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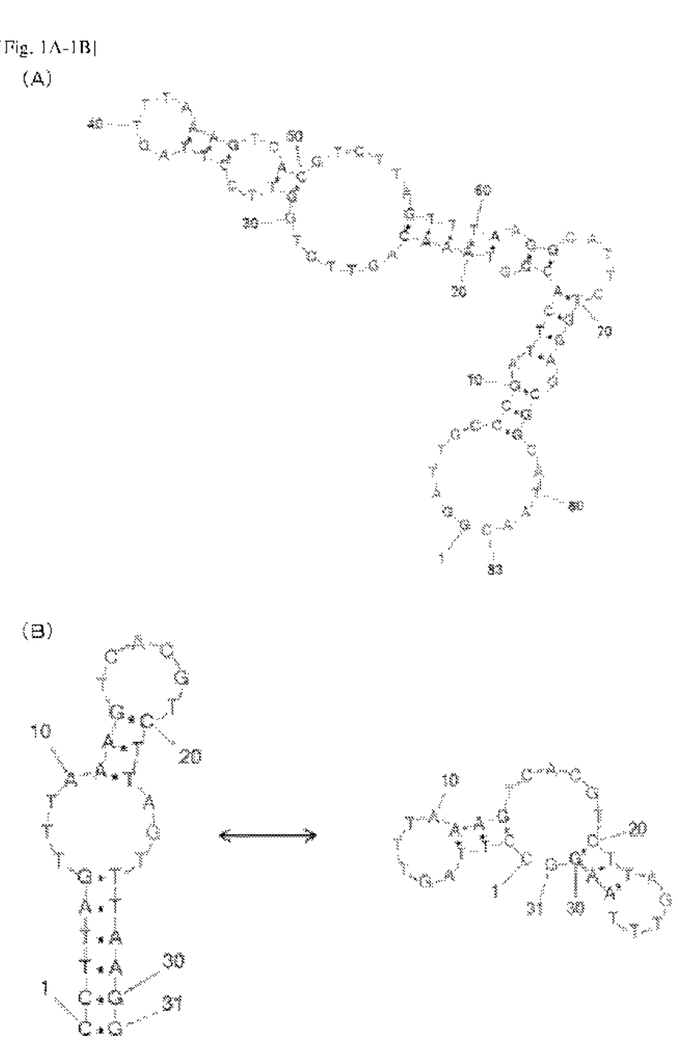
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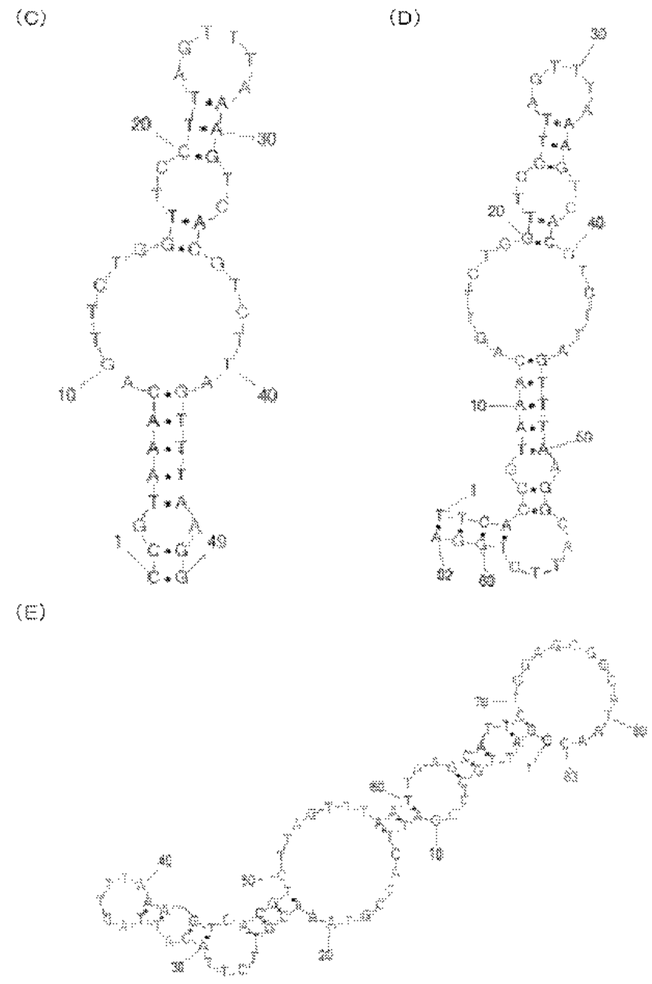
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(54) Title: METHOD FOR PRODUCING NUCLEIC ACID MOLECULE, BIOMATERIAL, AND METHOD FOR PRODUCING BIOMATERIAL

[Fig. 1A-1B]



[Fig. 1C-1E]



(57) Abstract: The present invention provides a method for producing a nucleic acid molecule that can obtain a nucleic acid molecule that binds to a target and does not inhibit a function of the target. The production method for a nucleic acid molecule of the present invention is a method for producing a nucleic acid molecule that binds to a first biological molecule and does not inhibit a function of the first biological molecule, the method including the steps of: (A) bringing a candidate nucleic acid molecule into contact with the first biological molecule to select a nucleic acid molecule that has bound to the first biological molecule as a first selected nucleic acid molecule; and (B) selecting the first selected nucleic acid molecule as an intended nucleic acid molecule.

[Continued on next page]

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Description

Title of Invention: METHOD FOR PRODUCING NUCLEIC ACID MOLECULE, BIOMATERIAL, AND METHOD FOR PRODUCING BIOMATERIAL

Technical Field

[0001] The present invention relates to a method for producing a nucleic acid molecule, a biomaterial, and a method for producing a biomaterial.

Background Art

[0002] Growth factors are endogenous proteins that stimulate cell proliferation and differentiation. It is known that a vascular endothelial growth factor (VEGF), which is one of the growth factors, binds as a ligand to a vascular endothelial growth factor receptor (VEGFR), thereby being involved in angiogenesis etc. (Non-Patent Document 1).

[0003] On the other hand, nucleic acid molecules that specifically bind to a target such as a protein are attracting attention.

Citation List

Patent Literature

[0004] [Non-Patent Document 1] Ferrara N. et al., Nat Med., 2003 Jun; 9(6): 669-76.

Summary of Invention

Technical Problem

[0005] However, heretofore, there has been a case in which the nucleic acid molecule inhibits the function of the target by binding to the target.

[0006] With the foregoing in mind, it is an object of the present invention to provide a method for producing a nucleic acid molecule that can obtain a nucleic acid molecule that binds to a target and does not inhibit a function of the target.

Solution to Problem

[0007] In order to achieve the above object, the present invention provides a method for producing a nucleic acid molecule that binds to a first biological molecule and does not inhibit a function of the first biological molecule, the method including the steps of:

(A) bringing a candidate nucleic acid molecule into contact with the first biological molecule to select a nucleic acid molecule that has bound to the first biological molecule as a first selected nucleic acid molecule; and

(B) selecting the first selected nucleic acid molecule as an intended nucleic acid molecule.

[0008] The present invention also provides a nucleic acid molecule that binds to VEGF, the nucleic acid molecule including:

any one of the following polynucleotides (a) and (b):

(a) a polynucleotide that consists of a base sequence of SEQ ID NO: 1 or a partial sequence of the base sequence of SEQ ID NO: 1; and

(b) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), binds to VEGF, and does not inhibit a function of the VEGF.

[0009] The present invention also provides a biomaterial including:

a binding nucleic acid molecule; and

a carrier,

the binding nucleic acid molecule being bound to the carrier,

wherein the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein.

[0010] The present invention also provides a method for producing a biomaterial, the method including the step of:

binding a binding nucleic acid molecule to a carrier, wherein

the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein.

Advantageous Effects of Invention

[0011] The present invention can provide a method for producing a nucleic acid molecule that can obtain a nucleic acid molecule that binds to a target and does not inhibit a function of the target.

Brief Description of Drawings

[0012] [fig.1A-1E]FIGs. 1A to 1E show the predicted secondary structures of aptamers 1 to 5 (SEQ ID NOs: 1-4 and 7), respectively.

