U	CC	GCGC	GG	A	GUCCC	UGUAAU	GGGGC	GUUU	GUCG
449:	CGAC	U	CC	GCGC	GG	A	GUCCC	UAUUUU	GGGGC	GUUU	GUCG
451:	CGAC	U	CC	GCGC	GG	A	GAGGAG	GUUA	CCCCUC	GUUU	GUCG
453:	CGAC	U	CC	GCGC	GG	A	GUCCC	UUGAUU	GGGGC	GUUU	GUCG
455:	CGAC	U	CC	GCGC	GG	A	GGGU	UACUGGCU	ACCC	GUUU	GUCG
457:	CGAC	U	CC	GCGC	GG	A	GUCCC	UAACAU	GGGGC	GUUU	GUCG
459:	CGAC	U	CC	GCGC	GG	A	GGGGAG	GCAA	CUUCCC	GUUU	GUCG
461:	CGAC	U	CC	GCGC	GG	A	GUCCC	UUUAUU	GGGGC	GUUU	GUCG
463:	CGAC	U	CC	GCGC	GG	A	GUCCC	UACAAU	GGGGC	GUUU	GUCG
465:	CGAC	U	CC	GCGC	GG	A	GUGCC	GUUUGA	GGUAC	GUUU	GUCG
467:	CGAC	U	CC	GCGC	GG	A	GGGCU	GAGGCAAUGCCC		GUUU	GUCG
469:	CGAC	U	CC	GCGC	GG	A	GUCCC	UACUUU	GGGGC	GUUU	GUCG
471:	CGAC	U	CC	GCGC	GG	A	GUCCC	UCACAU	GGGGC	GUUU	GUCG
473:	CGAC	U	CC	GCGC	GG	A	GGGU	UACAGGCU	ACCC	GUUU	GUCG
475:	CGAC	U	CC	GCGC	GG	A	GUCCC	UUUGUU	GGGGC	GUUU	GUCG
477:	CGAC	U	CC	GCGC	GG	A	GUCCC	UAAAAU	GGGGC	GUUU	GUCG

Sequences in bold indicated randomized positions in the library.

[00286] For the S3-L4 N14 library, we analyzed the sequence data from 1,644,139 reads. An alignment of the sequences corresponding to the top 20 stacks from this library are shown in **Table 22**. These 20 stacks correspond to the top 80% of the enriched library sequences.

[00287] As shown in **Table 22**, the sequences from the top 20 stacks from the S3-L4 N14 library strongly support the structural and sequence requirements summarized in **FIG. 7** in which the preferred identity of nucleotide in loop L1 is U, and the stem S3 is capable of forming 4 or 5 or base pairs with a corresponding loop L4 of 6 or 4 nucleotides respectively. We note that while some sequences appear capable of forming up to a 6 base pairs in stem S3 (M33-3, M33-10,

M33-13, M33-14, M33-19, M33-27, M33-28 and M33-29), such pairing would result the formation of an unfavorable two nucleotide loop L4. Thus, we have chosen to draw all these sequences with a 5 base pair stem S3. These results further support the finding that S3 is a stem as indicated by the degree of covariation and that stem S3 is not highly conserved in sequence identity except for the first base pair in the stem which prefers to be G:C pair. The consensus sequence for this randomized region (S3-L4) within the N14 library from the top 20 stacks is GKBHYDNNNKDBVC. When S3 is composed of 4 base pairs, the consensus sequence for the S3-L4 region is GKSH-UDRGBU-DSMC. When S3 is composed of 5 base pairs, consensus sequence for the S3-L4 region is GYBYC-DNWD-GRKRC. When stem S3 is 4 base pairs long, loop L4 is 6 nucleotides long and the consensus is 5'-UDRGBU-3'. When stem S3 is 5 base pairs long, loop L4 is 4 nucleotides long and the consensus is 5'-DNWD-3'.

Table 22. Top 20 sequence stacks identified in from the S3-L4 N14 library.

SEQ ID NO:	S1	L1	S2	L2	S2	L3	S3	L4	S3	L6	S1
479:	CGAC	U	CC	GCGC	GG	A	GGGU	UUGGCU	ACCC	GUUU	GUCG
481:	CGAC	U	CC	GCGC	GG	A	GGCU	UGAGGU	AGCC	GUUU	GUCG
483:	CGAC	U	CC	GCGC	GG	A	GUCCC	ACAU	GGGGC	GUUU	GUCG
485:	CGAC	U	CC	GCGC	GG	A	GGGA	UGAGGU	UCCC	GUUU	GUCG
487:	CGAC	U	CC	GCGC	GG	A	GGCA	UGAGGU	UGCC	GUUU	GUCG
489:	CGAC	U	CC	GCGC	GG	A	GUGC	UGAGGU	GCAC	GUUU	GUCG
491:	CGAC	U	CC	GCGC	GG	A	GUCCC	UAUU	GGGGC	GUUU	GUCG
493:	CGAC	U	CC	GCGC	GG	A	GGGU	UAGGCU	ACCC	GUUU	GUCG
495:	CGAC	U	CC	GCGC	GG	A	GCUUC	GGAU	GAGGC	GUUU	GUCG
497:	CGAC	U	CC	GCGC	GG	A	GUCCC	UCAU	GGGGC	GUUU	GUCG
499:	CGAC	U	CC	GCGC	GG	A	GUCCC	GUAU	GGGGC	GUUU	GUCG
501:	CGAC	U	CC	GCGC	GG	A	GUCCC	AAUU	GGGGC	GUUU	GUCG
503:	CGAC	U	CC	GCGC	GG	A	GGGU	UUGGUU	ACCC	GUUU	GUCG
505:	CGAC	U	CC	GCGC	GG	A	GUCCC	AUUU	GGGGC	GUUU	GUCG
507:	CGAC	U	CC	GCGC	GG	A	GUCCC	ACAA	GGGGC	GUUU	GUCG
509:	CGAC	U	CC	GCGC	GG	A	GCUUC	GGAA	GAGGC	GUUU	GUCG
511:	CGAC	U	CC	GCGC	GG	A	GUCCC	AUUA	GGGGC	GUUU	GUCG
513:	CGAC	U	CC	GCGC	GG	A	GUGCC	AACU	GGUAC	GUUU	GUCG
515:	CGAC	U	CC	GCGC	GG	A	GUCCC	UAUA	GGGGC	GUUU	GUCG
517:	CGAC	U	CC	GCGC	GG	A	GUCCC	UAAU	GGGGC	GUUU	GUCG

Sequences in bold indicated randomized positions in the library.

[00288] When combined the data both the S3-L4 N16 and S3-L4 N14 further broaden the observed consensus sequences for stem S3 and loop L4. When the data from the N16 selection is combined with data from the primary selection, degenerate selection and SAR analysis

broadens the observed consensus when stem S3 is 6 base pairs long expands to 5'-GRGGWN-3' on the 5' side and 5'-NH CYC-3' on the 3' side. When stem S3 is 5 base pairs long, the consensus from the outcome of the N16 and N14 selections combined with data from the primary selection, degenerate selection and SAR analysis broadens the observed consensus to 5'-GBBNY-3' on the 5' side and 5'-RNBNC-3' on the 3' side. When stem S3 is 4 base pairs long, the consensus from the outcome of the N16 and N14 selections combined with data from the primary selection, degenerate selection and SAR analysis broadens the observed consensus to 5'-GKGN-3' on the 5' side and 5'-NSMC-3' on the 3' side. Similarly, when combined with data from the primary selection, degenerate selection and SAR analysis, when loop L4 is 6 nucleotides long, 5 nucleotides long or 4 nucleotides long the consensus sequences expand to 5'UDUHRKYU-3', 5'KNNNNW-3' and 5'DNDN-3'.

[00289] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. An aptamer having a nucleic acid sequence, wherein said aptamer comprises a stem-loop secondary structure which specifically binds to and inhibits at least one of VEGF-A₁₂₁ and VEGF-A₁₁₀.
2. The aptamer of claim 1, wherein said aptamer comprises up to five loops.
3. The aptamer of claim 1 or 2, wherein said aptamer comprises up to three stems.
4. The aptamer of any one of claims 1-3, wherein said aptamer comprises up to one terminal stem.
5. The aptamer of any one of claims 1-4, wherein said aptamer comprises up to two internal stems.
6. The aptamer of any one of claims 1-5, wherein said aptamer comprises up to two terminal loops.
7. The aptamer of any one of claims 1-6, wherein said aptamer comprises up to three internal loops.
8. The aptamer of any one of claims 1-7, wherein said aptamer comprises, in a 5' to 3' direction: (i) a first side of a first base paired stem (S1); (ii) optionally, a first loop (L1); (iii) a first side of a second base paired stem (S2); (iv) a second loop (L2); (v) a second side of said second base paired stem (S2'); (vi) a third loop (L3); (vii) a first side of a third base paired stem (S3); (viii) a fourth loop (L4); (ix) a second side of said third base paired stem (S3'); (x) a fifth loop (L5); and (xi) a second side of said first base paired stem (S1').
9. The aptamer of claim 8, wherein said S1' forms at least one base pair with said S1.
10. The aptamer of claim 8 or 9, wherein said S1' forms from two to eight contiguous base pairs with said S1.
11. The aptamer of any one of claims 8-10, wherein said S1' comprises from one to three nucleotides that are mismatched with said S1.
12. The aptamer of any one of claims 8-11, wherein a 3' terminal nucleotide of said S1, and a 5' terminal nucleotide of said S1' form a base pair.

13. The aptamer of claim 12, wherein said base pair is C•G.
14. The aptamer of claim 12 or 13, wherein said base pair is separated from other base pairs in said S1 and said S1' by one or more mismatched nucleotides.
15. The aptamer of any one of claims 8-14, wherein said S1 comprises a consensus nucleic acid sequence of 5'-HNBYHDCC-3', and said S1' comprises a consensus nucleic acid sequence of 5'-GKYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; K is G or U; V is A, C, or G; and W is A or U.
16. The aptamer of any one of claims 8-14, wherein said S1 comprises a consensus nucleic acid sequence of 5'-HNBYHDNN-3', and said S1' comprises a consensus nucleic acid sequence of 5'-NNYNKVNW-3', where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; and D is A, G, or U.
17. The aptamer of any one of claims 8-16, wherein said L1 comprises up to one nucleotide.
18. The aptamer of any one of claims 8-17, wherein said L1 comprises a consensus nucleic acid sequence of 5'-N*-3', wherein N* is A, C, G, U, a 3-carbon non-nucleotidyl spacer, two 3 carbon non-nucleotidyl spacers, a 6-carbon non-nucleotidyl spacer, or a 9-carbon non-nucleotidyl spacer.
19. The aptamer of claim 18, wherein said 3-carbon non-nucleotidyl spacer is 1,3-propanediol.
20. The aptamer of claim 18, wherein said 6-carbon non-nucleotidyl spacer is 1,6-hexanediol.
21. The aptamer of claim 18, wherein said 9-carbon non-nucleotidyl spacer is triethyleneglycol.
22. The aptamer of any one of claims 8-21, wherein said S2' forms at least one base pair with said S2.
23. The aptamer of any one of claims 8-22, wherein said S2' forms up to two base pairs with said S2.
24. The aptamer of any one of claims 8-23, wherein said S2 comprises a consensus nucleic acid sequence of 5'-CC-3', and said S2' comprises a consensus nucleic acid sequence of 5'-GG-3'.

25. The aptamer of any one of claims 8-23, wherein said S2 comprises a consensus nucleic acid sequence of 5'-NN-3', and said S2' comprises a consensus nucleic acid sequence of 5'-NN-3', where N is A, C, G, or U.
26. The aptamer of any one of claims 8-25, wherein said L2 comprises up to four nucleotides.
27. The aptamer of any one of claims 8-26, wherein said L2 comprises a consensus nucleic acid sequence of 5'-GCGC-3'.
28. The aptamer of any one of claims 8-26, wherein said L2 comprises a consensus nucleic acid sequence of 5'-KNGC-3', where K is G or U; and N is A, C, G or U.
29. The aptamer of any one of claims 8-28, wherein said S3' forms at least one base pair with said S3.
30. The aptamer of any one of claims 8-29, wherein said S3' forms from four to six base pairs with said S3.
31. The aptamer of any one of claims 8-30, wherein said S3' forms six base pairs with said S3, and said S3 has one mismatch nucleotide at a 3' terminal nucleotide of said S3.
32. The aptamer of any one of claims 8-30, wherein said S3' forms four base pairs with said S3, and said L4 has six or eight nucleotides.
33. The aptamer of any one of claims 8-30, wherein said S3' forms five base pairs with said S3, and said L4 has four or six nucleotides.
34. The aptamer of any one of claims 8-30, wherein said S3' forms six base pairs with said S3, and said L4 has four nucleotides.
35. The aptamer of any one of claims 8-30, wherein said S3' forms six base pairs with S3, said S3 has one mismatch nucleotide at a 3' terminal nucleotide, and said L4 has three nucleotides.
36. The aptamer of any one of claims 8-35, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GRGRWN-3', and said S3' comprises a consensus nucleic acid sequence of 5'-NHYYC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U.

37. The aptamer of any one of claims 8-35, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGRUN3', and said S3' comprises a consensus nucleic acid sequence of 5'-NWyCCC-3', where R is A or G; N is A, C, G, or U; W is A or U; and Y is C or U.

38. The aptamer of any one of claims 8-35, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGRUN3', and said S3' comprises a consensus nucleic acid sequence of 5'-NAYCCC-3', where R is A or G; N is A, C, G, or U; and Y is C or U.

39. The aptamer of any one of claims 8-35, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGRUD-3', and said S3' comprises a consensus nucleic acid sequence of 5'-HWYCCC-3', wherein R is A or G; D is A, G, or U; H is A, C, or U; W is A or U; and Y is C or U.

40. The aptamer of any one of claims 8-30, or 32, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GKGn-3', and said S3' comprises a consensus nucleic acid sequence of 5'-NSMC-3'.

41. The aptamer of any one of claims 8-30, or 32, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGU-3', and said S3' comprises a consensus nucleic acid sequence of 5'-ACCC-3'.

42. The aptamer of any one of claims 8-30, or 32, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGCU-3', and said S3' comprises a consensus nucleic acid sequence of 5'-AGCC-3'.

43. The aptamer of any one of claims 8-30, or 32, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGG-3', and said S3' comprises a consensus nucleic acid sequence of 5'-CCCC-3'.

44. The aptamer of any one of claims 8-30, or 33, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGRU-3', and said S3' comprises a consensus nucleic acid sequence of 5'-AYCCC-3', where R is A or G; and Y is C or U.

45. The aptamer of any one of claims 8-30, or 33, wherein said S3 comprises a consensus nucleic acid sequence of 5'-SVVVK-, and said S3' comprises a consensus nucleic acid sequence of 5'-MBBBS-3', where S is G or C; V is A, C, or G; K is G or U; M is A or C; and B is C, G, or U.

46. The aptamer of any one of claims 8-30, or 34, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGGUD-3', and said S3' comprises a consensus nucleic acid sequence of 5'-HAUCCC-3', where D is A, G, or U; and H is A, C, or U.

47. The aptamer of any one of claims 8-30, or 35, wherein said S3 comprises a consensus nucleic acid sequence of 5'-GGGRUUR-3', and said S3' comprises a consensus nucleic acid sequence of 5'-UAUCCC-3', where the underlined U is the single mis-matched nucleotide, and R is A or G.

48. The aptamer of any one of claims 8-47, wherein said L3 comprises up to one nucleotide.

49. The aptamer of any one of claims 8-48, wherein said L3 comprises a consensus nucleic acid sequence of 5'-A-3'.

50. The aptamer of any one of claims 8-48, wherein said L3 comprises a consensus nucleic acid sequence of 5'-W-3', where W is A or U.

51. The aptamer of any one of claims 8-50, wherein said L4 has three, four, six, or eight nucleotides.

52. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-MAU-3', where M is A or C.

53. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-CUA-3'.

54. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-DNDN-3', where D is A, G, or U; N is A, C, G, or U; or a consensus sequence of 5'-DNWD-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U; or a consensus sequence of 5'-DNAH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U; or a consensus nucleic acid sequence of 5'-DNDH-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U.

55. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-KNNNNW-3'.

56. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-UDNDHU-3', where D is A, G, or U; N is A, C, G, or U; and H is A, C, or U.

57. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-UDNHRKYU-3'.

58. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-UUUCAUUU-3'.

59. The aptamer of any one of claims 8-51, wherein said L4 comprises a consensus nucleic acid sequence of 5'-UDRGBU-3'.

60. The aptamer of any one of claims 8-59, wherein said L5 comprises up to four nucleotides.

61. The aptamer of any one of claims 8-60, wherein said L5 comprises a consensus nucleic acid sequence of 5'-GYUU-3', where Y is C or U.

62. The aptamer of any one of claims 8-60, wherein said L5 comprises a consensus nucleic acid sequence of GNNN-3', where N is A, C, G, or U.

63. The aptamer of any one of claims 8-60, wherein said L5 comprises a consensus nucleic acid sequence of 5'-GNHW-3', where N is A, C, G, or U; H is A, C, or U; and W is A or U.

64. An aptamer comprising a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGGGRUDDNDHHWYCCCGYUUGKYNKVNW-3' (**SEQ ID NO: 1**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; W is A or U; K is G or U; and V is A, C, or G.