[fig.2]FIG. 2 is a schematic view of two mRNA splice variants which encode different isoforms of VEGF. The amino acid sequence of the heparin binding domain encoded by exon 7 is depicted (SEQ ID NO: 13).

[fig.3A-3E]FIGs. 3A to 3E are graphs respectively showing the binding properties of the aptamers 1 to 5 to VEGF165 in Example 1 of the present invention.

[fig.4A-4E]FIGs. 4A to 4E are graphs respectively showing the binding properties of the aptamers 1 to 5 to VEGF165 in Example 2 of the present invention.

[fig.5A-5E]FIGs. 5A to 5E are graphs respectively showing the binding properties of the aptamers 1 to 5 to VEGF165 in the presence of calcium ions in Example 2 of the present invention.

[fig.6A-6E]FIGs. 6A to 6E are graphs respectively showing the binding properties of the aptamers 1 to 5 to VEGF165 at 37°C in Example 3 of the present invention.

[fig.7A-7E]FIGs. 7A to 7E are graphs respectively showing the binding properties of

the aptamers 1 to 5 to VEGF165 at 37°C in the presence of calcium ions in Example 3 of the present invention.

[fig.8A-8E]FIGs. 8A to 8E are graphs respectively showing the binding properties of the aptamers 1 to 5 in Example 4 of the present invention.

[fig.9]FIG. 9 is a dose response curve for VEGF receptor phosphorylation as a function of VEGF concentration in Example 5 of the present invention.

[fig.10]FIG. 10 is a graph showing the phosphorylation of VEGF receptors in Example 5 of the present invention.

[fig.11]FIG. 11 is a graph showing the phosphorylation of VEGF receptors after treatment of cells with VEGF in the presence of aptamers in Example 5 of the present invention.

[fig.12]FIG. 12 is a graph showing the phosphorylation of VEGF receptors after treatment of cells with VEGF in the presence of aptamers in Example 5 of the present invention.

[fig.13]FIG. 13 is a schematic view showing an example of a target substance and an example of a non-target substance in the second example embodiment of the present invention.

Description of Embodiments

[0013] Hereinafter, the present invention will be described by way of example embodiments. It is to be noted, however, that the present invention is not limited to the following example embodiments. Unless otherwise stated, the descriptions regarding the respective example embodiments may be referenced to each other.

First Example Embodiment

[0014] (1) Nucleic acid molecule

The nucleic acid molecule of the present invention is, as described above, a nucleic acid molecule that binds to VEGF, including: any one of the following polynucleotides (a) and (b):

(a) a polynucleotide that consists of a base sequence of SEQ ID NO: 1 or a partial sequence of the base sequence of SEQ ID NO: 1; and

(b) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), binds to VEGF, and does not inhibit a function of the VEGF.

[0015] In the present invention, a vascular endothelial growth factor (VEGF) is a target. The binding ability of the nucleic acid molecule of the present invention to VEGF can be checked using a commercially available VEGF, for example. A specific example of the commercially available VEGF is a VEGF165 (PeproTech, Cat: 100-20). In the present invention, VEGF165 also may be referred to simply as “VEGF” hereinafter. The

VEGF is, for example, VEGF-A. The origin of VEGF is not particularly limited and is, for example, human.

[0016] The nucleic acid molecule of the present invention can bind to VEGF, as described above. In the present invention, the expression "binds to VEGF" (and grammatical variations thereof) also is referred to as "has binding properties to VEGF" or "has binding activity to VEGF", for example. The binding between the nucleic acid molecule of the present invention and VEGF can be determined by surface plasmon resonance (SPR) analysis or the like, for example. The analysis can be performed using a BIACORE 3000 (trade name, GE Healthcare UK Ltd.), for example. Since the nucleic acid molecule of the present invention binds to VEGF, it can be used for detection of VEGF, for example.

[0017] The dissociation constant, which indicates the binding force, of the nucleic acid molecule of the present invention to VEGF is, for example, 1.71×10^{-17}, 4.73×10^{-21}, 1.07×10^{-10}, 7.35×10^{-15}, or 2.45×10^{-17} mol/L in the absence of calcium ions and 1.43×10^{-16}, 3.66×10^{-17}, 2.17×10^{-21}, 3.32×10^{-16}, or 2.16×10^{-16} mol/L in the presence of calcium ions.

[0018] The nucleic acid molecule of the present invention also is referred to as a DNA molecule or a DNA aptamer. The nucleic acid molecule of the present invention may be a molecule consisting of or including the polynucleotide (a) or (b), for example.

[0019] The polynucleotide (a) may be, for example, a polynucleotide including or consisting of the base sequence of SEQ ID NO: 1 or a polynucleotide including or consisting of a partial sequence of the base sequence of SEQ ID NO: 1. The polynucleotide consisting of the base sequence of SEQ ID NO: 1 is shown below. In the base sequence of SEQ ID NO: 1 and base sequences of SEQ ID NOs: 2 to 4 and 7 to be described below, sequences corresponding to motif sequences (SEQ ID NOs: 5 and 6) to be described below are underlined.

[0020] VEGF746CaP_R8m1 (SEQ ID NO: 1)
GGATTGCCCGATTCACCGTAAACAGTTCTGGTTCCTTAGTTTAAAGTCAC
GTCTTAGTTTAAGGCATTCTGGAGCGGCATAAC

[0021] In sequence analysis performed after nucleic acid molecule selection according to the SELEX method, for example, the base sequence of SEQ ID NO: 1 is present at a frequency of 6.2% in all the sequences. In the sequence analysis, for example, when a predetermined sequence is present at a frequency of 5% or more in all the sequences, it can be determined that the sequence has been enriched sufficiently by the selection.

[0022] The partial sequence is not particularly limited, and examples thereof include the base sequences of SEQ ID NOs: 2 to 4.

[0023] VEGF746_CaP_5s31 (SEQ ID NO: 2)
CCTTAGTTTAAAGTCACGTCTTAGTTTAAGG

VEGF746_CaP_5s49 (SEQ ID NO: 3)
CCGTAAACAGTTCTGGTTCCTTAGTTTAAAGTCACGTCTTAGTTTAAGG
VEGF746_CaP_5s62 (SEQ ID NO: 4)
TTCACCGTAAACAGTTCTGGTTCCTTAGTTTAAAGTCACGTCTTAGTTTAAG
GCATTCTGGA

[0024] Regarding the polynucleotide (b), the "identity" is not particularly limited as long as it is in a range where the polynucleotide (b) binds to VFGF, for example. The identity is, for example, at least 80%, preferably at least 85%, more preferably at least 90%, still more preferably at least 95%, at least 96%, and at least 97%, particularly preferably at least 98%, and most preferably at least 99%. The identity can be calculated with analysis software such as BLAST or FASTA using default parameters, for example (the same applies hereinafter).

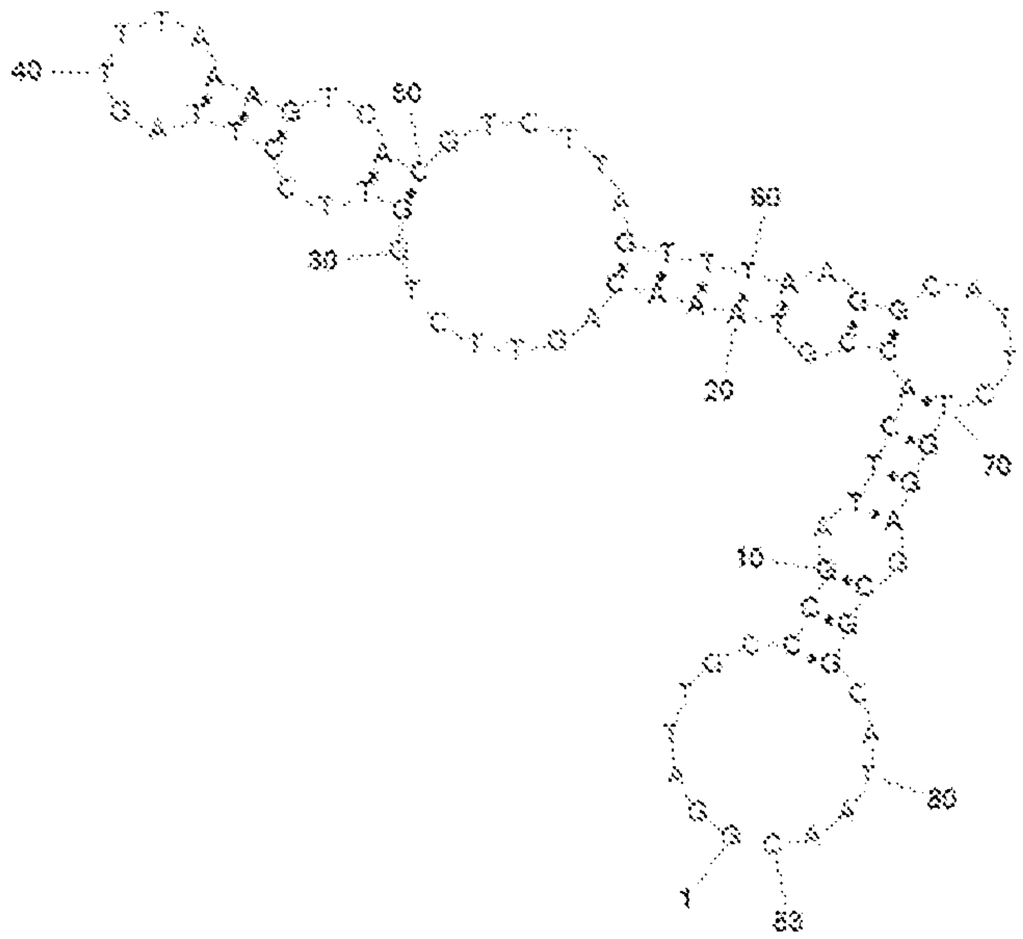
[0025] Regarding the polynucleotide (b), the "function of the VEGF" is, for example, a function of binding to a VEGF receptor. Examples of the VEGF receptor include VEGFR-1 (FLT-1) and VEGFR-2 (KDR/FLK-1). Also, the "function of the VEGF" is, for example, a function of regulating activity of the VEGF receptor by binding to the VEGF receptor, specifically, a function of activating the VEGF receptor and a function of phosphorylating the VEGF receptor.

[0026] The polynucleotide (b) may be the following polynucleotide (b1), for example. In this case, the nucleic acid molecule of the present invention may be a molecule consisting of or including the polynucleotide (b1), for example.

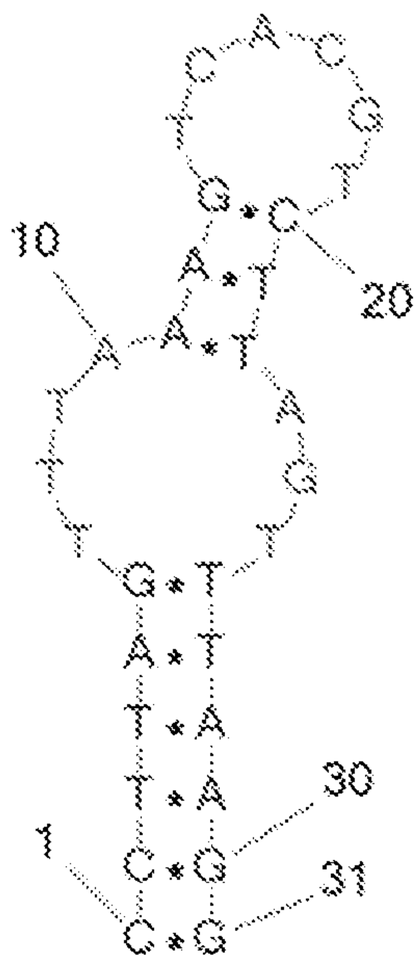
(b1) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), includes a base sequence of any one of SEQ ID NOs: 2 to 4, binds to the VEGF, and does not inhibit the function of the VEGF.

[0027] The polynucleotide in the nucleic acid molecule of the present invention may be the following polynucleotide (b2), for example. In this case, the nucleic acid molecule of the present invention may be a molecule consisting of or including the polynucleotide (b2), for example.

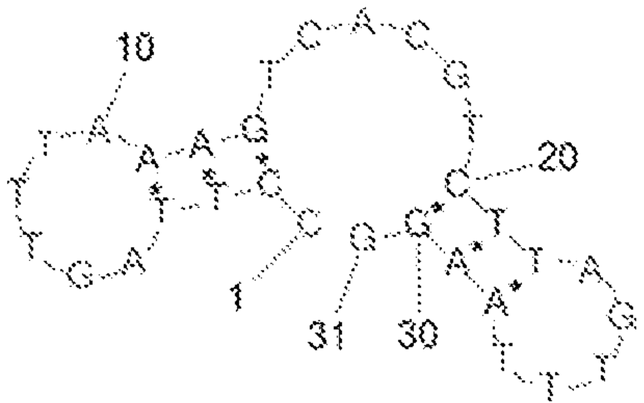
(b2) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), can form a secondary structure represented by any one of the following formulae (1) to (5), binds to the VEGF, and does not inhibit the function of the VEGF.



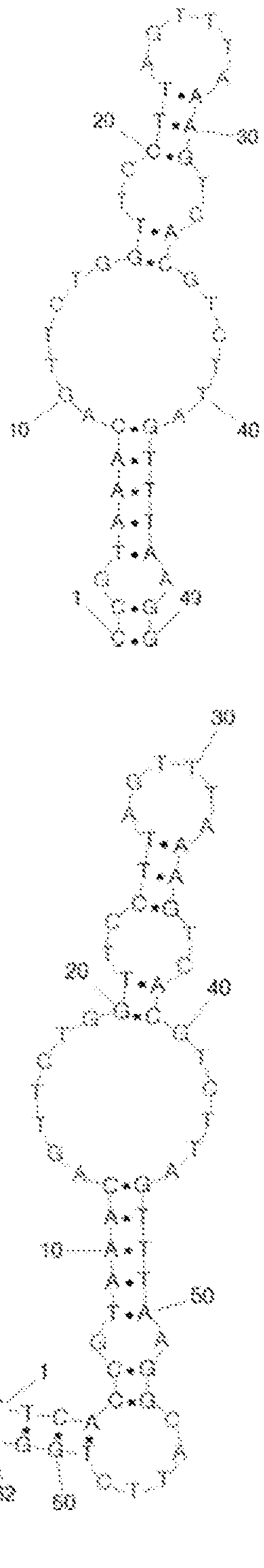
... (1)



... (2)



... (3)



... (4)

... (5)

[0028] Regarding the above formulae, the formula (1) corresponds to SEQ ID NO: 1, the formulae (2) and (3) correspond to SEQ ID NO: 2, the formula (4) corresponds to SEQ ID NO: 3, and the formula (5) corresponds to SEQ ID NO: 4. It is speculated that the secondary structures represented by the formulae (2) and (3) are in equilibrium. It is to be noted, however, that the present invention is not limited thereto.

[0029] Specifically, the polynucleotide (b2) is, for example: a polynucleotide that consists of

a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 1, can form the secondary structure represented by the formula (1), binds to the VEGF, and does not inhibit the function of the VEGF; a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 2, can form the secondary structure represented by the formula (2) or (3), binds to the VEGF, and does not inhibit the function of the VEGF; a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 3, can form the secondary structure represented by the formula (4), binds to the VEGF, and does not inhibit the function of the VEGF; or a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 4, can form the secondary structure represented by the formula (5), binds to the VEGF, and does not inhibit the function of the VEGF.

[0030] Specifically, the polynucleotide (b2) is, for example, a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 1, can form the secondary structure represented by any one of the formulae (2) to (5), binds to the VEGF, and does not inhibit the function of the VEGF.

[0031] Regarding the polynucleotide (b2), the expression "can form a secondary structure" means that, for example, the polynucleotide (b2) can form the stem structure and the loop structure represented by the above formulae. The stem structure and the loop structure will be described below. The "secondary structure" may be, for example, an actually formed secondary structure, or a secondary structure when simulation is performed by a known method.

[0032] The polynucleotide in the nucleic acid molecule of the present invention may be the following polynucleotide (b3), for example. In this case, the nucleic acid molecule of the present invention may be a molecule consisting of or including the polynucleotide (b3), for example.

(b3) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), includes a base sequence of at least one of SEQ ID NOs: 5 and 6, binds to the VEGF, and does not inhibit the function of the VEGF.