65. An aptamer comprising a consensus nucleic acid sequence of 5'-HNBYHDCCUCCGCGCGGAGGGRUUDNDHUAYCCCGYUUGKYNKVNW-3' (**SEQ ID NO: 2**), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; D is A, G, or U; R is A or G; K is G or U; V is A, C, or G; and W is A or U.

66. An aptamer comprising a consensus nucleic acid sequence of 5'-WNKYHDCCUCCGCGCGGAGGGGUDDNAHHAUCCCGUUUGGYBKMHW-3' (**SEQ ID NO: 3**), where W is A or U; N is A, C, G, or U; K is G or U; Y is C or U; H is A, C, or U; D is A, G, or U; B is C, G, or U; and M is A or C.

67. An aptamer comprising a consensus nucleic acid sequence of 5'-
AUGCCGCCUCCGCGCGGAGGGGUUCAUUUCCCCGUUUGGCUGCAU-3' (SEQ ID NO:
4).
68. An aptamer comprising a consensus nucleic acid sequence of 5'-
HNBYHDCCUCCGCGCGGAGDSBHDNNNNHNNBNCGYUUGKYNKVNW-3' (SEQ ID NO:
436).
69. An aptamer comprising a consensus nucleic acid sequence of 5'-
HNBYHDCCUCCGCGCGGAGKBHYDNNNKDBVCGYUUGKYNKVNW -3' (SEQ ID NO:
437).
70. An aptamer comprising a consensus nucleic acid sequence of 5'-
HNBYHDCCUCCGCGCGGAGKSHUDRGBUDSMCGYUUGKYNKVNW-3' -3' (SEQ ID NO:
438).
71. An aptamer comprising a consensus nucleic acid sequence of 5'-
HNBYHDNNN*NNKNGCNNWGGGRUNDNDHNWYCCCGNNNNNNYNKVNW-3' (SEQ ID
NO: 5), where H is A, C, or U; N is A, C, G, or U; B is C, G, or U; Y is C or U; N* is A, C, G, U,
deleted entirely, or a non-nucleotidyl spacer 3 modification, a 6 carbon alkyl linker (1,6-hexanediol),
or a spacer 9 (triethyleneglycol) modification; D is A, G, or U; K is G or U; W is A or U; R is A or
G; and V is A, C, or G.
72. The aptamer of any one of claims 1-71, wherein less than 50% of pyrimidines present in said
nucleic acid sequence comprise a C-5 modified pyrimidine.
73. The aptamer of any one of claims 1-72, wherein less than 25% of pyrimidines present in said
nucleic acid sequence comprise a C-5 modified pyrimidine.
74. The aptamer of any one of claims 1-73, wherein less than 10% of pyrimidines present in said
nucleic acid sequence comprise a C-5 modified pyrimidine.
75. The aptamer of any one of claims 1-74, wherein said nucleic acid sequence does not comprise
any C-5 modified pyrimidines.
76. The aptamer of any one of claims 72-75, wherein said C-5 modified pyrimidine comprises a C-5
modified cytosine or a C-5 modified uridine.

77. The aptamer of any one of claims 72-76, wherein said C-5 modified pyrimidine comprises a C-5 hydrophobic modification.

78. The aptamer of any one of claims 1-77, wherein less than 100% of uridines present in said nucleic acid sequence comprise a C-5 modified uridine.

79. The aptamer of any one of claims 1-78, wherein less than 50% of uridines present in said nucleic acid sequence comprise a C-5 modified uridine.

80. The aptamer of any one of claims 1-79, wherein less than 25% of uridines present in said nucleic acid sequence comprise a C-5 modified uridine.

81. The aptamer of any one of claims 1-80, wherein less than 10% of uridines present in said nucleic acid sequence comprise a C-5 modified uridine.

82. The aptamer of any one of claims 1-81, wherein no uridines present in said nucleic acid sequence comprise a C-5 modified uridine.

83. The aptamer of any one of claims 78-82, wherein said C-5 modified uridine comprises a C-5 hydrophobic modification.

84. The aptamer of any one of claims 78-83, wherein said C-5 modified uridine is selected from the group consisting of: 5-(N-benzylcarboxamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxamide)-2'-O-methyluridine, 5-(N-benzylcarboxamide)-2'-fluorouridine, 5-(N-phenethylcarboxamide)-2'-deoxyuridine (PEdU), 5-(N-thiophenylmethylcarboxamide)-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxamide)-2'-deoxyuridine (iBudU), 5-(N-tyrosylcarboxamide)-2'-deoxyuridine (TyrdU), 5-(N-3,4-methylenedioxybenzylcarboxamide)-2'-deoxyuridine (MBndU), 5-(N-4-fluorobenzylcarboxamide)-2'-deoxyuridine (FBndU), 5-(N-3-phenylpropylcarboxamide)-2'-deoxyuridine (PPdU), 5-(N-imidizolylethylcarboxamide)-2'-deoxyuridine (ImdU), 5-(N-isobutylcarboxamide)-2'-O-methyluridine, 5-(N-isobutylcarboxamide)-2'-fluorouridine, 5-(N-tryptaminocarboxamide)-2'-deoxyuridine (TrpdU), 5-(N-R-threoninylcarboxamide)-2'-deoxyuridine (Thrdu), 5-(N-tryptaminocarboxamide)-2'-O-methyluridine, 5-(N-tryptaminocarboxamide)-2'-fluorouridine, 5-(N-[1-(3-trimethylammonium)propyl]carboxamide)-2'-deoxyuridine chloride, 5-(N-naphthylmethylcarboxamide)-2'-deoxyuridine (NapdU), 5-(N-naphthylmethylcarboxamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxamide)-2'-fluorouridine, 5-(N-[1-(2,3-

dihydroxypropyl)]carboxamide)-2'-deoxyuridine), 5-(N-2-naphthylmethylcarboxamide)-2'-deoxyuridine (2NapdU), 5-(N-2-naphthylmethylcarboxamide)-2'-O-methyluridine, 5-(N-2-naphthylmethylcarboxamide)-2'-fluorouridine, 5-(N-1-naphthylethylcarboxamide)-2'-deoxyuridine (NEdU), 5-(N-1-naphthylethylcarboxamide)-2'-O-methyluridine, 5-(N-1-naphthylethylcarboxamide)-2'-fluorouridine, 5-(N-2-naphthylethylcarboxamide)-2'-deoxyuridine (2NEdU), 5-(N-2-naphthylethylcarboxamide)-2'-O-methyluridine, 5-(N-2-naphthylethylcarboxamide)-2'-fluorouridine, 5-(N-3-benzofuranylethylcarboxamide)-2'-deoxyuridine (BFdU), 5-(N-3-benzofuranylethylcarboxamide)-2'-O-methyluridine, 5-(N-3-benzofuranylethylcarboxamide)-2'-fluorouridine, 5-(N-3-benzothiophenylethylcarboxamide)-2'-deoxyuridine (BTdU), 5-(N-3-benzothiophenylethylcarboxamide)-2'-O-methyluridine, and 5-(N-3-benzothiophenylethylcarboxamide)-2'-fluorouridine.

85. The aptamer of any one of claims 78-84, wherein said C-5 modified uridine is 5-(N-naphthylmethylcarboxamide)-2'-deoxyuridine (NapdU).

86. The aptamer of any one of claims 1-85, wherein said aptamer selectively binds to a receptor binding face or receptor binding domain of VEGF-A₁₂₁ or VEGF-A₁₁₀.

87. The aptamer of claim 86, wherein said receptor binding domain comprises at least one of residues 1-109 of **SEQ ID NO: 6-10**.

88. The aptamer of claim 86 or 88, wherein said receptor binding domain comprises at least one of residues Phe17, Ile43, Ile46, Glu64, Gln79, Ile83, Lys84, Pro85, Arg82, His86, Asp63, and Glu67 of **SEQ ID NO: 6-10**.

89. The aptamer of any one of claims 1-88, wherein said aptamer inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 50 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis.

90. The aptamer of any one of claims 1-89, wherein said aptamer inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 25 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis.

91. The aptamer of any one of claims 1-90, wherein said aptamer inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 10 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis.
92. The aptamer of any one of claims 1-91, wherein said aptamer inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 5 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis.
93. The aptamer of any one of claims 1-92, wherein said aptamer inhibits VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with an IC₅₀ of less than about 1 nM as measured by a VEGF-A:KDR competition binding assay, a KDR phosphorylation AlphaLISA[®] assay, or an *in vitro* model of VEGF-A-induced angiogenesis.
94. The aptamer of any one of claims 1-93, wherein said aptamer binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 50 nM as measured by surface plasmon resonance assay.
95. The aptamer of any one of claims 1-94, wherein said aptamer binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 25 nM as measured by surface plasmon resonance assay.
96. The aptamer of any one of claims 1-95, wherein said aptamer binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 10 nM as measured by surface plasmon resonance assay.
97. The aptamer of any one of claims 1-96, wherein said aptamer binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 5 nM as measured by surface plasmon resonance assay.
98. The aptamer of any one of claims 1-97, wherein said aptamer binds to VEGF-A₁₂₁, VEGF-A₁₁₀, or both, with a K_d of less than about 1 nM as measured by surface plasmon resonance assay.
99. The aptamer of any one of claims 1-98, wherein said aptamer selectively binds to and inhibits at least one of VEGF-A₁₆₅, VEGF-A₁₈₉, and VEGF-A₂₀₆.
100. The aptamer of any one of claims 1-99, wherein said aptamer inhibits or reduces an interaction of VEGF-A with KDR.

101. The aptamer of any one of claims 1-100, wherein said aptamer inhibits or reduces VEGF-A-induced KDR phosphorylation.
102. The aptamer of any one of claims 1-101, wherein said aptamer comprises RNA or sugar-modified RNA.
103. The aptamer of any one of claims 1-102, wherein said aptamer comprises DNA or sugar-modified DNA.
104. The aptamer of any one of claims 1-103, wherein at least 50% of said nucleic acid sequence comprises sugar-modified nucleotides.
105. The aptamer of any one of claims 1-104, wherein 100% of said nucleic acid sequence comprises sugar-modified nucleotides.
106. The aptamer of claim 104 or 105, wherein said sugar-modified nucleotides comprise a 2'F-modified nucleotide, a 2'OMe-modified nucleotide, or both.
107. The aptamer of any one of claims 104-106, wherein said sugar-modified nucleotides are selected from the group consisting of: 2'F-G, 2'OMe-G, 2'OMe-U, 2'OMe-A, 2'OMe-C, and any combination thereof.
108. The aptamer of any one of claims 1-107, wherein said aptamer further comprises a 3' terminal inverted deoxythymidine.
109. The aptamer of any one of claims 1-108, wherein said aptamer comprises a nuclease-stabilized nucleic acid backbone.
110. The aptamer of any one of claims 1-109, wherein said nucleic acid sequence comprises from about 30 to about 90 nucleotides, wherein said nucleotides are unmodified nucleotides, modified nucleotides, or a combination of modified nucleotides and unmodified nucleotides.
111. The aptamer of any one of claims 1-110, wherein said aptamer is conjugated to a polyethylene glycol (PEG) molecule.

112. The aptamer of claim 111, wherein said PEG molecule has a molecular weight selected from the group consisting of: less than 5 kDa, less than 10 kDa, less than 20 kDa, less than 40 kDa, less than 60 kDa, and less than 80 kDa.

113. The aptamer of any one of claims 1-112, wherein up to five nucleotides of said aptamer comprise a phosphate-backbone modification.

114. The aptamer of claim 113, wherein said phosphate-backbone modification is a phosphorothioate substitution.

115. An aptamer according to any aptamer described in Table 1 of the specification.

116. An aptamer having at least 70% sequence identity with any aptamer described in Table 1 of the specification.

117. The aptamer of claim 116, having at least 70% modification identity with any aptamer described in Table 1 of the specification.

118. An aptamer of any one of claims 1-117, for use in treating an ocular disease or disorder in a subject in need thereof.

119. The aptamer of claim 108, wherein one or more symptoms of said ocular disease or disorder are treated.

120. A method of treating an ocular disease or disorder in a subject in need thereof, comprising administering to said subject an aptamer of any one of claims 1-109, thereby treating said ocular disease or disorder.

121. The method of claim 120, wherein said ocular disease or disorder is selected from the group consisting of: diabetic retinopathy, retinopathy of prematurity, central retinal vein occlusion, macular edema, choroidal neovascularization, neovascular age-related macular degeneration, myopic choroidal neovascularization, punctate inner choroidopathy, ocular histoplasmosis syndrome, familial exudative vitreoretinopathy, and retinoblastoma.

122. The method of claim 120 or 121, wherein said ocular disease or disorder exhibits elevated levels of VEGF-A.

123. Use of an aptamer of any one of claims 1-119 in a formulation of a medicament for treatment of an ocular disease or disorder.

124. Use of an aptamer of any one of claims 1-119 for treatment of an ocular disease or disorder.

125. A method for modulating vascular endothelial growth factor-A (VEGF-A) in a biological system, said method comprising: administering to said biological system an aptamer according to any one of claims 1-119, thereby modulating VEGF-A in said biological system.

126. The method of claim 125, wherein said biological system comprises a biological tissue or biological cells.

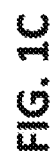
127. The method of claim 125, wherein said biological system is a subject.

128. The method of claim 127, wherein said subject is a human.

129. The method of any one of claims 125-128, wherein said modulating comprises inhibiting a function associated with VEGF-A.

130. The method of any one of claims 125-129, wherein said modulating comprises preventing or reducing an association of VEGF-A with one or more of Flt-1, KDR, or Nrp-1.