SEQ ID NO: 5

AGTTTAAAGT

SEQ ID NO: 6

AGTTTWAD

In the above sequence, W indicates adenine or thymine, and D indicates adenine, guanine, or thymine.

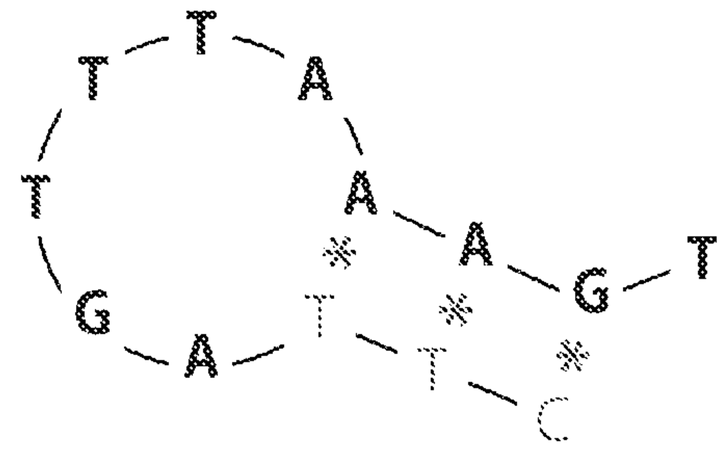
[0033] Specifically, the polynucleotide (b3) is, for example, a polynucleotide that consists of a base sequence having at least 80% identity to any one of the base sequences of SEQ

ID NOs: 1 to 4, includes a base sequence of at least one of SEQ ID NOs: 5 and 6, binds to the VEGF, and does not inhibit the function of the VEGF.

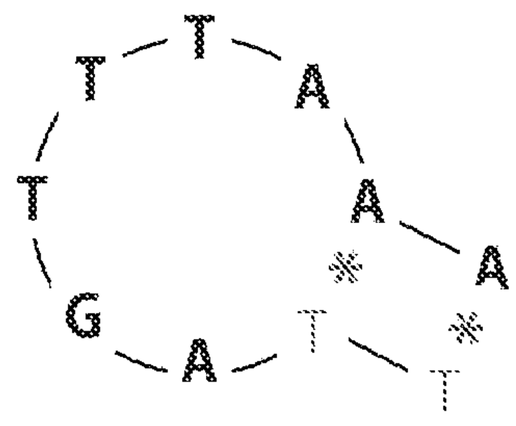
[0034] The base sequences of SEQ ID NOs: 5 and 6 are motif sequences in the base sequences of SEQ ID NOs: 1 to 4 and 7, for example. The motif sequences were determined by performing alignment analysis with respect to sequences present at a frequency of 5% or more in all the sequences in the sequence analysis performed after nucleic acid molecule selection according to the SELEX method.

[0035] The polynucleotide in the nucleic acid molecule of the present invention may be the following polynucleotide (b4), for example. In this case, the nucleic acid molecule of the present invention may be a molecule consisting of or including the polynucleotide (b4), for example.

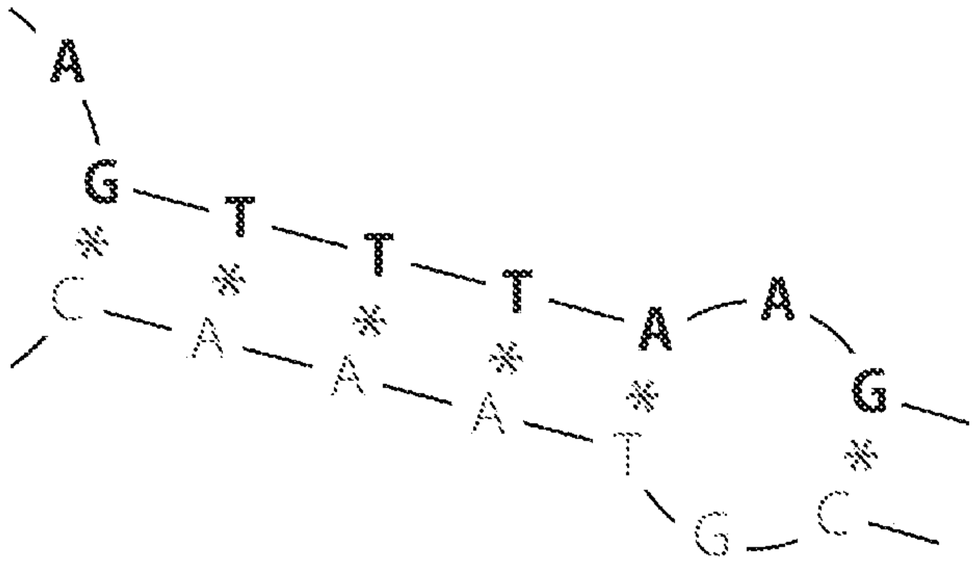
(b4) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), can form a secondary structure represented by at least one of the following formulae (6) to (8), binds to the VEGF, and does not inhibit the function of the VEGF.



...(6)



...(7)



...(8)

[0036] The formula (6) shows the secondary structure corresponding to the base sequence of SEQ ID NO: 5. The formulae (7) and (8) show the secondary structures corresponding to the base sequence of SEQ ID NO: 6. In the formulae (6) to (8), bases corresponding to SEQ ID NOs: 5 and 6 are indicated in boldface.

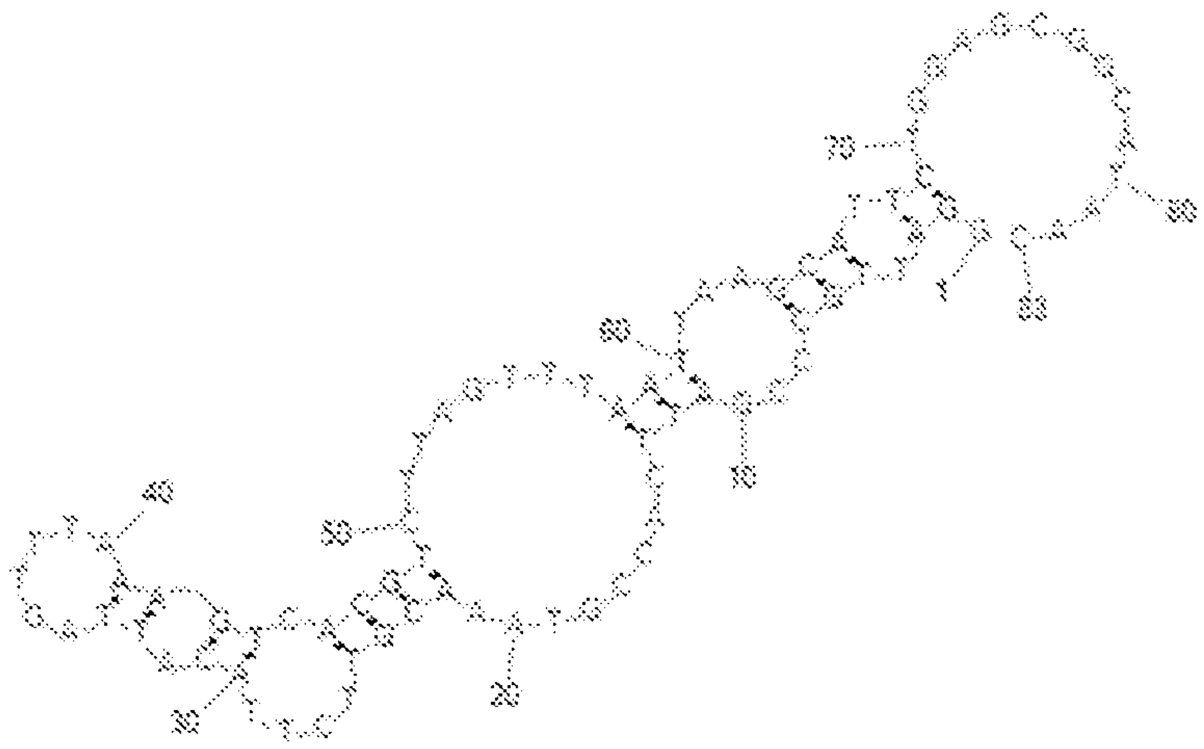
[0037] Specifically, the polynucleotide (b4) is, for example: a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 1, can form the secondary structure of the formula (6) or (7) and the secondary structure of the formula (8), binds to the VEGF, and does not inhibit the function of the VEGF; a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 2, can form the secondary structure of the formula (6) or (7), binds to the VEGF, and does not inhibit the function of the VEGF; a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 3, can form the secondary structure of the formula (6) or (7) and the secondary structure of the formula (8), binds to the VEGF, and does not inhibit the function of the VEGF; or a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of SEQ ID NO: 4, can form the secondary structure the formula (6) or (7) and the secondary structure of the formula (8), binds to the VEGF, and does not inhibit the function of the VEGF.

[0038] The polynucleotide (b) may be a polynucleotide consisting of the base sequence of SEQ ID NO: 7, for example. The base sequence of SEQ ID NO: 7 is a base sequence having 89.5% identity to the base sequence of SEQ ID NO: 1.

[0039] VEGF746CaP_R8m49 (SEQ ID NO: 7)

GGATTGCCCGATTCACCGTAAACGTTCTTACATTAGTTTAAAGTCACGTCT TAGTTTAATTAAGCATTCTGGAGCGGCATAAC

[0040] The base sequence of SEQ ID NO: 7 can form a secondary structure represented by the following formula (10). The base sequence of SEQ ID NO: 7 includes the base sequences of SEQ ID NOs: 5 and 6. The base sequence of SEQ ID NO: 7 can form a secondary structure represented by the formula (7).



... (10)

[0041] The polynucleotide in the nucleic acid molecule of the present invention may be the following polynucleotide (c), for example. In this case, the nucleic acid molecule of the present invention may be a molecule consisting of or including the polynucleotide (c), for example.

(c) a polynucleotide that consists of a base sequence complementary to a polynucleotide hybridizing to the base sequence of the polynucleotide (a) under stringent conditions, binds to VEGF, and does not inhibit a function of the VEGF.

[0042] Regarding the polynucleotide (c), the "polynucleotide hybridizing to" is not particularly limited, and is, for example, a polynucleotide perfectly or partially complementary to the base sequence of the polynucleotide (a). The hybridization can be detected by various types of hybridization assay, for example. The hybridization assay is not particularly limited. For example, it is possible to employ a method described in "Molecular Cloning: A Laboratory Manual 2nd Ed." edited by Sambrook et al. (Cold Spring Harbor Laboratory Press [1989]) or the like.

[0043] Regarding the polynucleotide (c), the "stringent conditions" may be any of low stringency conditions, moderate stringency conditions, and high stringency conditions, for example. The "low stringency conditions" are, for example, conditions where 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 50% formamide are used at 32°C. The "moderate stringency conditions" are, for example, conditions where 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 50% formamide are used at 42°C. The "high stringency conditions" are, for example, conditions where 5 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 50% formamide, are used at 50°C. Those skilled in the art can set the degree of stringency by, for example, setting the conditions such as the temperature, the salt concentration, the concentration and length of a probe, the ionic strength, the time, etc. as appropriate. As the "stringent conditions", it is also possible to employ conditions described in the above- described "Molecular Cloning: A

Laboratory Manual 2nd Ed." edited by Sambrook et al. (Cold Spring Harbor Laboratory Press [1989]) or the like, for example.

[0044] The polynucleotide in the nucleic acid molecule of the present invention may be the following polynucleotide (d), for example. In this case, the nucleic acid molecule of the present invention may be a molecule consisting of or including the polynucleotide (d), for example.

(d) a polynucleotide that consists of a base sequence obtained by deletion, substitution, insertion, and/or addition of one or more bases in the base sequence of the polynucleotide (a), binds to VEGF, and does not inhibit a function of the VEGF.

[0045] Regarding the polynucleotide (d), the phrase "one or more" is not limited as long as, for example, it is in a range where the polynucleotide (d) binds to VEGF. The "one or more" bases in the base sequence of the polynucleotide (a) are, for example, 1 to 10 bases, preferably 1 to 7 bases, more preferably 1 to 5 bases, still more preferably 1 to 3 bases, and particularly preferably 1 base or 2 bases. In the present invention, the numerical range regarding the number of bases, sequences, or the like discloses, for example, all the positive integers falling within that range. That is, for example, the description "one to five bases" discloses all of "one, two, three, four, and five bases" (the same applies hereinafter).

[0046] The nucleic acid molecule may include, for example, any one sequence selected from the polynucleotides (a) to (d), or a plurality of sequences selected from the polynucleotides (a) to (d). In the latter case, it is preferable that the plurality of polynucleotide sequences are linked to each other to form a single-stranded polynucleotide. The plurality of polynucleotide sequences may be linked to each other directly, or may be linked to each other indirectly with a linker, for example. It is preferable that the polynucleotide sequences are linked to each other directly or indirectly at their ends. The plurality of polynucleotide sequences may be the same or different from each other, for example. Preferably, they are the same. When the nucleic acid molecule of the present invention includes the plurality of polynucleotide sequences, the number of the sequences is not particularly limited, and is, for example, 2 or more, preferably 2 to 12, more preferably 2 to 6, and still more preferably 2.

[0047] The linker is a polynucleotide, for example. The building blocks of the linker are nucleotide residues, for example. Examples of the nucleotide residues include ribonucleotide residues and deoxyribonucleotide residues, for example. The length of the linker is not particularly limited, and is, for example, 1- to 24-mer, preferably 12- to 24-mer, more preferably 16- to 24-mer, and still more preferably 20- to 24-mer.

[0048] In the nucleic acid molecule of the present invention, the polynucleotide preferably is a single-stranded polynucleotide. It is preferable that the single-stranded polynucleotide can form a stem structure and a loop structure by self-annealing, for example.

It is preferable that the polynucleotide can form a stem-loop structure, an internal loop structure, and/or a bulge structure, for example.

[0049] The nucleic acid molecule may be a double strand, for example. When the nucleic acid molecule is a double strand, for example, one of single-stranded polynucleotides is any of the polynucleotides (a) to (d), and the other single-stranded polynucleotide is not limited. The other single-stranded polynucleotide may be, for example, a polynucleotide consisting of a base sequence complementary to any of the polynucleotides (a) to (d). When the nucleic acid molecule of the present invention is a double strand, it is preferable to dissociate the double strand to single-stranded polynucleotides by denaturation or the like before use, for example. Also, it is preferable that the dissociated single-stranded polynucleotide, which is any of the polynucleotides (a) to (d), is forming a stem structure and a loop structure as described above, for example.

[0050] In the present invention, the expression "can form a stem structure and a loop structure" encompasses that, for example, a stem structure and a loop structure are formed actually, and also, even if a stem structure and a loop structure are not formed, they can be formed depending on conditions. The expression "can form a stem structure and a loop structure" encompasses, for example, both the cases where the formation thereof has been confirmed through an experiment and where the formation thereof is predicted through simulation using a computer or the like.

[0051] The length of the nucleic acid molecule is not particularly limited. The lower limit of the length is, for example, 15-mer, 75-mer, or 80-mer, and the upper limit of the length is, for example, 1000-mer, 200-mer, 100-mer, or 90-mer.

[0052] The building blocks of the nucleic acid molecule are nucleotide residues, for example. Examples of the nucleotide residues include deoxyribonucleotide residues and ribonucleotide residues. Examples of the nucleic acid molecule of the present invention include DNA consisting of deoxyribonucleotide residues only and DNA including one or more ribonucleotide residues. In the latter case, the phrase "one or more" is not particularly limited. For example, the number of the ribonucleotide residues in the polynucleotide is, for example, 1 to 3.

[0053] The polynucleotide may include a modified base(s). The modified base is not particularly limited, and may be, for example, a modified natural base (non-artificial base), which preferably has a similar function to the natural base. The natural base is not particularly limited, and may be, for example, a purine base with a purine skeleton, a pyrimidine base with a pyrimidine skeleton, or the like. The purine base is not particularly limited, and examples thereof include adenine (a) and guanine (g). The pyrimidine base is not particularly limited, and examples thereof include cytosine (c), thymine (t), and uracil (u). The modified site in the base is not particularly limited. When the base is a purine base, the modified site in the purine base is the 7-position or

the 8-position in the purine skeleton, for example. When the base is a pyrimidine base, the modified site in the pyrimidine base is the 5-position or the 6-position in the pyrimidine skeleton, for example. When the pyrimidine skeleton has "=O" bound to the carbon at the 4-position and a group that is not "-CH_3" or "-H" bound to the carbon at the 5-position, the modified base can be referred to as modified uracil or modified thymine.