GGGAGUGUGUACGAGGCAUU-AGGGCGCC-N30-GGGGGCUU-UGAUACUUGAUCGCCCUAGAACG



13.6.4

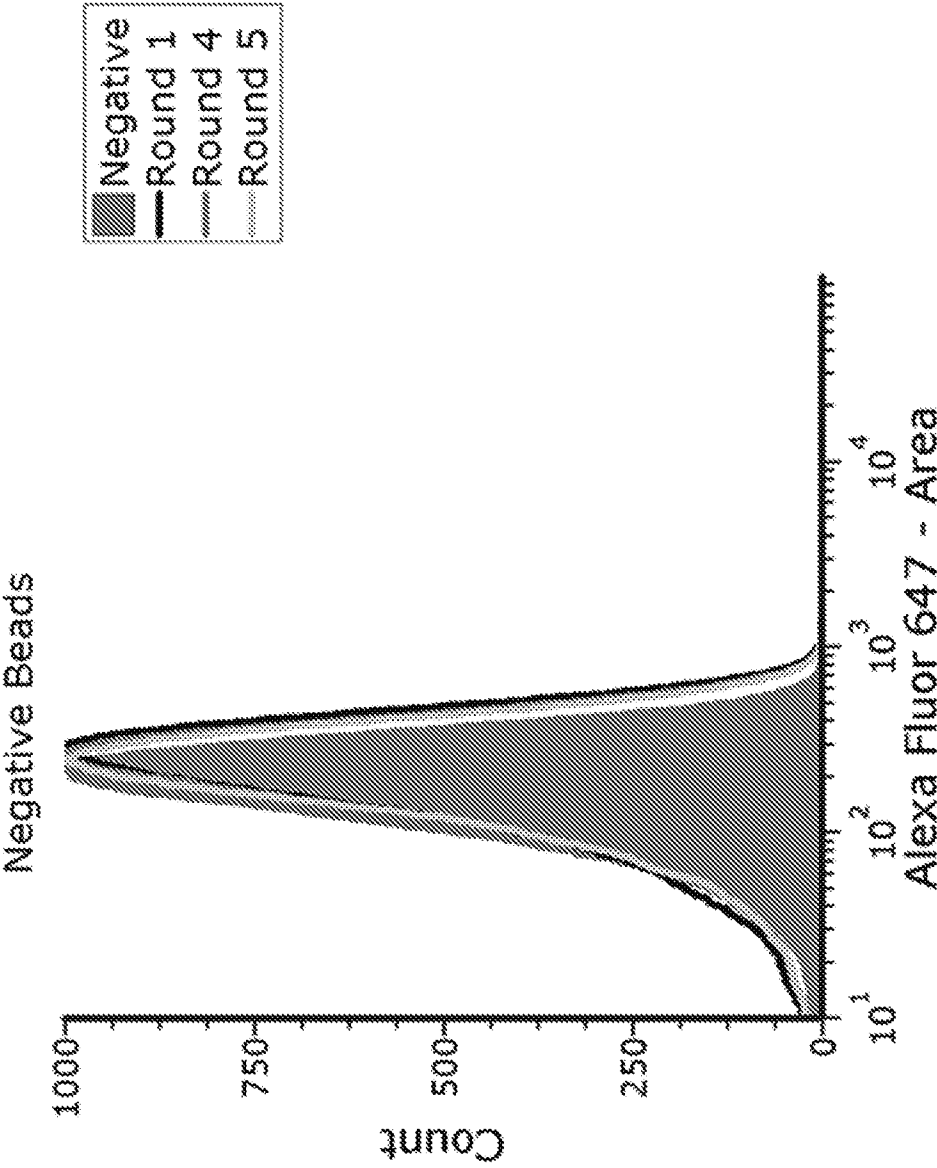


FIG. 2A

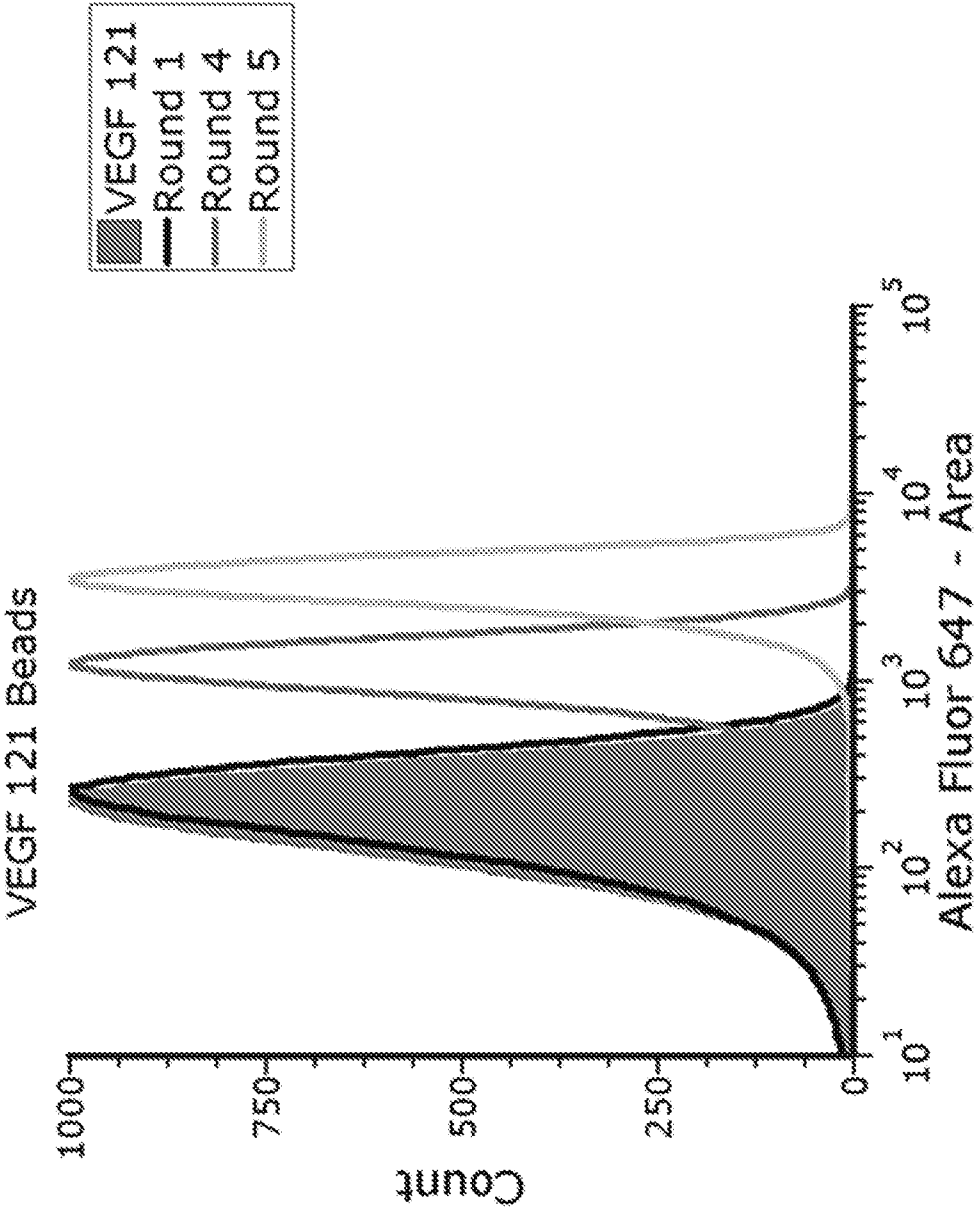


FIG. 2B

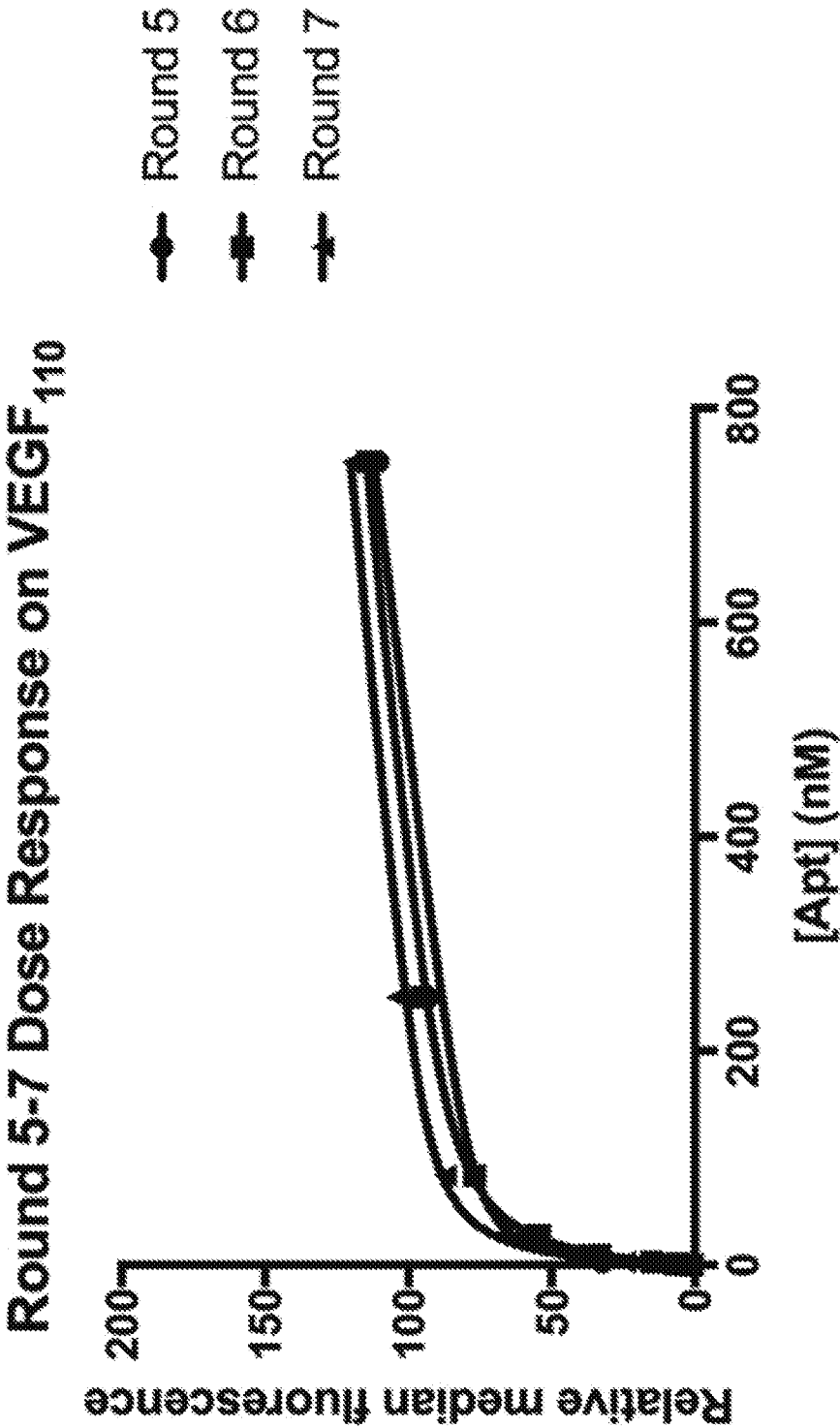


FIG. 2C

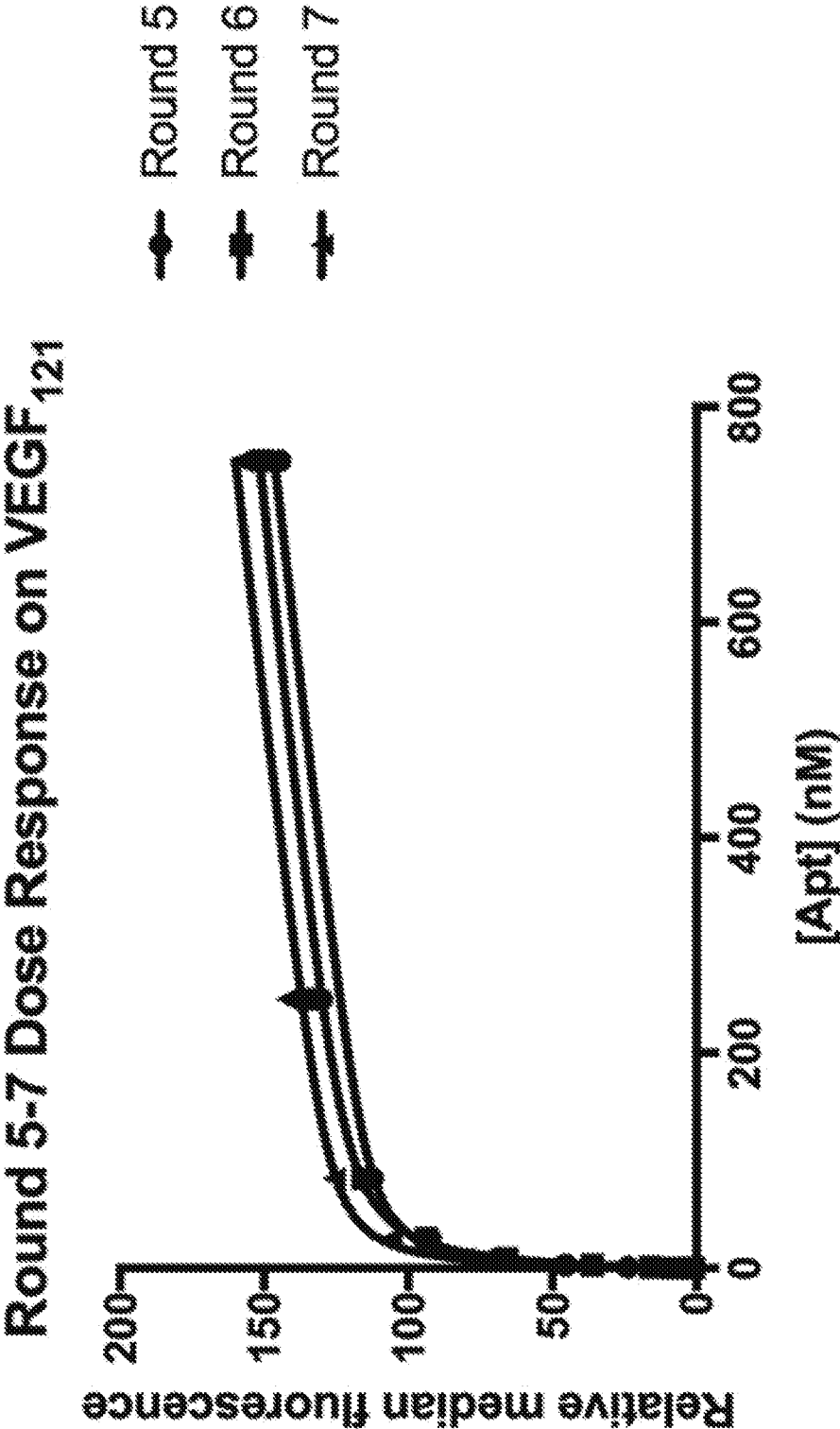


FIG. 2D

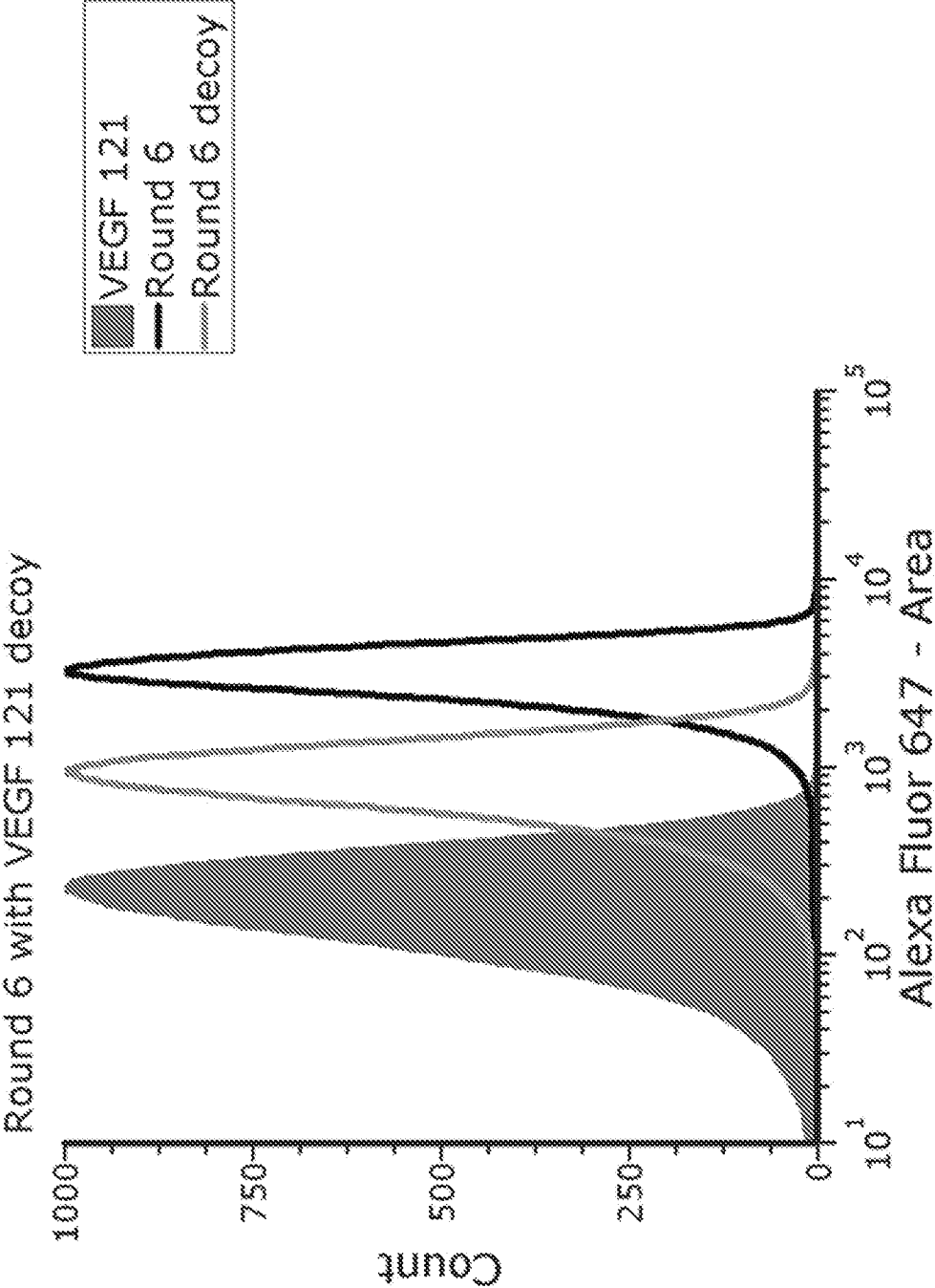