[0054] The nucleic acid molecule of the present invention may include, for example, one or more artificial nucleic acid monomer residues. The phrase "one or more" is not particularly limited, and may be, for example, 1 to 80, preferably 1 to 70, more preferably 1 to 50, still more preferably 1 to 40, particularly preferably 1 to 30, and most preferably 1 to 21 in the polynucleotide. The artificial nucleic acid monomer residue is, for example, a peptide nucleic acid (PNA), a locked nucleic acid (LNA), or a 2'-O,4'-C-ethylenebridged nucleic acid (ENA). The nucleic acid in the monomer residue is the same as described above, for example. The number of the artificial nucleic acid monomer residues in the full length of the nucleic acid molecule including the polynucleotide also is not particularly limited, and is, for example, the same as those described above.

[0055] It is preferable that the nucleic acid molecule of the present invention is resistant to nuclease, for example. In order to allow the nucleic acid molecule to have nuclease resistance, it is preferable that the nucleic acid molecule of the present invention includes the modified nucleotide residue(s) and/or the artificial nucleic acid monomer residue(s), for example. Also, in order to allow the nucleic acid molecule to have nuclease resistance, the nucleic acid molecule of the present invention may have polyethylene glycol (PEG) of several tens of kDa, deoxythymidine, or the like bound to the 5' end or the 3' end thereof, for example.

[0056] The nucleic acid molecule of the present invention may further include an additional sequence, for example. Preferably, the additional sequence is bound to at least one of the 5' end and the 3' end, more preferably to the 3' end of the nucleic acid molecule, for example. The additional sequence is not particularly limited. The length of the additional sequence is not particularly limited, and is, for example, 1- to 200-mer, preferably 1- to 50-mer, more preferably 1- to 25-mer, and still more preferably 18- to 24-mer. The building blocks of the additional sequence are, for example, nucleotide residues, examples of which include deoxyribonucleotide residues and ribonucleotide residues. The additional sequence is not particularly limited, and examples thereof include polynucleotides such as DNA consisting of deoxyribonucleotide residues and DNA including a ribonucleotide residue(s). Specific examples of the additional sequence include poly(dT) and poly(dA).

[0057] The nucleic acid molecule of the present invention can be used in the state where it is

immobilized on a carrier, for example. For example, either the 5' end or the 3' end of the nucleic acid molecule of the present invention may be immobilized. When the nucleic acid molecule of the present invention is immobilized, the nucleic acid molecule may be immobilized either directly or indirectly to the carrier, for example. In the latter case, it is preferable to immobilize the nucleic acid molecule via the additional sequence, for example. The carrier may be beads, a plate, a filter, a column, a substrate, or a container, for example.

[0058] The nucleic acid molecule of the present invention may further include a labeling substance, for example. Specifically, the nucleic acid molecule may have the labeling substance bound thereto. The nucleic acid molecule having the labeling substance bound thereto also can be referred to as a nucleic acid sensor of the present invention, for example. The labeling substance may be bound to at least one of the 5' end and the 3' end of the nucleic acid molecule, for example. Labelling with the labeling substance may be achieved through binding or chemical modification, for example. The labeling substance is not particularly limited, and is, for example, an enzyme, a fluorescent substance, a dye, an isotope, a drug, a toxin, or an antibiotic. Examples of the enzyme include luciferase and SA-Lucia luciferase. Examples of the fluorescent substance include fluorophores such as pyrene, TAMRA, fluorescein, a Cy3 dye, a Cy5 dye, a FAM dye, a rhodamine dye, a Texas Red dye, JOE, MAX, HEX, and TYE. Examples of the dye include Alexa dyes such as Alexa 488 and Alexa 647. The labeling substance may be linked to the nucleic acid molecule directly, or indirectly via a linker, for example. The linker is not particularly limited, and may be a polynucleotide, for example.

[0059] The method for producing the nucleic acid molecule of the present invention is not particularly limited. For example, the nucleic acid molecule of the present invention can be synthesized by known methods such as: nucleic acid synthesis utilizing chemical synthesis methods; and genetic engineering procedures. The nucleic acid molecule of the present invention can be produced by, for example, a method for producing a nucleic acid molecule according to the present invention to be described below.

[0060] The nucleic acid molecule of the present invention exhibits binding properties to VEGF, as described above. Thus, use of the nucleic acid molecule of the present invention is not particularly limited, as long as it is the use utilizing the binding properties of the nucleic acid molecule to VEGF. The nucleic acid molecule of the present invention can be used in various methods as a substitute for, e.g., an antibody against VEGF.

[0061] The nucleic acid molecule of the present invention can detect VEGF. The method for detecting VEGF is not particularly limited, and VEGF can be detected by detecting the

binding between the VEGF and the nucleic acid molecule.

[0062] (2) Detection reagent and kit

A detection reagent of the present invention is a reagent for detecting VEGF, characterized in that it contains the nucleic acid molecule of the present invention. It is only necessary that the detection reagent of the present invention contains the nucleic acid molecule of the present invention, and other configurations are not limited by any means. With the use of the detection reagent of the present invention, it is possible to detect VEGF as described above, for example. The detection reagent of the present invention also can be referred to as a binding agent to VEGF, for example.

[0063] The detection reagent of the present invention may further contain a labeling substance, and the labeling substance may be bound to the nucleic acid molecule, for example. For example, the description regarding the labeling substance provided above in connection with the nucleic acid molecule of the present invention also applies to the labeling substance in the detection reagent. Also, the detection reagent of the present invention may further contain a carrier, and the nucleic acid molecule may be immobilized on the carrier, for example. For example, the description regarding the carrier provided above in connection with the nucleic acid molecule of the present invention also applies to the carrier in the detection reagent.

[0064] A detection kit of the present invention includes the nucleic acid molecule of the present invention or the detection reagent of the present invention. The detection kit of the present invention may further include a component(s) in addition to the nucleic acid molecule or the detection reagent of the present invention, for example. Examples of the component include a buffer solution for preparing a sample and instructions for use. In the case where a detection method of the present invention to be described below is a detection method carried out using a nucleic acid sensor obtained by binding luciferase as a labeling substance to the nucleic acid molecule and a labeled carrier, the detection kit of the present invention may include the nucleic acid sensor and a VEGF labeled carrier, for example.

[0065] As to the detection reagent and the detection kit of the present invention, reference can be made to the above description regarding the nucleic acid molecule of the present invention, for example. Also, as to the method for using the detection reagent and the detection kit of the present invention, reference can be made to the above description regarding the nucleic acid molecule of the present invention and the following description regarding the detection method of the present invention.

[0066] (3) Detection method

A method for detecting VEGF according to the present invention is a method for detecting VEGF, including the steps of: bringing the nucleic acid molecule of the present invention or the detection reagent of the present invention into contact with a

sample to form a complex of VEGF in the sample and the nucleic acid molecule or the detection reagent; and detecting the complex. The detection method of the present invention is characterized in that the nucleic acid molecule or the detection reagent according to the present invention is used therein, and other steps, conditions, or the like are not particularly limited. The detection method of the present invention will be described below with reference to an example where the nucleic acid molecule of the present invention is used in the detection method. It should be noted, however, that, in the following example, the nucleic acid molecule of the present invention is interchangeable with the detection reagent of the present invention.

[0067] The nucleic acid molecule of the present invention specifically binds to VEGF. Thus, according to the present invention, VEGF in a sample can be detected specifically by detecting the binding between the VEGF and the nucleic acid molecule or the detection reagent, for example. Specifically, for example, since the amount of the VEGF in the sample can be analyzed, qualitative analysis or quantitative analysis also is possible.

[0068] In the present invention, the sample is not particularly limited. The sample is a biological sample, for example. Specific examples of the biological sample include plasma, serum, lymph fluid, whole blood, and tissue culture solutions.

[0069] The sample may be a liquid sample or a solid sample, for example. The sample preferably is a liquid sample from the viewpoint of ease of handling, because the liquid sample can be brought into contact with the nucleic acid molecule more easily, for example. In the case of the solid sample, a mixed solution, a liquid extract, a solution, or the like of the solid sample prepared using a solvent may be used, for example. The solvent is not particularly limited, and may be water, physiological saline, or a buffer solution, for example.

[0070] In the above-described complex formation step, the method for causing the contact between the sample and the nucleic acid molecule is not particularly limited. The contact between the sample and the nucleic acid molecule preferably is achieved in a liquid, for example. The liquid is not particularly limited, and may be, for example, water, physiological saline, or a buffer solution.

[0071] In the complex formation step, the conditions under which the contact between the sample and the nucleic acid molecule is caused are not particularly limited. The contact temperature is, for example, 4°C to 37°C, preferably 18°C to 25°C. The contact time is, for example, 10 to 120 minutes, preferably 30 to 60 minutes.

[0072] In the complex formation step, the nucleic acid molecule may be an immobilized nucleic acid molecule immobilized on a carrier (solid-phase carrier) or an unimmobilized nucleic acid molecule in a free state, for example. In the latter case, for example, the nucleic acid molecule is brought into contact with the sample in a container. In the former case, the carrier is not particularly limited, and may be a plate,

a filter, a column, a substrate, beads, or a container, for example. The container may be a microplate or a tube, for example. The immobilization of the nucleic acid molecule is as described above, for example. In the complex formation step, the sample may be immobilized on a carrier, for example.

[0073] The detection step is the step of detecting the binding between the VEGF in the sample and the nucleic acid molecule, as described above. By detecting the presence or absence of the binding between the VEGF and the nucleic acid molecule, it is possible to analyze the presence or absence of the VEGF in the sample (qualitative analysis), for example. Also, by detecting the degree of the binding (the amount of the binding) between the VEGF and the nucleic acid molecule, it is possible to analyze the amount of the VEGF in the sample (quantitative analysis), for example.

[0074] The method for detecting the binding between the VEGF and the nucleic acid molecule is not particularly limited. The method may be a conventionally known method for detecting the binding between substances, for example. Specific examples of the method include the above-described SPR.

[0075] If binding between VEGF and the nucleic acid molecule is not detected, it is likely that VEGF is not present in the sample. When the binding is detected, it can be determined that the VEGF is present in the sample. Also, by determining the correlation between the concentrations of VEGF and the amounts of the binding beforehand, it becomes possible to analyze the concentration of the VEGF in the sample from the measured amount of the binding on the basis of the correlation.

[0076] As an example of the method for detecting the binding between VEGF and the nucleic acid molecule, a detection method carried out using a nucleic acid sensor obtained by binding luciferase as a labeling substance to the nucleic acid molecule and a VEGF labeled carrier will be described below.

[0077] First, the nucleic acid sensor is mixed with the sample. When VEGF is present in the sample, the nucleic acid molecule in the nucleic acid sensor binds to the VEGF as a target. On the other hand, when the VEGF is not presence in the sample, the nucleic acid molecule in the nucleic acid sensor remains in a state of being unbound to the target.

[0078] Subsequently, the mixture is brought into contact with the VEGF labeled carrier, and then, the VEGF labeled carrier is removed. The carrier may be beads, for example. When the nucleic acid sensor is bound to the VEGF in the mixture, the nucleic acid molecule in the nucleic acid sensor cannot bind to VEGF on the VEGF labeled carrier. Thus, when a substrate for luciferase is added to a fraction from which the VEGF labeled carrier has been removed so as to cause a luminescent reaction, light emission is caused by the catalytic reaction of the luciferase in the nucleic acid sensor. On the other hand, when the nucleic acid sensor in the mixture is in a state of being unbound

to VEGF, the nucleic acid molecule in the nucleic acid sensor binds to the VEGF on the VEGF labeled carrier. Accordingly, when the VEGF labeled carrier is removed, the nucleic acid sensor bound to the VEGF labeled carrier is removed together with the VEGF labeled carrier. Thus, when a substrate for luciferase is added to a fraction from which the VEGF labeled carrier has been removed so as to cause a luminescent reaction, light emission caused by the catalytic reaction of the luciferase does not occur because the nucleic acid sensor is not present. Therefore, on the basis of the presence or absence of light emission, it is possible to analyze the presence or absence of the VEGF in the sample (qualitative analysis). Further, the amount of the VEGF in the sample correlates with the amount of the nucleic acid sensor remaining in the fraction after the VEGF labeled carrier has been removed. Therefore, on the basis of the intensity of the light emission, it is possible to analyze the amount of the VEGF in the sample (quantitative analysis).

Second Example Embodiment

[0079] The method for producing the nucleic acid molecule according to the present invention is, as described above, a method for producing a nucleic acid molecule that binds to a first biological molecule and does not inhibit a function of the first biological molecule, including the steps of:

(A) bringing a candidate nucleic acid molecule into contact with the first biological molecule to select a nucleic acid molecule that has bound to the first biological molecule as a first selected nucleic acid molecule; and

(B) selecting the first selected nucleic acid molecule as an intended nucleic acid molecule.

[0080] The production method of the present invention, for example, further includes, after the step (A), the step of:

(C) bringing a second biological molecule into contact with a complex of the first selected nucleic acid molecule and the first biological molecule to detect a function of the second biological molecule, wherein

after the step (C), in the step (B), the first selected nucleic acid molecule of the complex with which the function of the second biological molecule is detected is selected as an intended nucleic acid molecule.

[0081] In the production method of the present invention, the function of the first biological molecule is, for example, a function of binding to the second biological molecule. The function of the first biological molecule is, for example, a function of regulating activity of the second biological molecule by binding to the second biological molecule. The function of regulating activity of the second biological molecule may be, for example, a function of activating or deactivating the second biological molecule. The activation may be, for example, phosphorylation.

[0082] In the production method of the present invention, for example, the first biological molecule is a growth factor and the second biological molecule is a receptor to which the growth factor binds. In this case, the production method of the present invention is a method for producing a nucleic acid molecule that binds to a growth factor and does not inhibit a function of the growth factor, including the steps of:

(A) bringing a candidate nucleic acid molecule into contact with the growth factor to select a nucleic acid molecule that has bound to the growth factor as a first selected nucleic acid molecule; and

(B) selecting the first selected nucleic acid molecule as an intended nucleic acid molecule.

[0083] In this case, the production method of the present invention, for example, further includes, after the step (A), the step of:

(C) bringing a receptor to which the growth factor binds into contact with a complex of the first selected nucleic acid molecule and the growth factor to detect activation of the receptor, wherein

after the step (C), in the step (B), the first selected nucleic acid molecule of the complex with which the activation of the receptor is detected is selected as an intended nucleic acid molecule.

[0084] The production method of the present invention will be described below with reference to an example where the first biological molecule is a growth factor and the second biological molecule is a receptor to which the growth factor binds. It is to be noted, however, that the present invention is not limited to this illustrative example. The combination of the first biological molecule and the second biological molecule is, for example, a combination of a protein and a receptor for the protein. In this case, the combination may be, for example, the combination of proteins in any protein complex. Examples of the combination include the combination of a cytokine and a cytokine receptor and the combination of an enzyme and a receptor for the enzyme (substrate).

[0085] In the present invention, the growth factor is a vascular endothelial growth factor (VEGF), cytokine, or a heparin-binding growth factor, for example. In the method for producing the nucleic acid molecule according to the present invention, a commercially available VEGF can be used as VEGF to be brought into contact with a candidate nucleic acid molecule, for example. A specific example of the commercially available VEGF is VEGF165 (PeproTech, Cat: 100-20). In the present invention, VEGF165 also may be referred to simply as “VEGF” hereinafter.

[0086] The step (A) is, for example, the step of bringing a candidate nucleic acid molecule into contact with the growth factor to select a nucleic acid molecule that has bound to the growth factor as a first selected nucleic acid molecule.

[0087] In the present invention, the expression “binds to a growth factor” (and grammatical

variations thereof) also is referred to as “having binding properties to a growth factor” or “binding activity to a growth factor”, for example.

[0088] The candidate nucleic acid molecule is not particularly limited. For example, a library containing nucleic acids with random sequences can be used.

[0089] In the step (A), the method for causing the contact between the candidate nucleic acid molecule and the growth factor is not particularly limited. The contact between the candidate nucleic acid molecule and the growth factor preferably is achieved in a liquid, for example. The liquid is not particularly limited, and may be, for example, water, physiological saline, or a buffer solution. The buffer solution may be a SB1T buffer, for example.