FIG. 2E

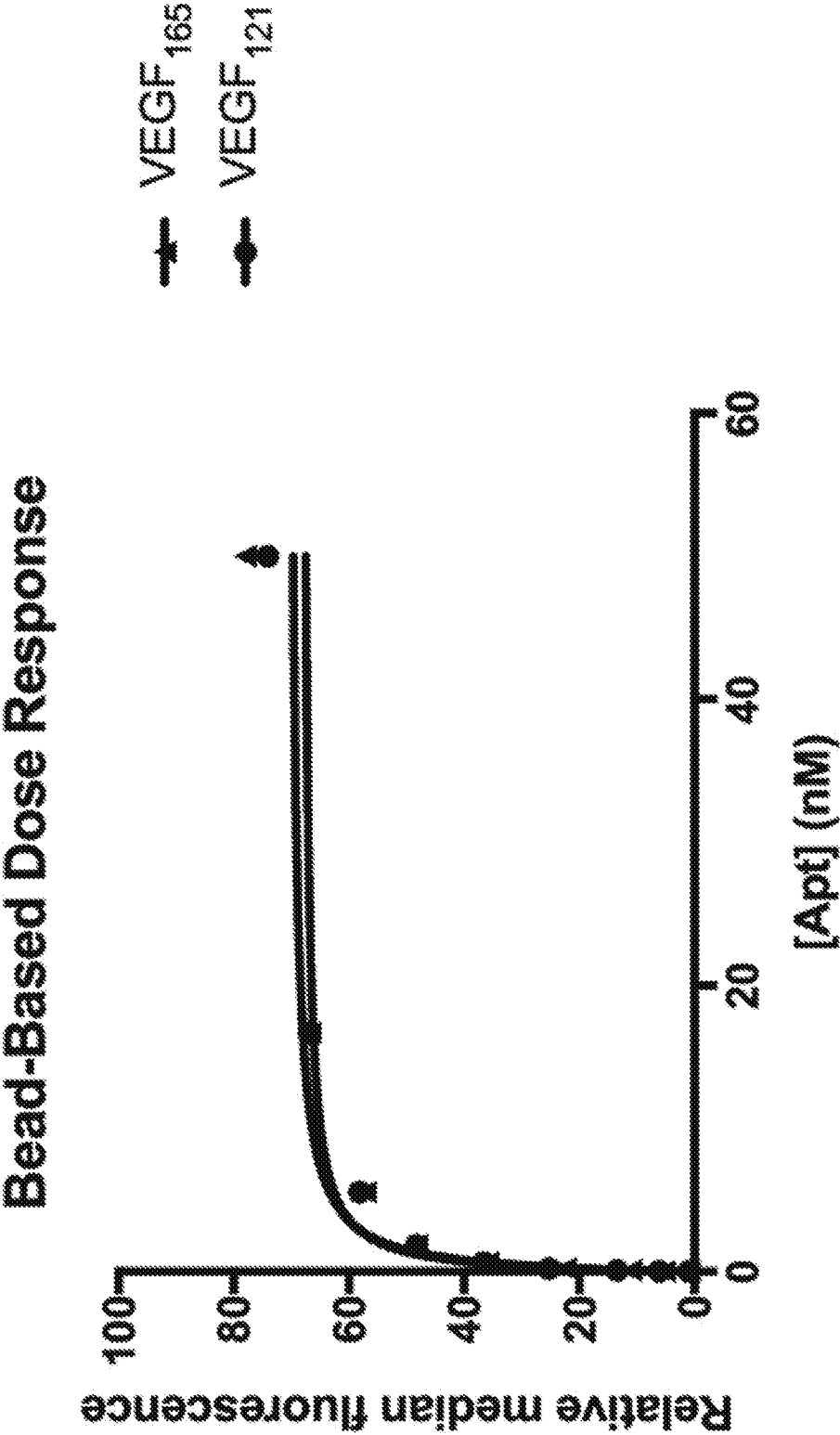


FIG. 3

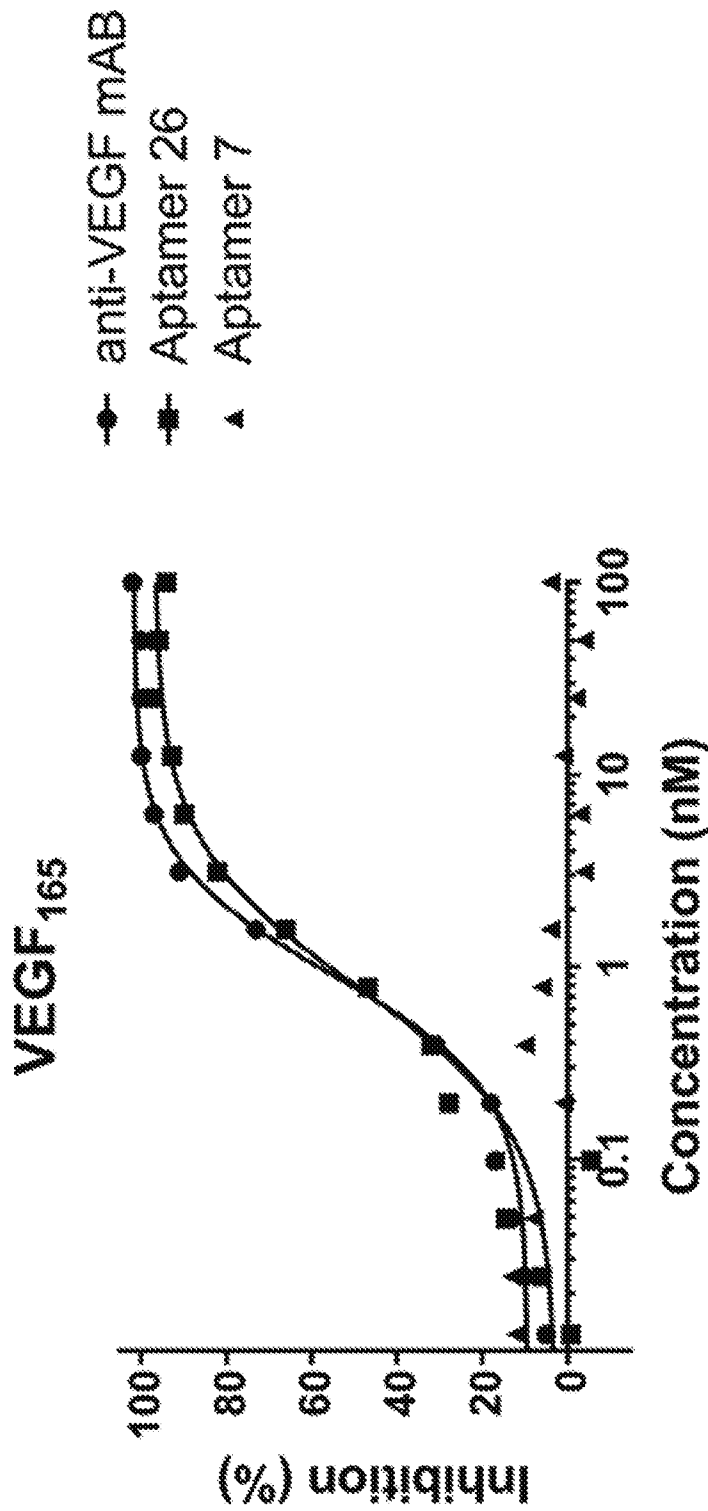


FIG. 4A

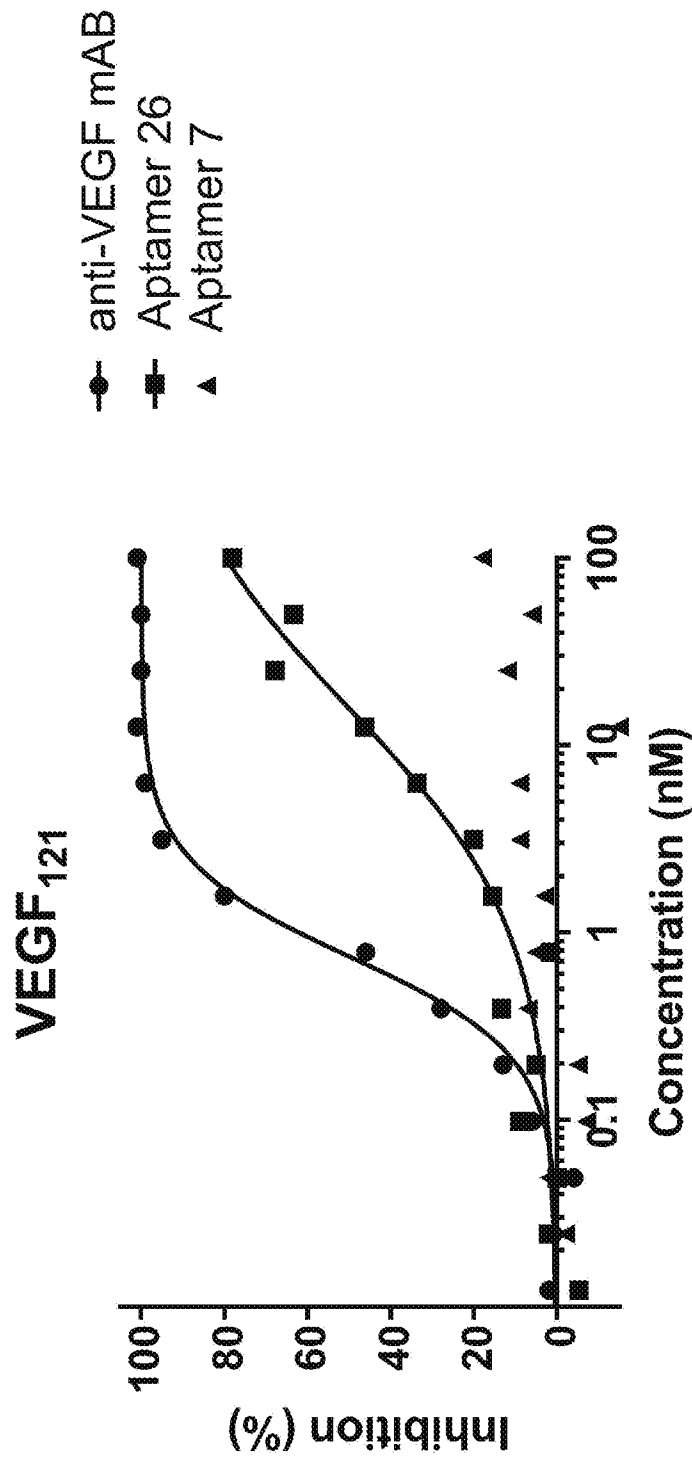


FIG. 4B

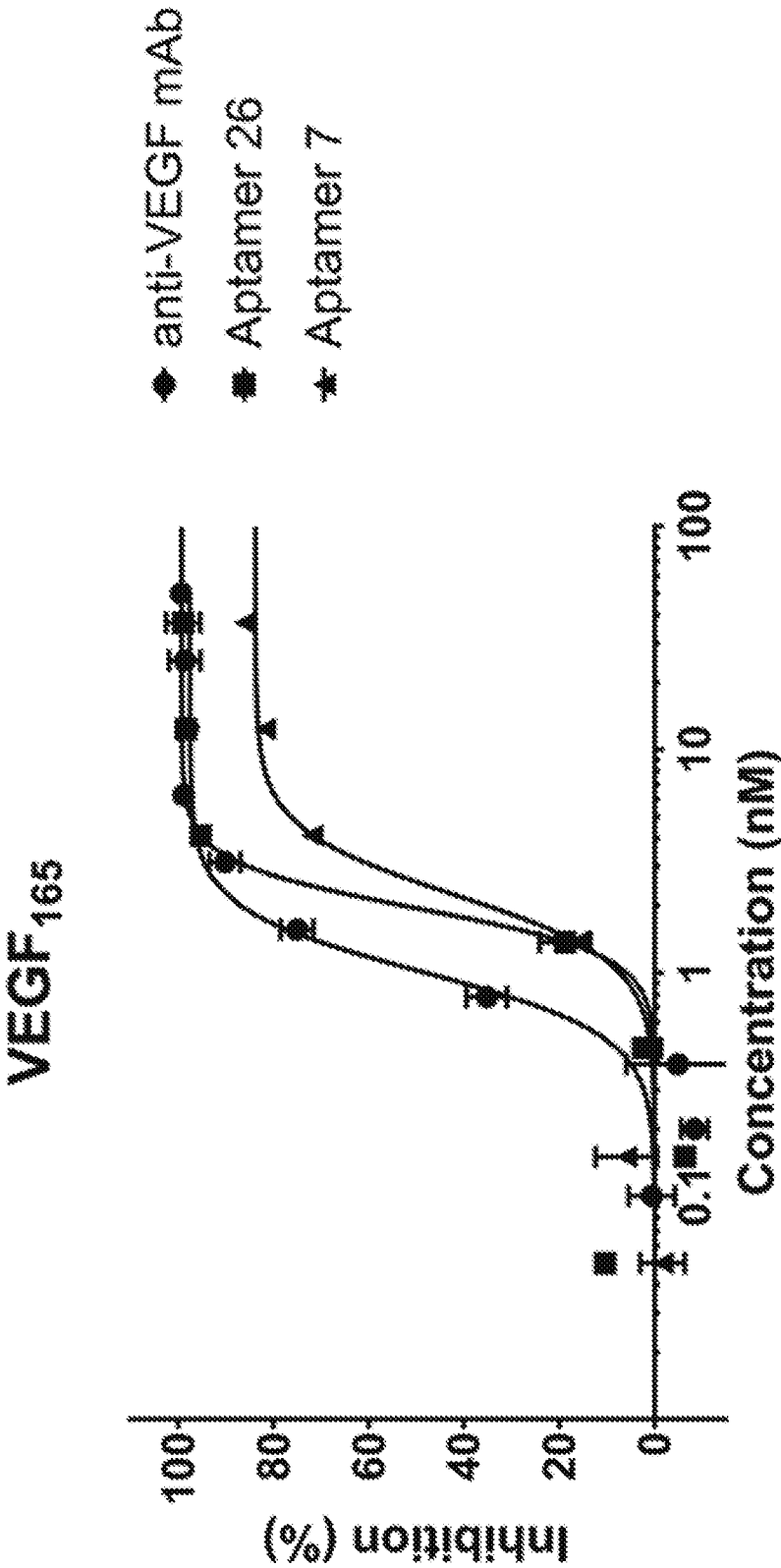


FIG. 5A

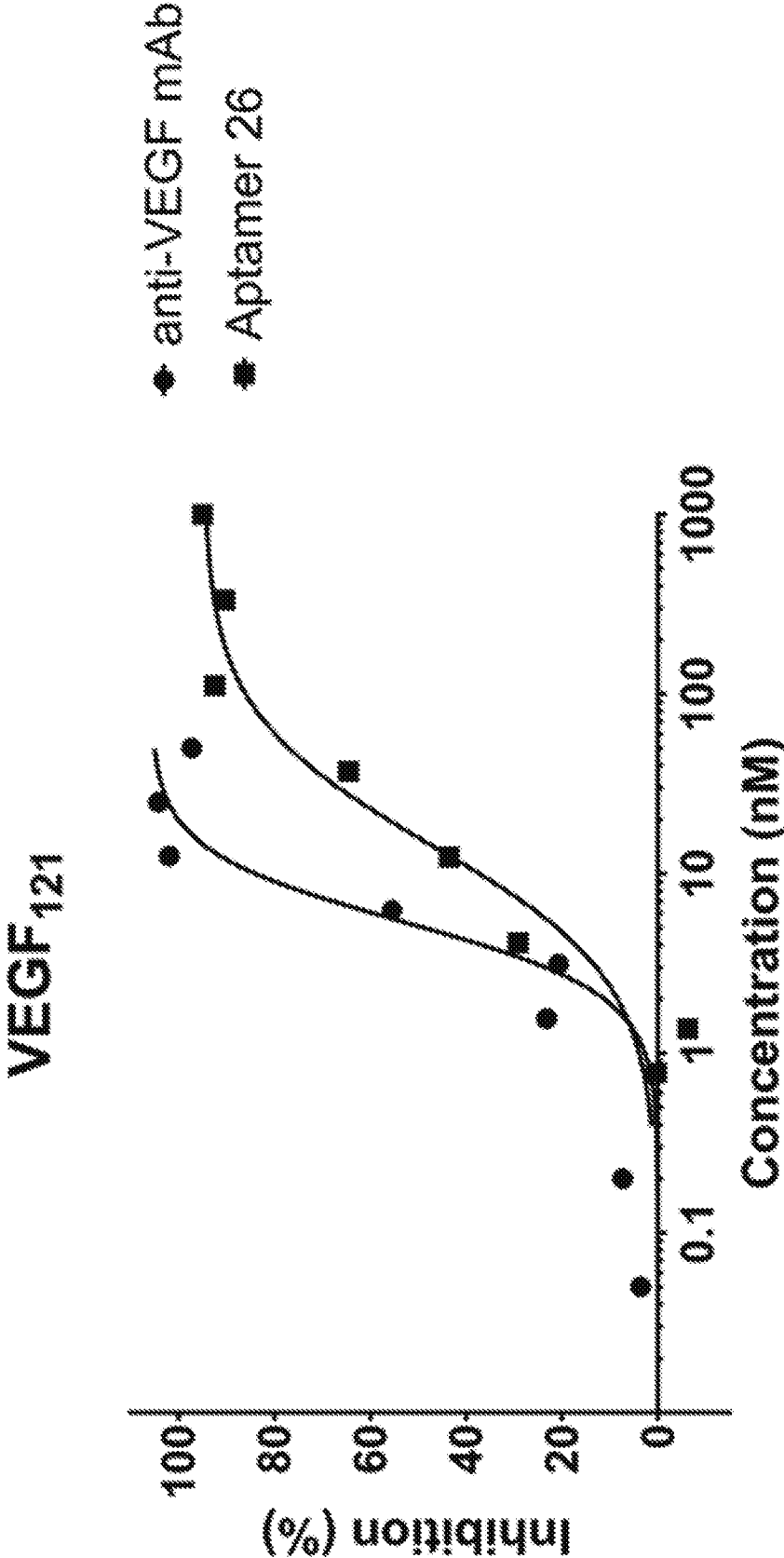
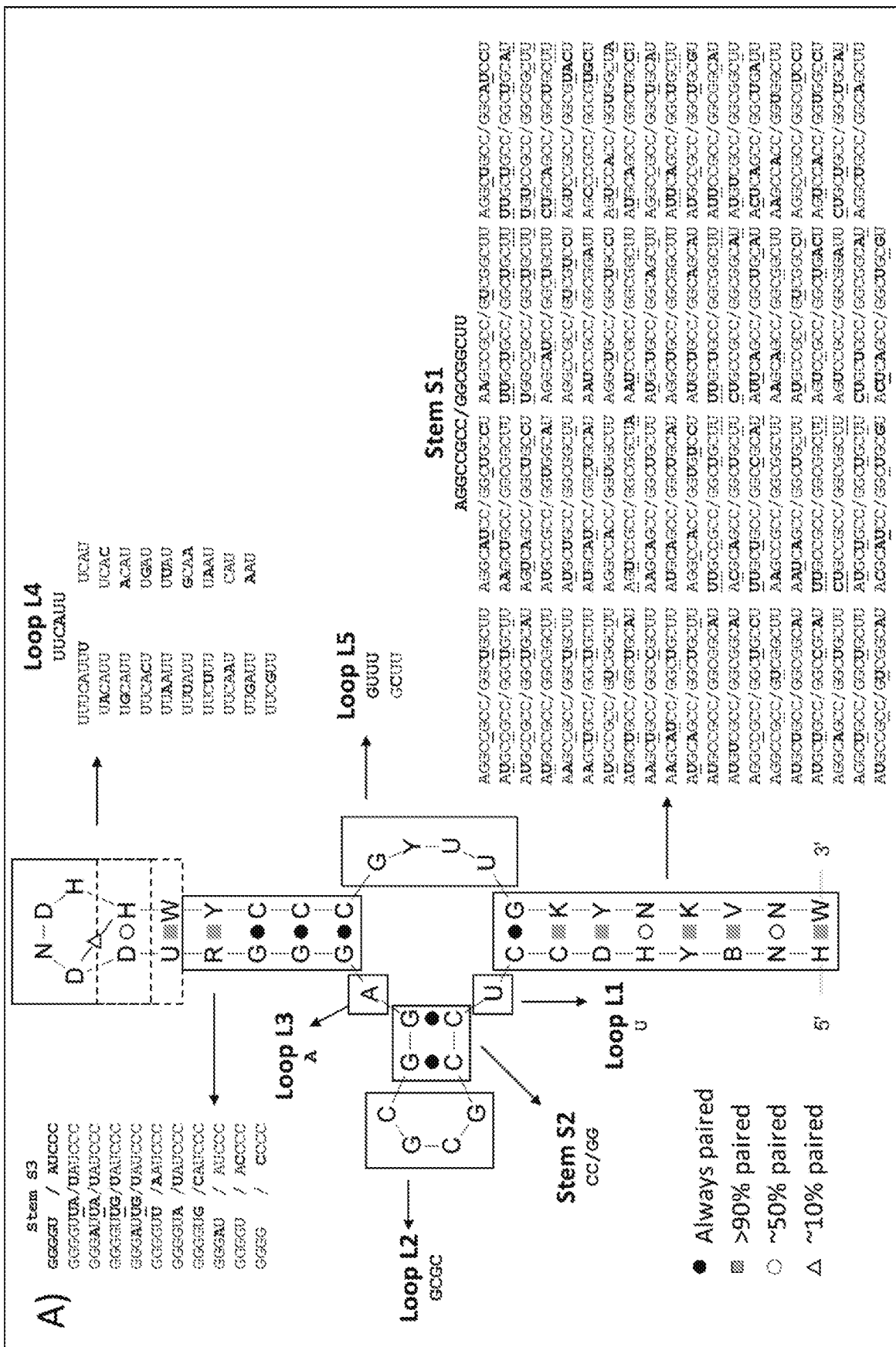


FIG. 5B

Structure:	S1												S2				L1				L2				S2				L3				S3			
Position:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23													
Aptamer 16:	A	U	G	C	C	G	C	C	U	C	C	G	C	G	C	G	G	A	G	G	G	G	U													
A	34.0%	32.5%	0.0%	0.0%	14.8%	1.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	21.5%	0.0%													
C	0.0%	1.3%	0.1%	97.7%	93.6%	0.0%	100.0%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%													
G	0.0%	29.3%	36.6%	0.0%	0.0%	91.6%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	75.5%	0.0%													
U	2.5%	36.2%	4.4%	2.3%	21.7%	4.3%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%													

Structure:	L4												S3				L5				S1			
Position:	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	
Aptamer 26:	U	U	C	A	U	U	A	U	C	C	C	G	U	U	U	G	G	C	G	G	C	U	U	
A	33.9%	11.5%	1.5%	35.0%	0.0%	34.0%	39.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	34.2%	0.0%	
C	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	99.1%	0.0%	0.0%	
G	1.3%	2.5%	1.5%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	93.6%	0.0%	75.5%	93.6%	0.1%	3.5%	0.0%	
U	35.0%	85.0%	0.7%	0.1%	95.9%	64.0%	0.0%	91.6%	0.0%	0.0%	0.0%	0.0%	99.7%	100.0%	100.0%	0.0%	4.3%	2.5%	97.7%	1.7%	0.0%	99.1%	99.0%	

FIG. 6



7
G
L

FIG 8

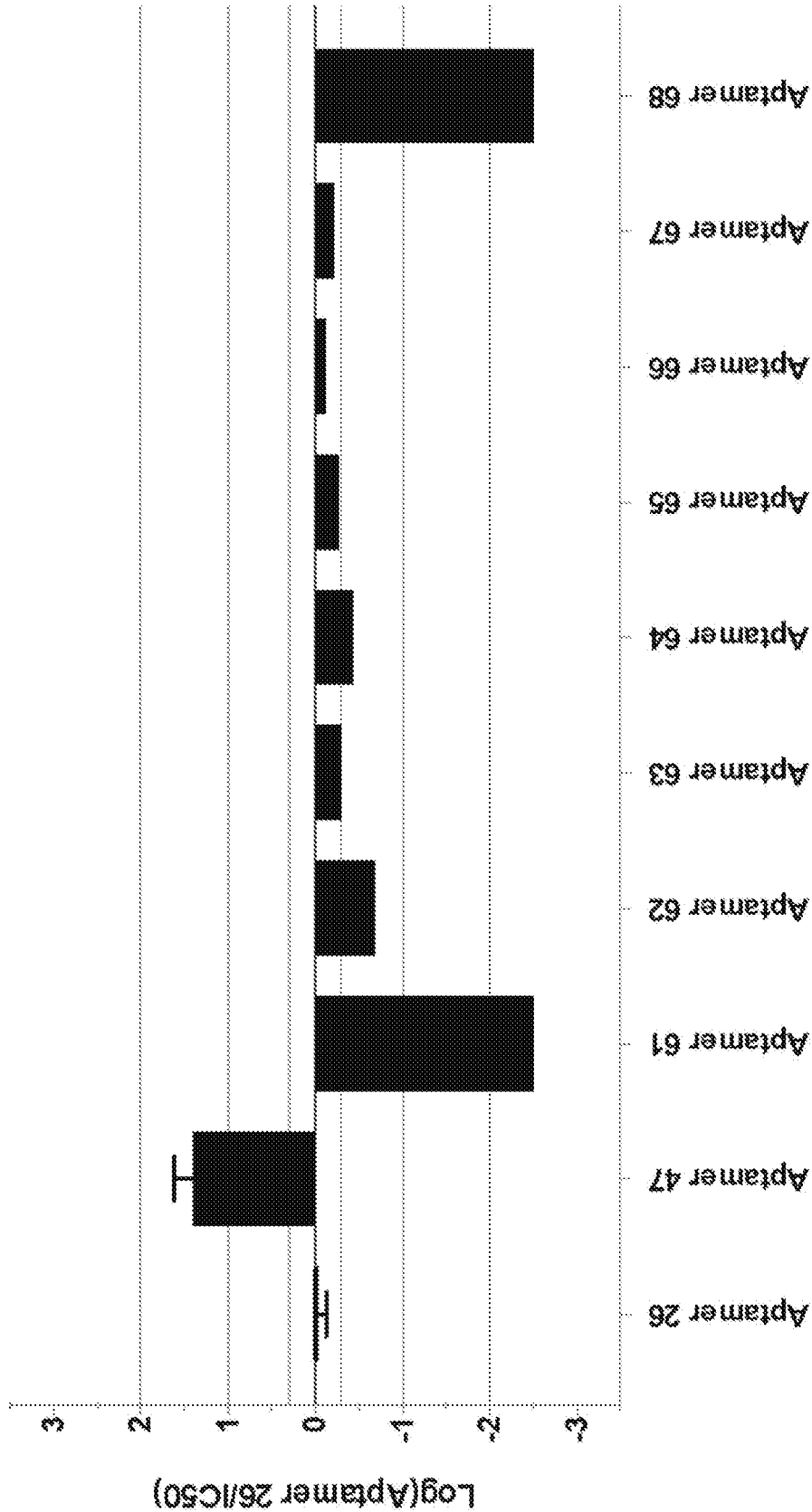


FIG. 8

FIG. 9

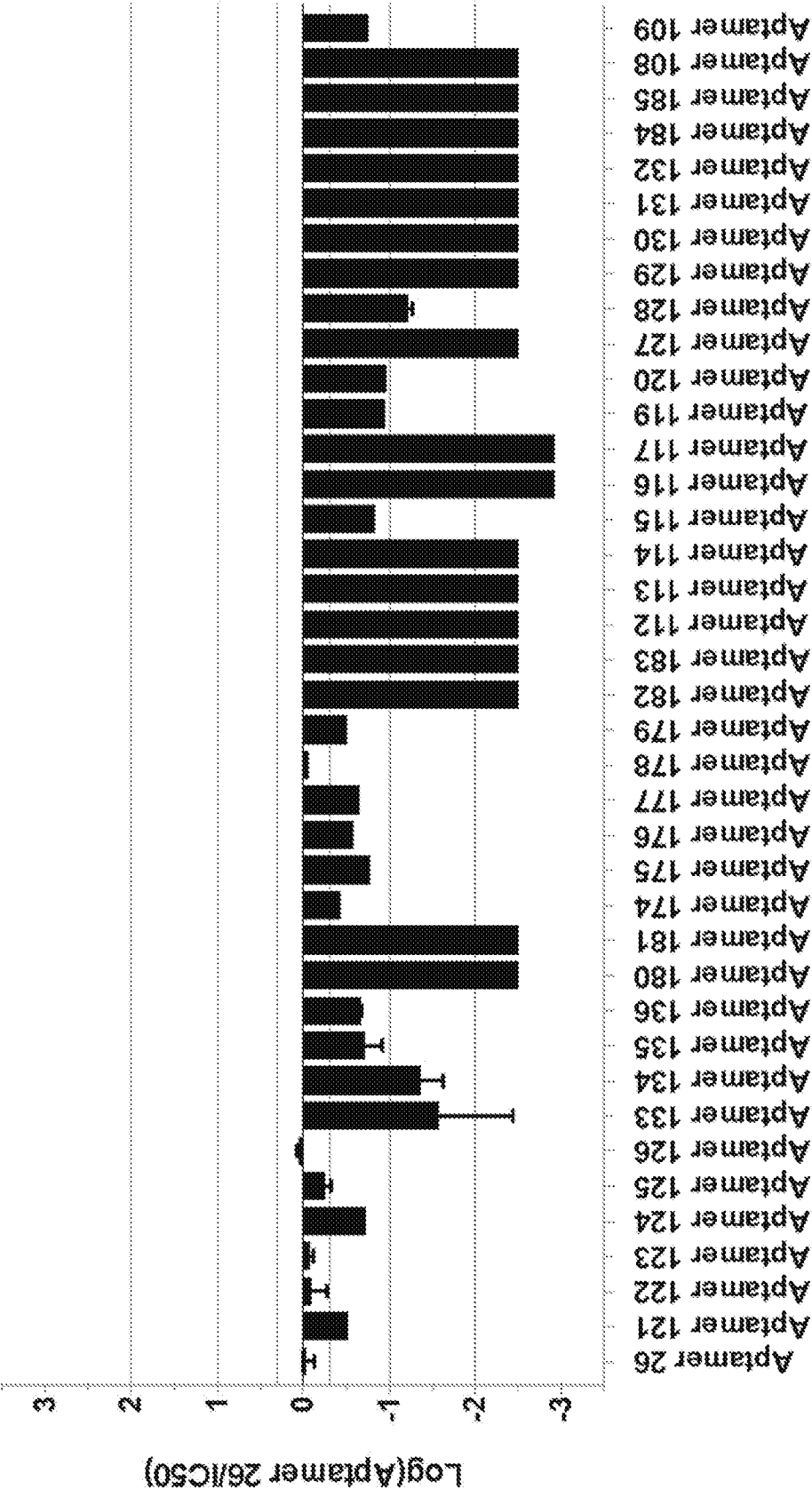


FIG. 9

FIG 10

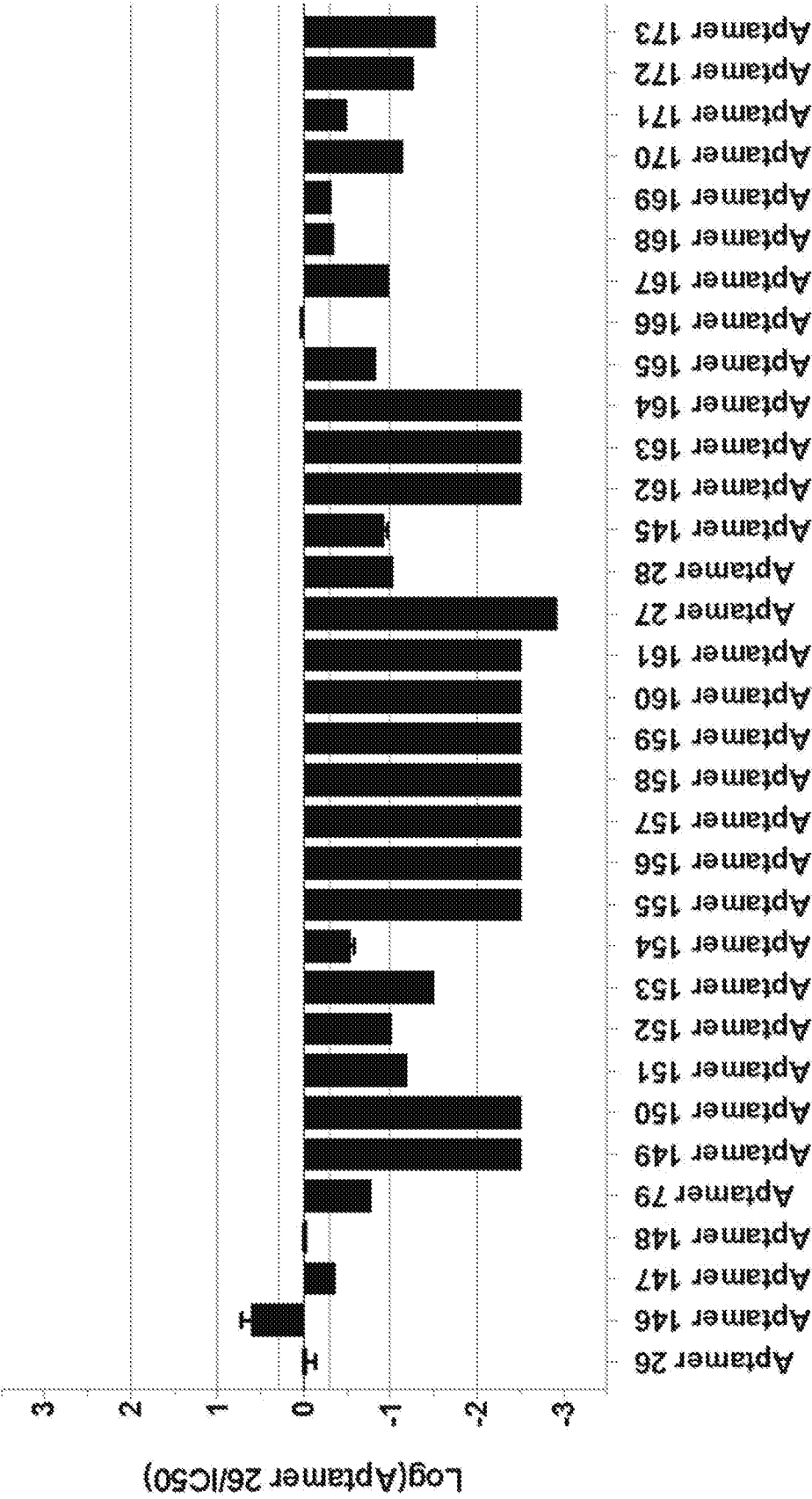


FIG. 10

FIG 11

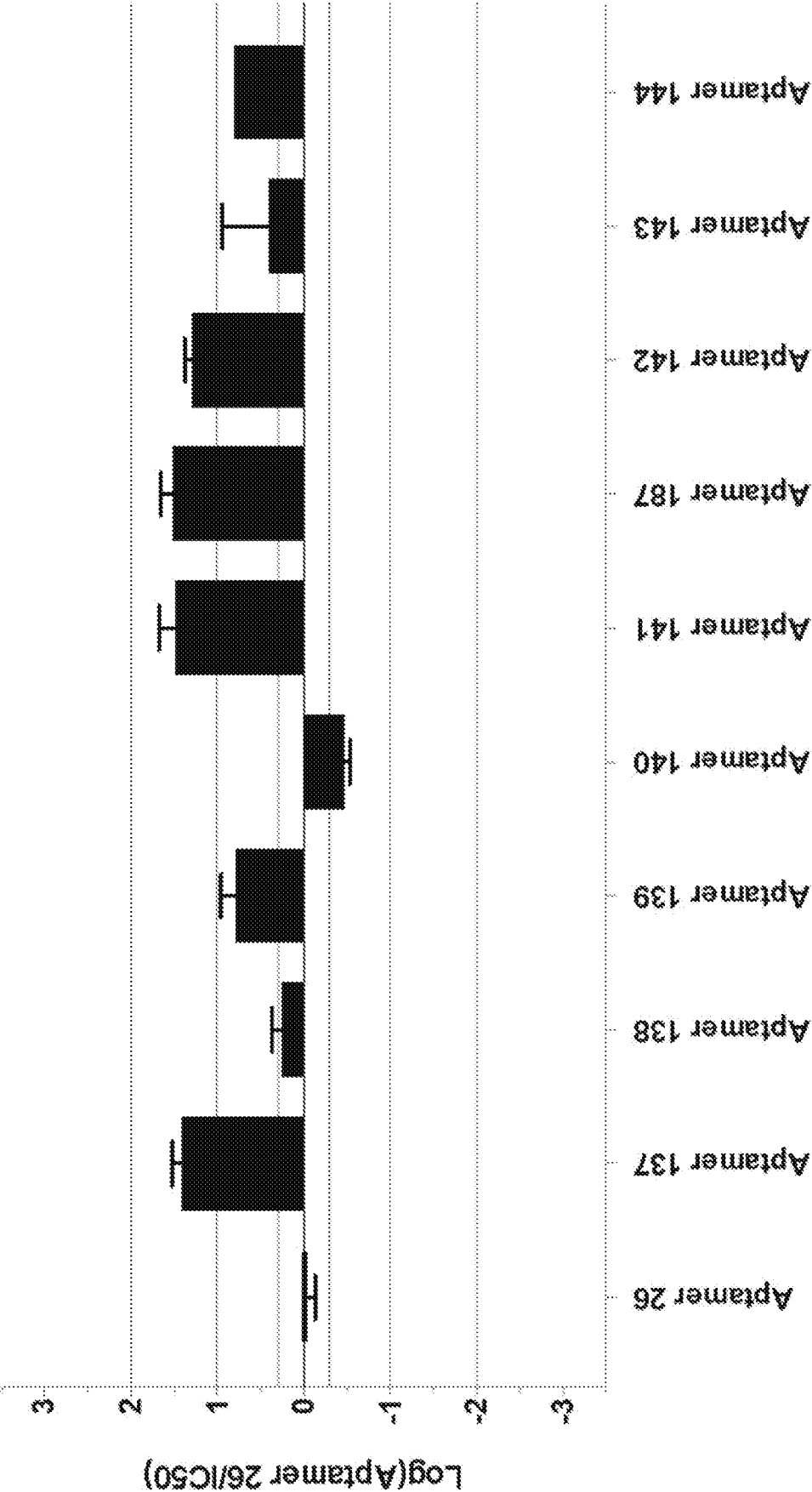


FIG. 11

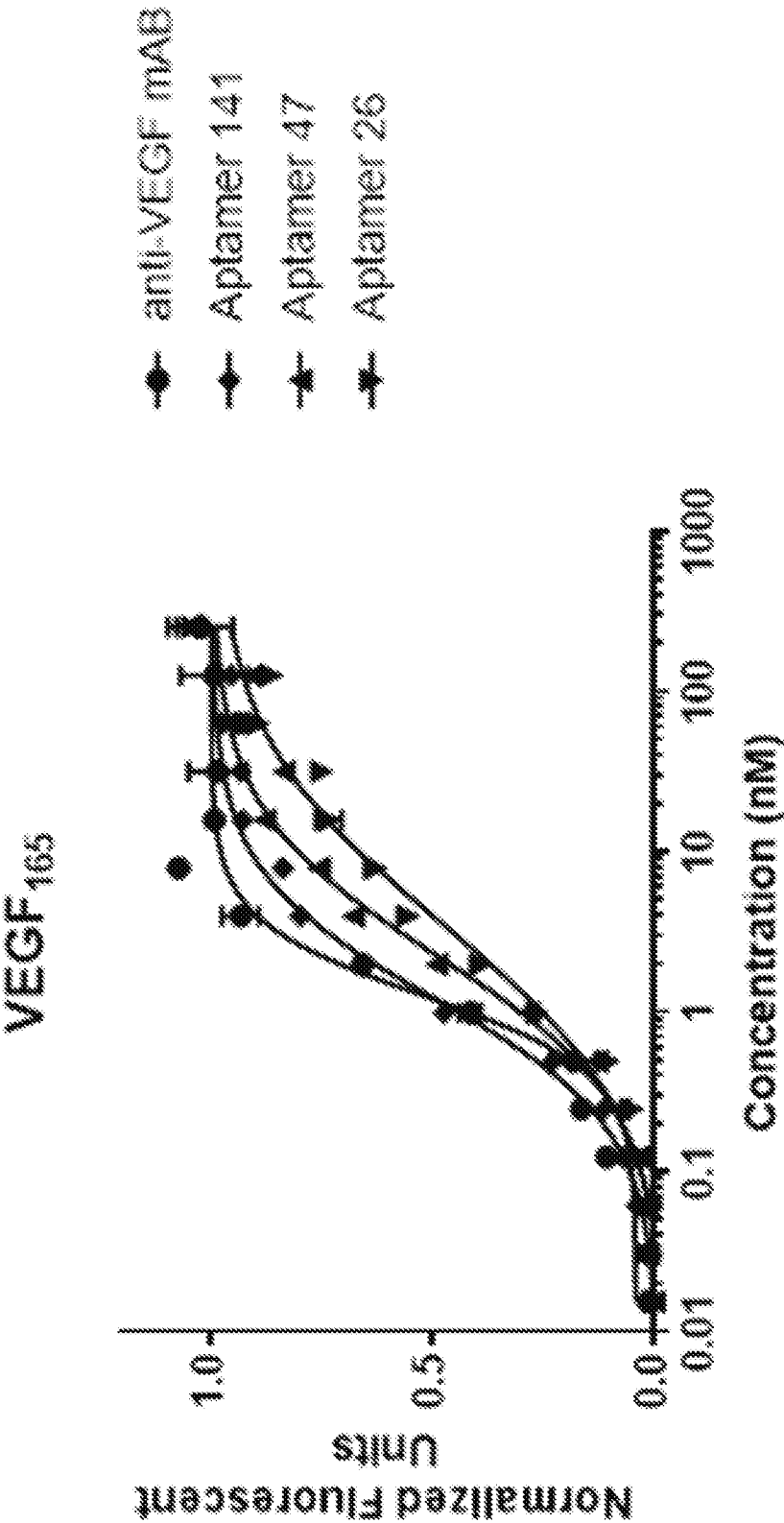


FIG. 12A

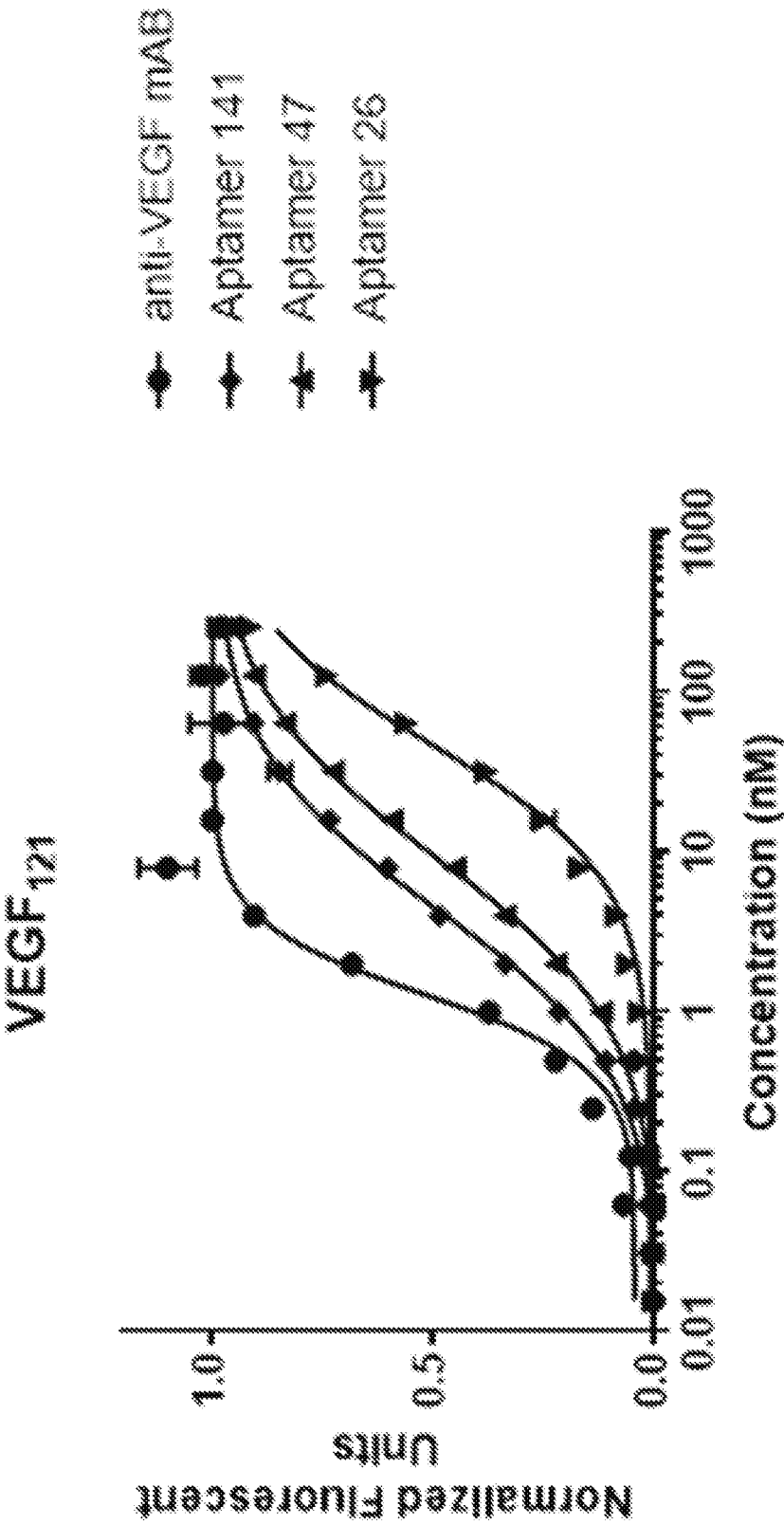


FIG. 12B

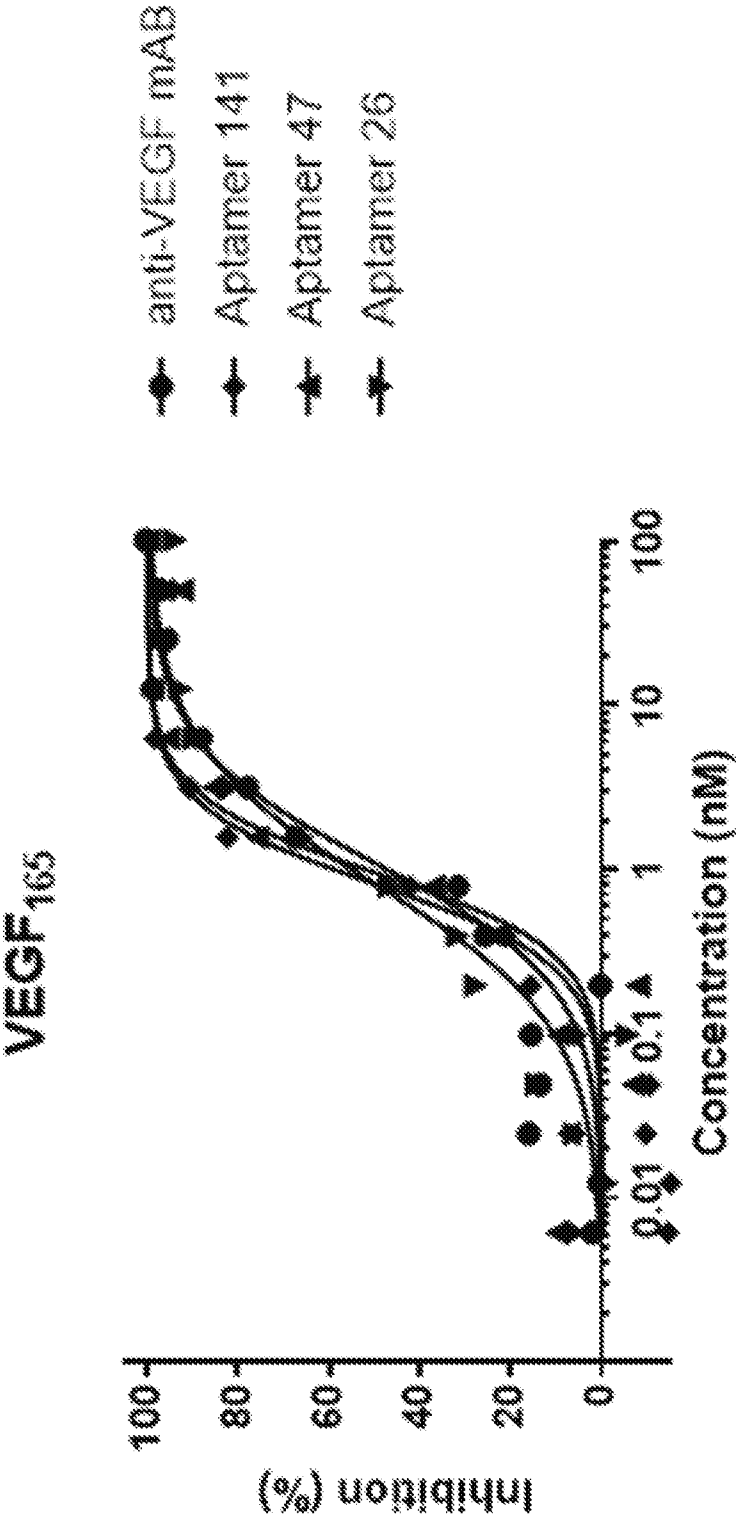


FIG. 13A

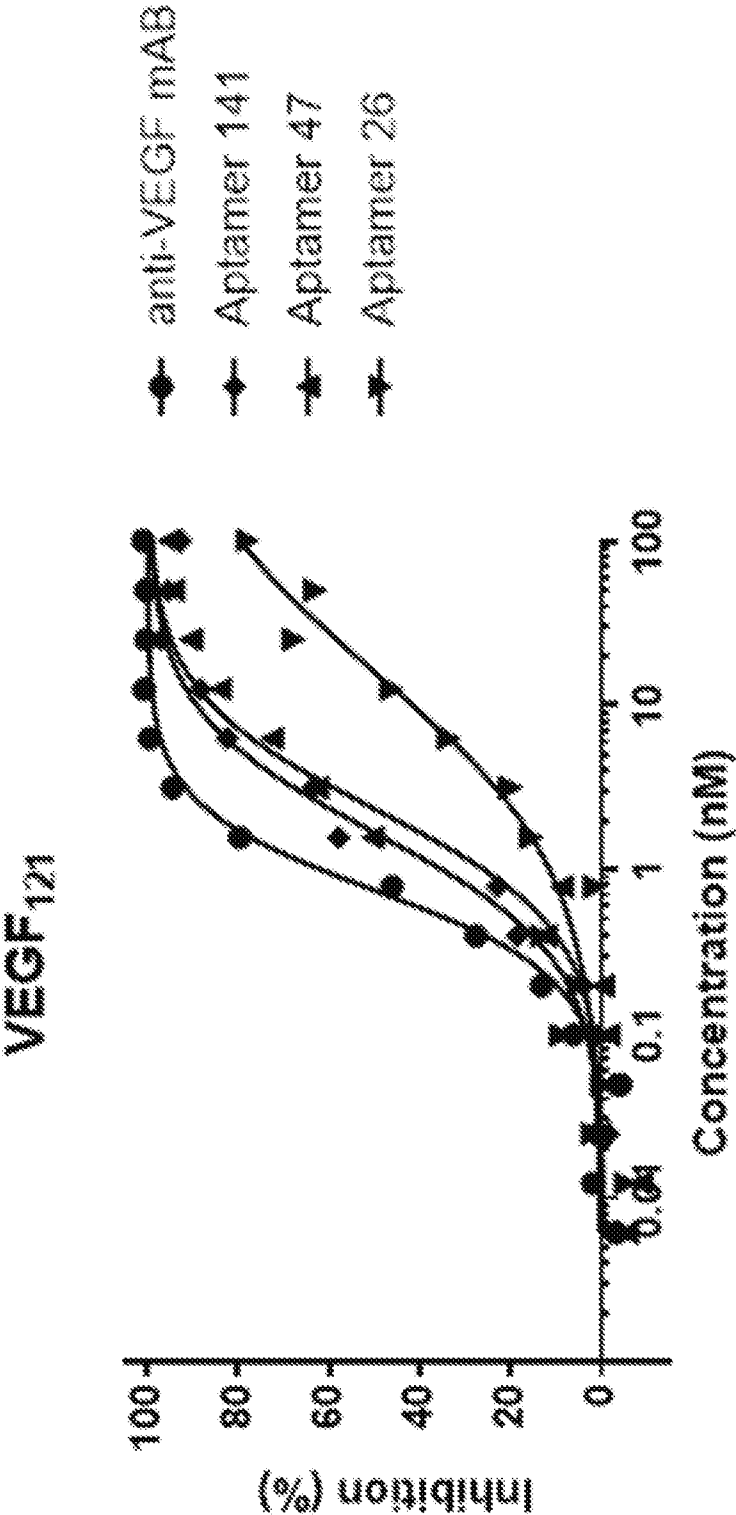


FIG. 13B

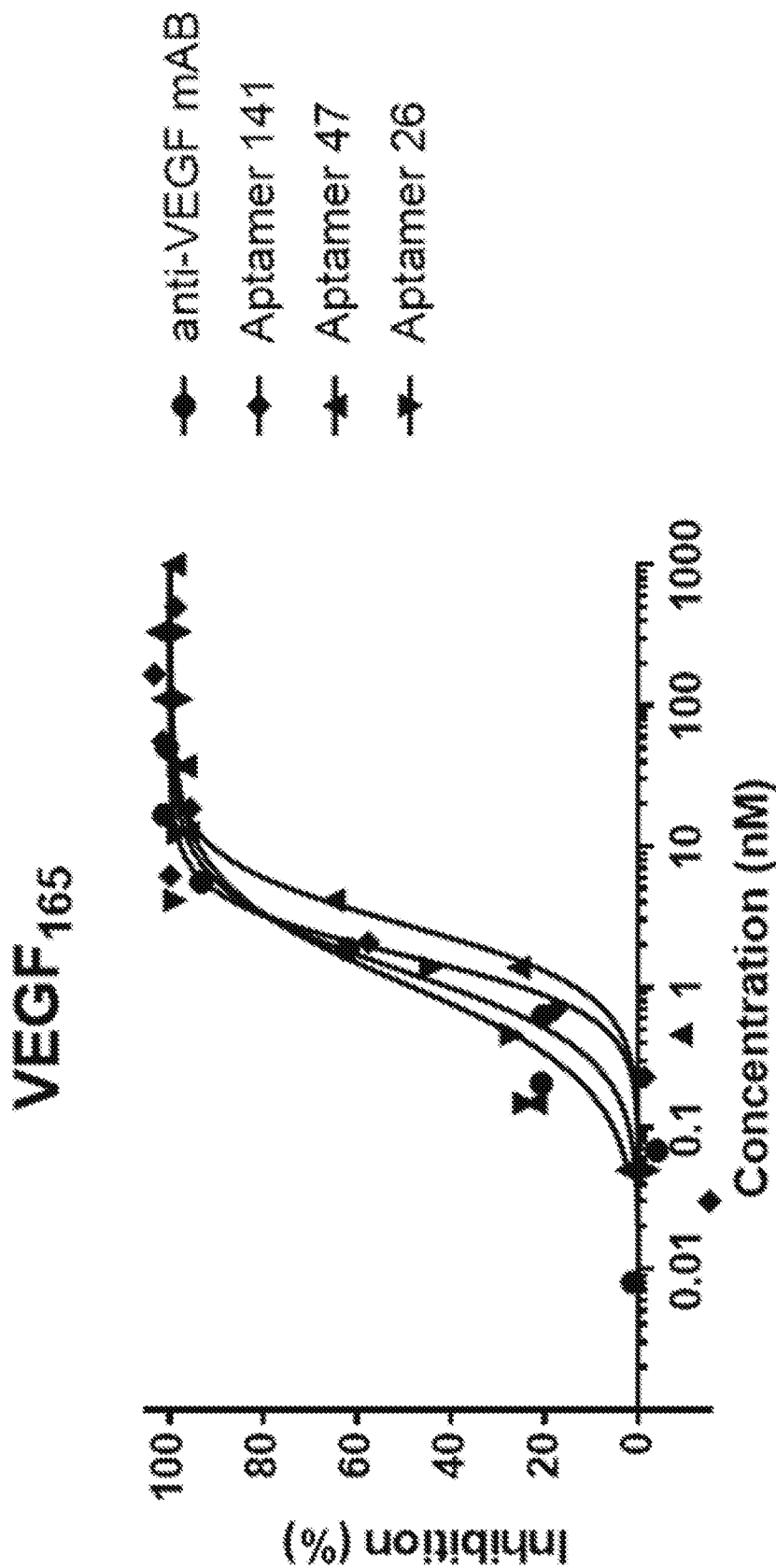


FIG. 14

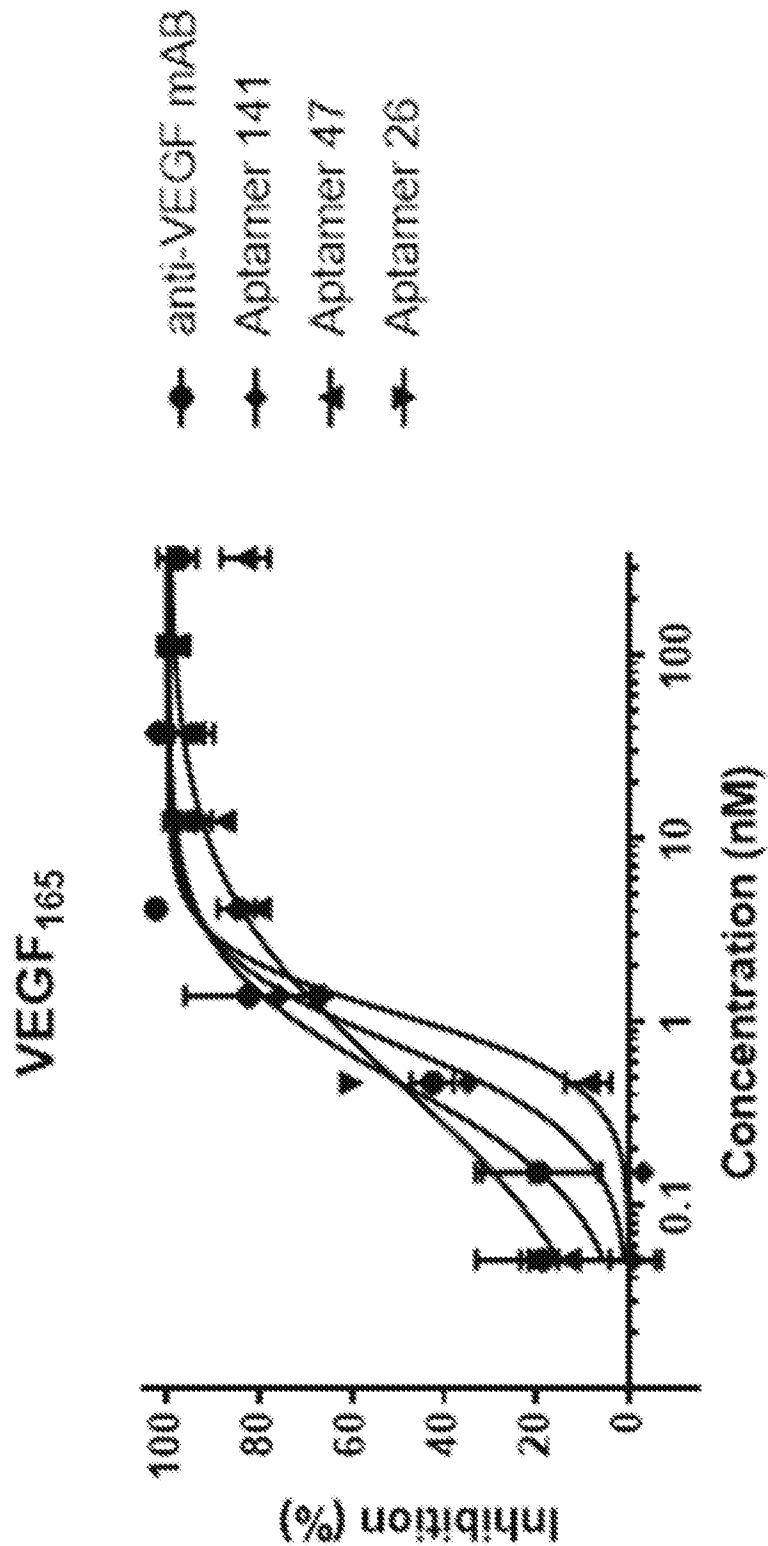


FIG. 15A

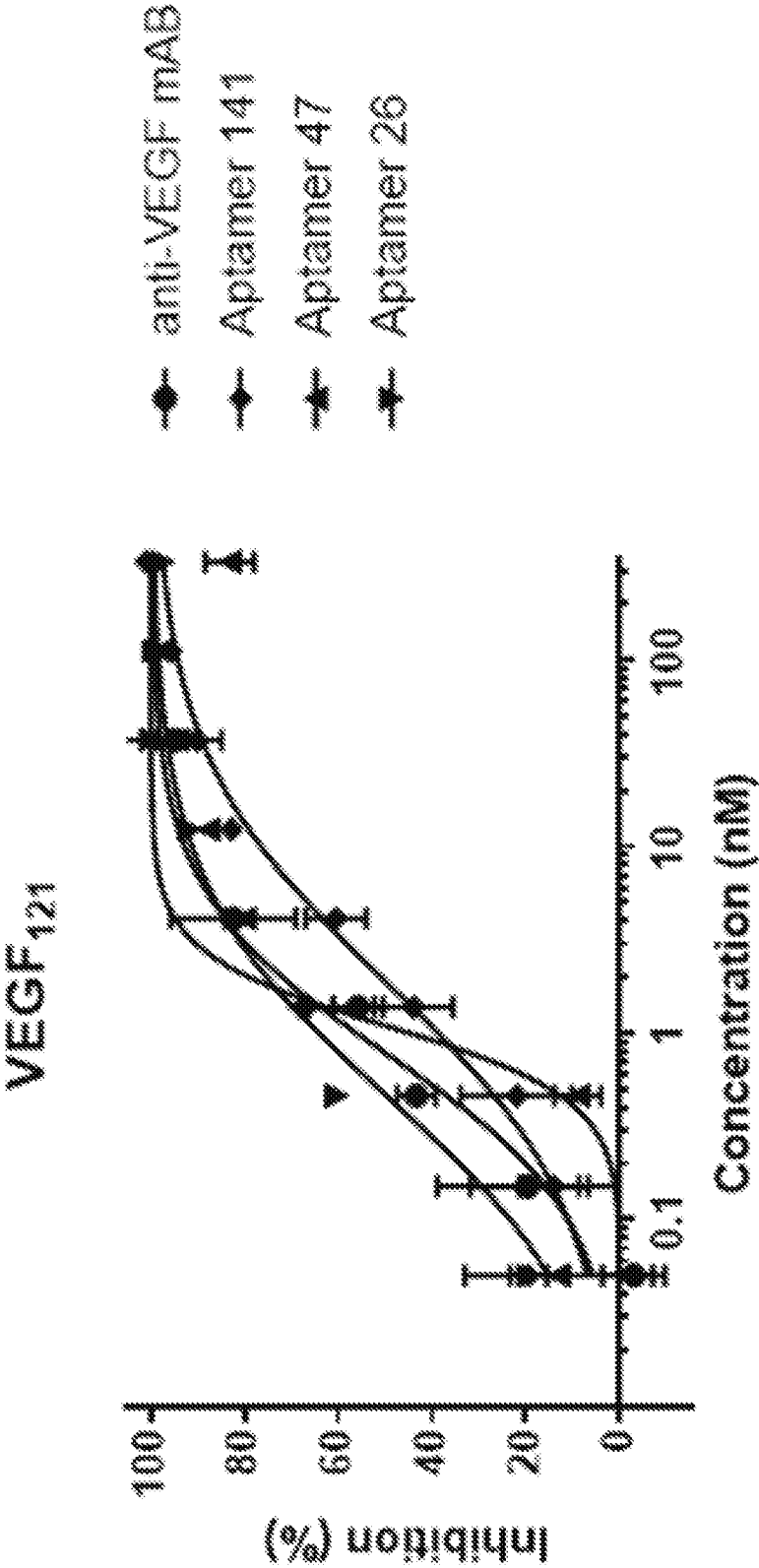
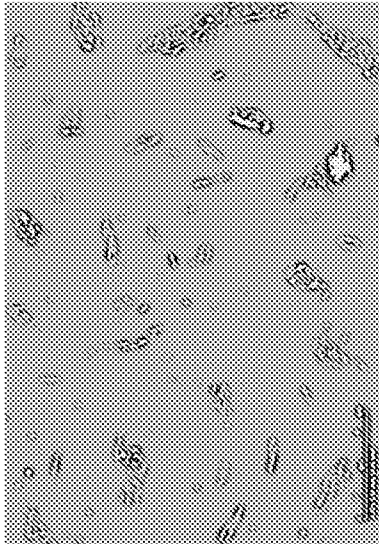
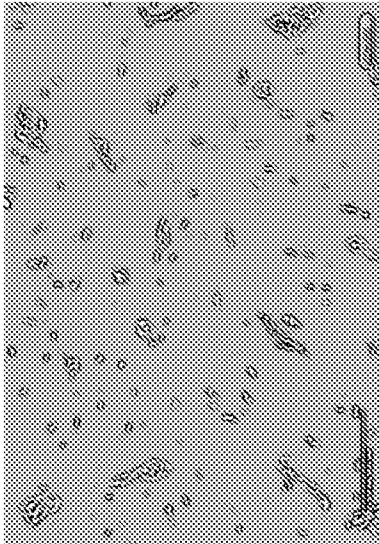


FIG. 15B

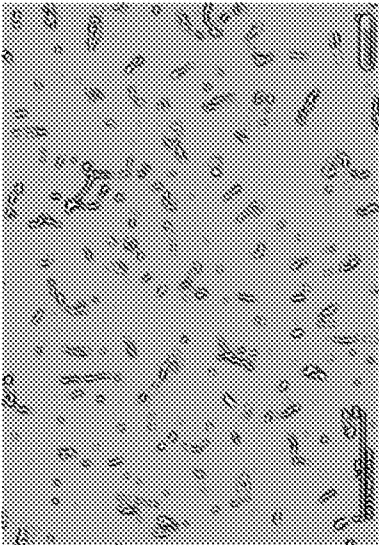
VEGF165 + Aptamer 47



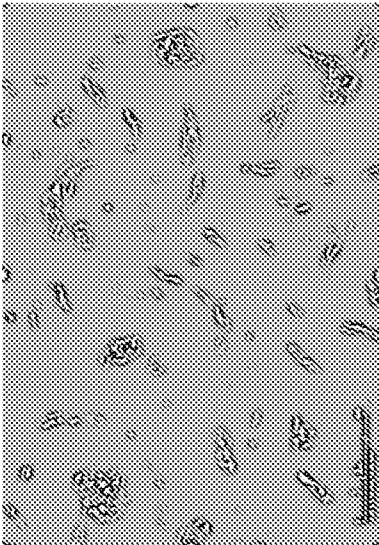
VEGF165 + Aptamer 26



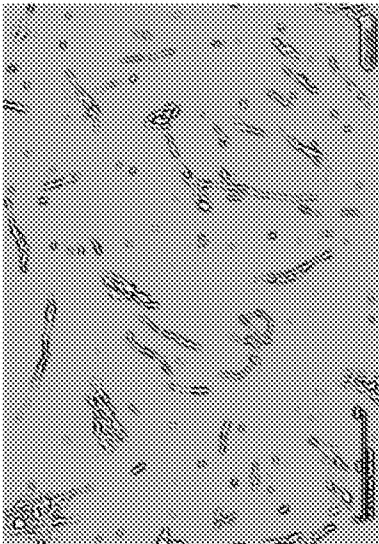
VEGF165 + anti-VEGF mAb



VEGF165 + Aptamer 141



No VEGF



VEGF165 Only

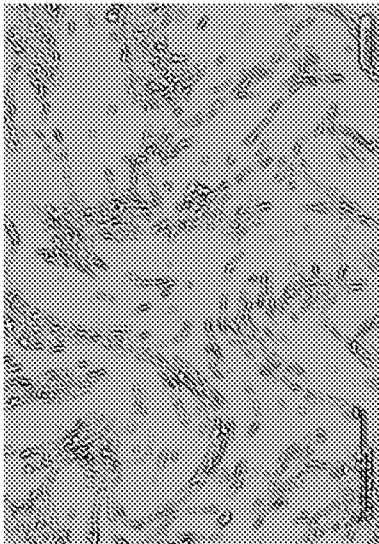


FIG. 16A

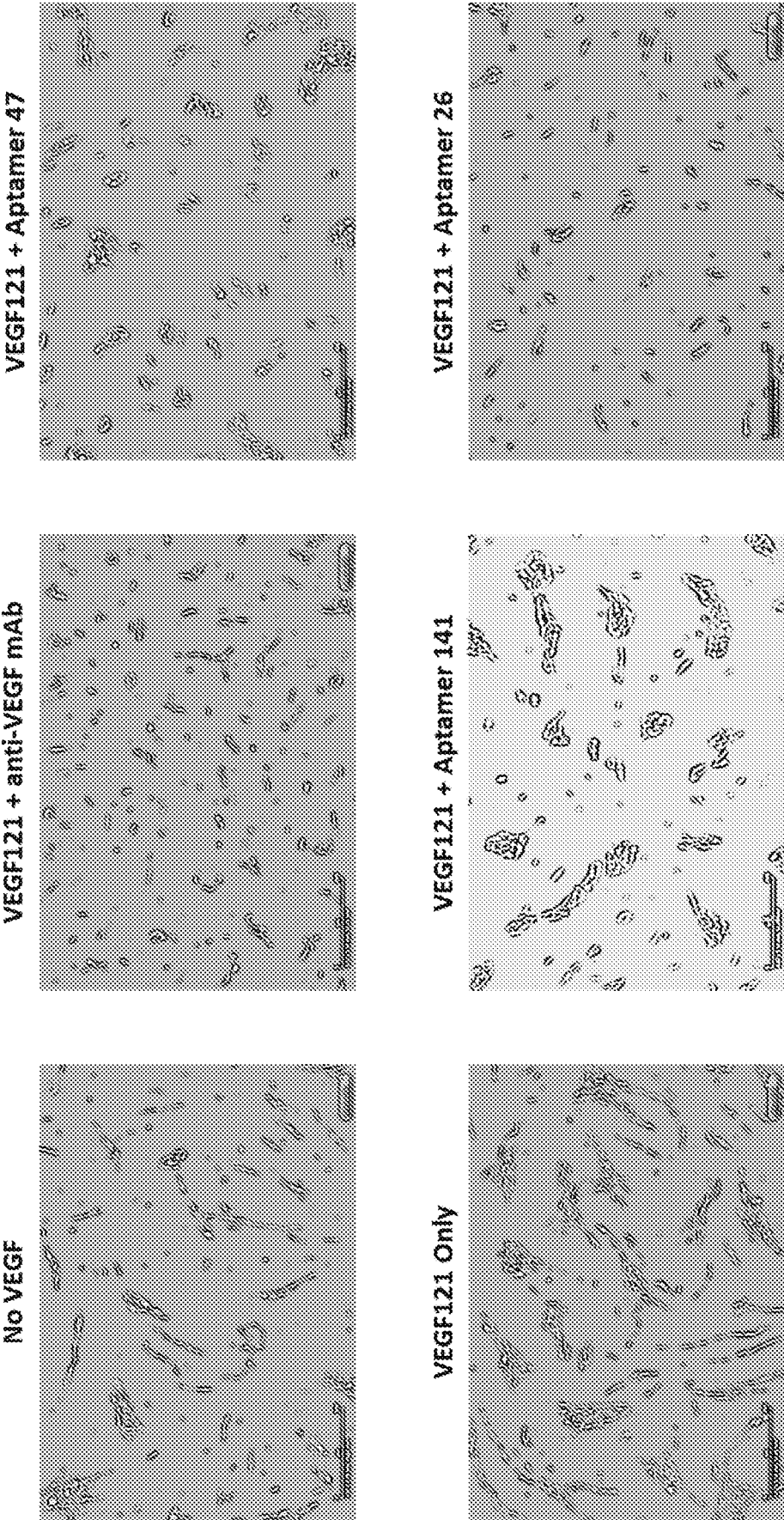


FIG. 16B

FIG 17

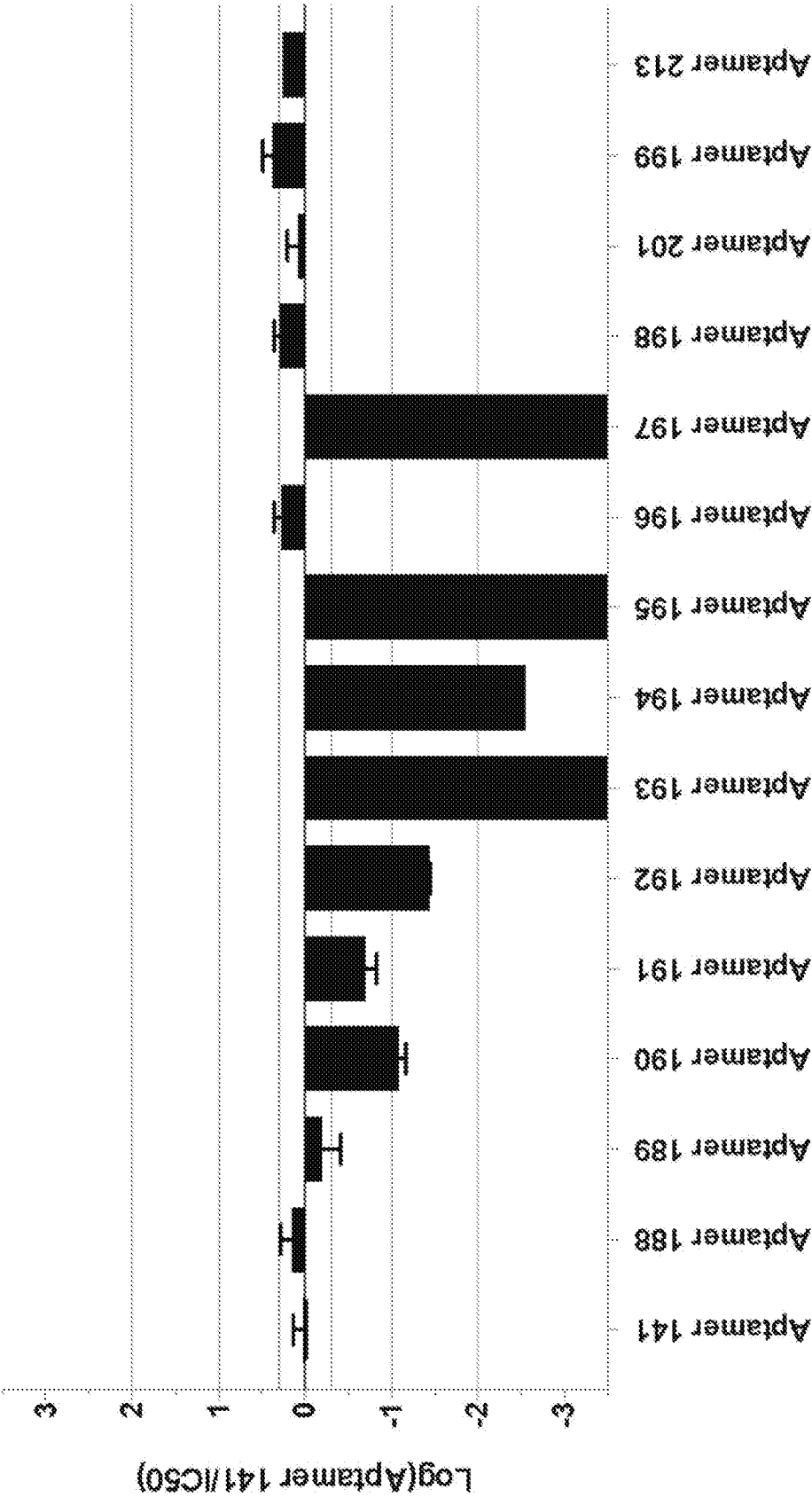


FIG. 17

FIG 18

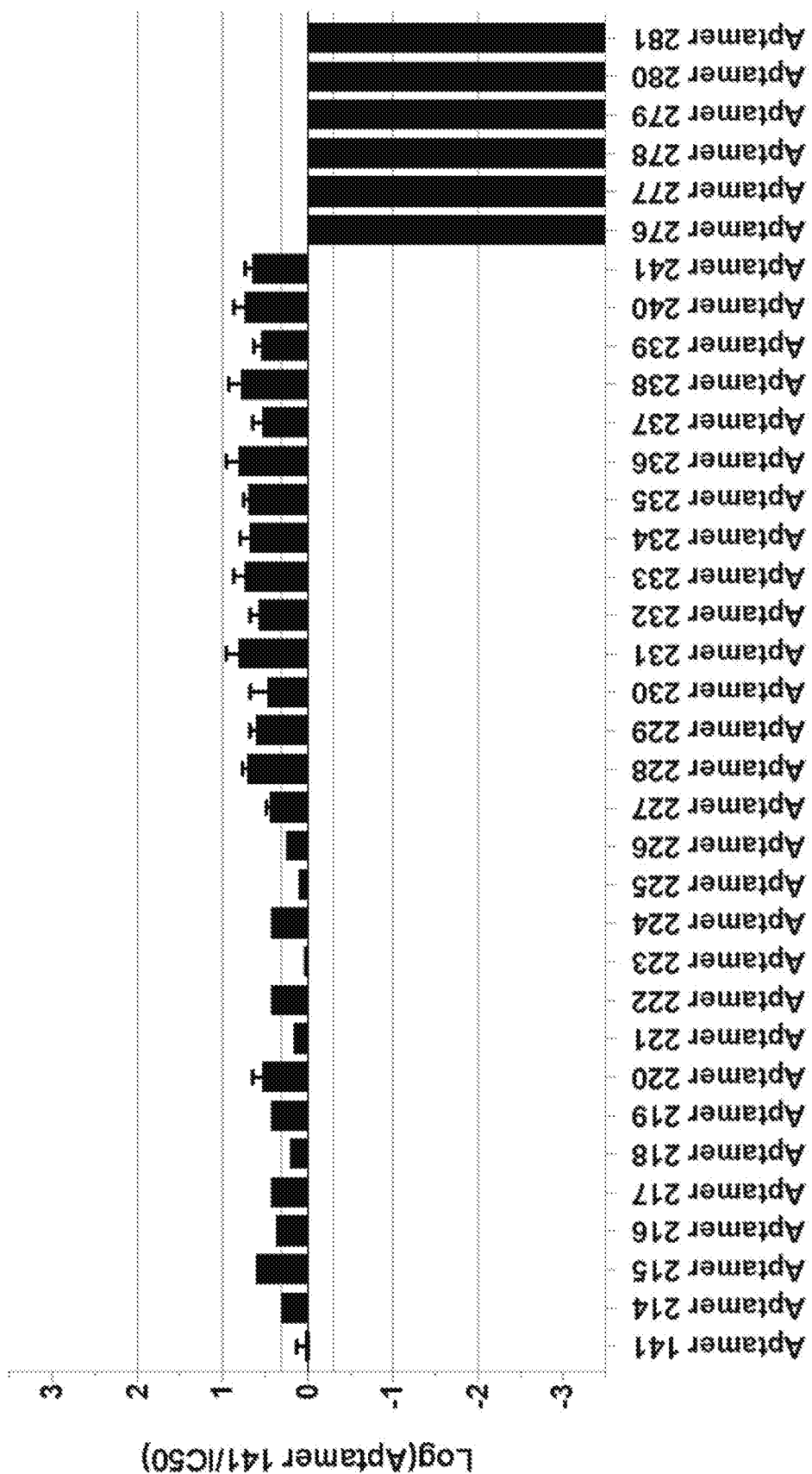


FIG. 18

FIG. 20

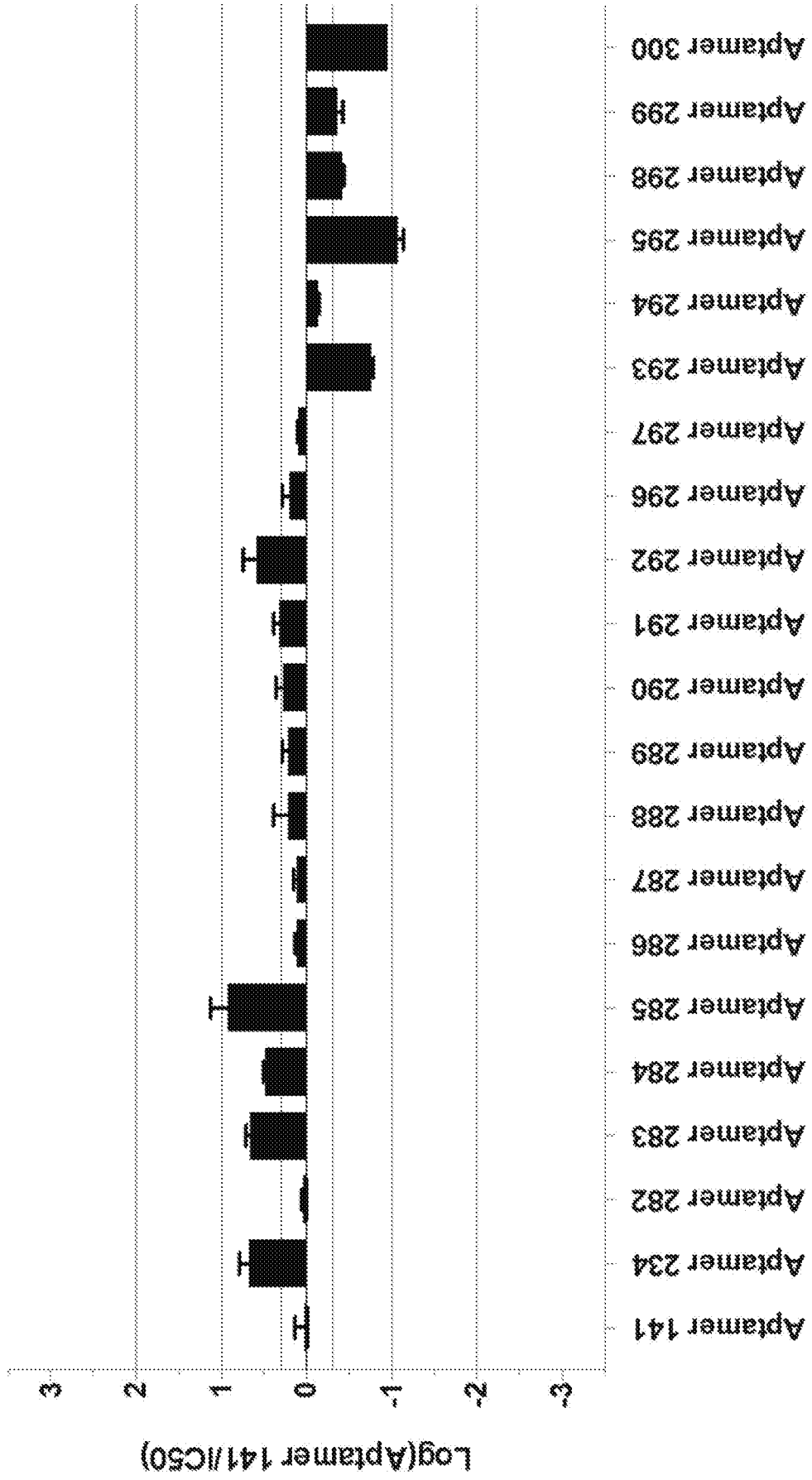


FIG 20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/13192

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/115, A61P 35/00, A61P 27/02 (2020.01)

CPC - A61K 38/1866, C12N 2310/16, A61P 27/12, C12N 2310/315, A61K 38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NONAKA et al. Screening and Improvement of an Anti-VEGF DNA Aptamer. Molecules, 7 January 2010, vol 15, no 1, pp 215-225. Especially abstract, pg 215, para 1 - pg 216, para 1, para 6, pg 217, para 1-2, pg 218, para 3, pg 222, para 1	1-3
Y	US 2015/0037889 A1 (THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY) 5 February 2015 (05.02.2015) abstract, claim 3, SEQ ID NO: 1	1-3

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 May 2020

Date of mailing of the international search report

23 JUN 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/13192

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-63, 72-114 and 118-130
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

----- See Extra Sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, limited to an aptamer that binds SEQ ID NO: 9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 20/13192

Continuation of Box No. III, Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-3 directed to an aptamer having a nucleic acid sequence, wherein said aptamer comprises a stem-loop secondary structure which specifically binds to and inhibits a VEGF-A polypeptide. The aptamer will be searched to the extent that the aptamer binds to and inhibits VEGF-A-121 (SEQ ID NO: 9, see para [0092], instant application). It is believed that claims 1-3 limited to an aptamer binds to and inhibits VEGF-A-121 (SEQ ID NO: 9) encompass this first named invention, and thus these claims will be searched without fee to the extent that the aptamer binds said polypeptide. Additional VEGF-A-binding aptamers will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected oligonucleotides. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an aptamer that binds to and inhibits VEGF-A-110 (SEQ ID NO: 10 see para [0093], instant application) (claims 1-3).

Group II+: Claims 64-71, directed to a VEGF-A-binding aptamer (see instant abstract) comprising a consensus nucleic acid sequence. Group II+ will be searched upon payment of additional fees. The VEGF-A-binding aptamer comprising a consensus nucleic acid sequence may be searched, for example, to the extent that the aptamer comprises SEQ ID NO: 1, where His A; N is A; Bis C; Y is C; D is A; R is A; W is A; K is G; and V is A. It is believed that claim 64 limited to said VEGF-A-binding aptamer, read on this exemplary invention. Additional VEGF-A-binding aptamers will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an aptamer that comprises SEQ ID NO: 2, where His A; N is A; Bis C; Y is C; D is A; R is A; K is G; V is A, and W is A (Claim 65).

Group III+: Claims 115-117, directed to a modified VEGF-A-binding aptamer described in Table I (instant specification, para [00125]). Group III+ will be searched upon payment of additional fees. The modified VEGF-A-binding aptamer may be searched, for example, to the extent that the aptamer encompasses R6-1, truncated, SEQ ID NO: 11, where G is 2'F; and A, C, & U are 2'O:Me modified RNA. It is believed that claims 115-117 limited to said SEQ ID NO: 11 aptamer read on this exemplary invention. Additional modified VEGF-A-binding aptamers will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be the aptamer 4.2, SEQ ID NO: 219, where G is 2'F; A, C, & U are 2'OMe modified RNA; C6SS is a disulfide linker; and idT is an inverted deoxythymidine residue. (see pg. 62, instant application) (Claims 115-117).

The inventions listed as Groups I+, II+ and III+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

No technical features are shared between the aptamer sequences of Group I+, the aptamer sequences of Group II+ and, the aptamer sequences of Group III+ and, accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Groups II+ and III+ do not require an aptamer that comprises a stem-loop secondary structure which specifically binds to and inhibits a VEGF-A polypeptide, as required by group I+.

Groups I+ and III+ do not require a VEGF-A-binding aptamer comprising a consensus nucleic acid sequence, as required by group II+.

Group I+ and II+ do not require a modified VEGF-A-binding aptamer described in Table I, as required by group III+.

Common Technical Features

The inventions of Groups I+, II+ and III+ share the technical feature of a VEGF-A-binding aptamer. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2005/0096257 A1 to Shima et al. (hereinafter 'Shima'). Shima teaches aptamers that bind to VEGF-A (para [0017] "the method of the invention involves administration of a VEGF antagonist that is a VEGF-A antagonist.", para [0018] "the method of the invention involves administration of a VEGF antagonist that is a nucleic acid molecule, an aptamer", para [0145] "A particularly useful antagonist to VEGF-A is EYE001 ... which is a modified, PEGylated aptamer that binds with high and specific affinity to the major soluble human VEGF isoform").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Groups I+, II+ and III+ therefore lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.