[0090] In the step (A), the conditions under which the contact between the candidate nucleic acid molecule and the growth factor is caused are not particularly limited. The contact temperature is, for example, 4°C to 37°C, preferably 18°C to 25°C. The contact time is, for example, 10 to 120 minutes, preferably 30 to 60 minutes.

[0091] In the contact between the candidate nucleic acid molecule and the growth factor, the candidate nucleic acid molecule may be an immobilized nucleic acid molecule immobilized on a carrier (solid-phase carrier) or an unimmobilized nucleic acid molecule in a free state, for example. In the latter case, for example, the nucleic acid molecule is brought into contact with the growth factor in a container. In the former case, the carrier is not particularly limited, and may be a plate, a filter, a column, a substrate, beads, or a container, for example. The container may be a microplate or a tube, for example. The immobilization of the candidate nucleic acid molecule is as described above, for example. In the contact between the candidate nucleic acid molecule and the growth factor, the first biological molecule, the second biological molecule, the complex, and a target substance and non-target substance to be described below may be immobilized on a carrier, for example.

[0092] In the step (A), the method for detecting the binding between the candidate nucleic acid molecule and the growth factor is not particularly limited. The method may be a conventionally known method for detecting the binding between substances, for example. Specifically, the binding between the candidate nucleic acid molecule and the growth factor can be determined by surface plasmon resonance (SPR) analysis, for example. In the analysis, a BIACORE 3000 (trade name, GE Healthcare UK Ltd.) can be used, for example.

[0093] Then, for example, when the binding between the candidate nucleic acid molecule and the growth factor is not detected, it can be determined that the candidate nucleic acid molecule does not bind to the growth factor, and when the binding is detected, it can be determined that the candidate nucleic acid molecule binds to the growth factor.

[0094] In the step (A), a nucleic acid molecule that exhibit a dissociation constant against

the growth factor (which indicates the binding force to the growth factor) of not more than a few nM can be selected as the first selected nucleic acid molecule, for example.

[0095] In the step (A), for example, the candidate nucleic acid molecules may be brought into contact with the growth factor, which is a target substance, and a non-target substance, and among the candidate nucleic acid molecules, a candidate nucleic acid molecule that has bound to the target substance and has not bound to the non-target substance may be selected as the first selected nucleic acid molecules.

[0096] In this case, the non-target substance may be, for example, a receptor to which the growth factor binds. The non-target substance may be, for example, a polypeptide including a sequence corresponding to a binding site of the amino acid sequence of a receptor to which the growth factor binds with the growth factor. Thus, the nucleic acid molecule that has not bound to the receptor can be selected as a first selected nucleic acid molecule.

[0097] Also, the non-target substance may be, for example, a polypeptide including a sequence corresponding to a binding site of the amino acid sequence of the growth factor with the receptor for the growth factor. Thus, when the growth factor and the nucleic acid molecule are bound, the nucleic acid molecule that does not inhibit the function of the bound growth factor of further binding to the receptor can be selected as the first selected nucleic acid molecule.

[0098] When the target substance is an intended growth factor, the non-target substance may be a growth factor other than the intended growth factor, for example. Thus, the nucleic acid molecule that binds to an intended growth factor and does not bind to a growth factor other than the intended growth factor can be selected as the first selected nucleic acid molecule.

[0099] As shown in FIG. 13, the target substance may be a polypeptide that includes a sequence (S_R) corresponding to a binding site of an amino acid sequence of the growth factor with the receptor for the growth factor and also includes a freely-selected nucleic acid binding sequence (S_A) that is different from the sequence corresponding to the binding site with the receptor for the growth factor and the non-target substance may be a polypeptide that includes the sequence (S_R) and does not include the nucleic acid binding sequence (S_A). For example, the non-target substance may be a polypeptide that includes a sequence in common with the target substance except that it does not include the sequence (S_A). In the target substance and the non-target substance, the position and the order of the sequence (S_R) and the sequence (S_A) are not particularly limited. The target substance and the non-target substance may be, for example, proteins in a splicing variant relationship with each other. In this case, the sequence (S_A) may be, for example, an amino acid sequence encoded by a freely-selected exon. The sequence (S_A) may be, for example, a sequence that includes a

heparin binding domain (HBD) to be described below. Thus, the nucleic acid molecule that binds to the sequence (S_A) and does not bind to the sequence (S_R) in an intended growth factor can be selected as the first selected nucleic acid molecule.

[0100] When the intended growth factor is, for example, a growth factor having a sequence including HBD to be described below, the growth factor other than the intended growth factor is, for example, a growth factor having a sequence not including HBD. The growth factor having a sequence including HBD is, for example, VEGF165, and the growth factor having a sequence not including HBD is, for example, VEGF121 to be described below.

[0101] When the target substance is, for example, VEGF165, the non-target substance is, for example, a receptor to which the VEGF165 binds. Examples of the receptor to which the VEGF165 binds include VEGFR-1 (FLT-1) and VEGFR-2 (KDR/FLK-1). Examples of a polypeptide including a sequence corresponding to a binding site of the amino acid sequence of VEGFR-1 with VEGF165 include a second Ig-like domain (aa:129-229) represented by the following SEQ ID NO: 8. Examples of a polypeptide including a sequence corresponding to a binding site of the amino acid sequence of VEGFR-2 with VEGF165 include a second Ig-like domain (aa:141-207) represented by the following SEQ ID NO: 9 and a third Ig-like domain (aa:224-320) represented by the following SEQ ID NO: 10. Examples of a polypeptide including a sequence corresponding to a binding site of the amino acid sequence of VEGF165 with VEGFR-1 include exon 3 represented by the following SEQ ID NO: 11. Examples of a polypeptide including a sequence corresponding to a binding site of the amino acid sequence of VEGF165 with VEGFR-2 include exon 4 represented by the following SEQ ID NO: 12.

[0102] Second Ig-like domain (SEQ ID NO: 8)

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRI IWDSRK GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTI

Second Ig-like domain (SEQ ID NO: 9)

NKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMIS YAGMVFCEAKINDE

Third Ig-like domain (SEQ ID NO: 10)

DVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRD LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNST

Exon 3 (SEQ ID NO: 11)

VKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLE CVPTEESNITMQ

Exon 4 (SEQ ID NO: 12)

IMRIKPHQGQHIGEMSFLQHNKCECR

[0103] The step (C) is, for example, the step of bringing the receptor to which the growth factor binds into contact with a complex of the first selected nucleic acid molecule and the growth factor to detect the activation of the receptor.

[0104] In the step (C), for example, a commercially available VEGF receptor can be used as the receptor to be brought into contact with the complex of the first selected nucleic acid molecule and the VEGF. A specific example of the commercially available VEGF receptor is a VEGF receptor (R&D systems, Cat. No: 357-KD-050/CF). The VEGF is, for example, VEGF-A. The origin of VEGF is not particularly limited and is, for example, human.

[0105] In the step (C), the method for causing the contact between the complex of the first selected nucleic acid molecule and the growth factor and the receptor to which the growth factor binds or the conditions under which the contact between them is caused are not particularly limited, for example, and they are the same as the contact method and the contact conditions for the candidate nucleic acid molecule and the growth factor in the step (A).

[0106] In the step (C), the method for detecting the activation of the receptor to which the growth factor binds is not particularly limited. The detection can be performed by, for example, measuring the phosphorylation of the receptor to which the growth factor binds. Specifically, the detection can be performed by ELISA or Western blotting, for example.

[0107] Then, for example, when activation of the receptor to which the growth factor binds is not detected, it can be determined that the complex does not activate the receptor, and when the activation is detected, it can be determined that the complex activates the receptor.

[0108] In the step (C), activation of the receptor to which the growth factor binds may be detected indirectly by, for example, detecting the binding between the complex of the first selected nucleic acid molecule and the growth factor and the receptor to which the growth factor binds.

[0109] The method for detecting the binding is not particularly limited. For example, the detection can be performed by SPR, capillary electrophoresis, or Native-PAGE.

[0110] The above description uses the growth factor VEGF to illustrate the method. It will be recognized by a person skilled in the art that the method can be applied to other proteins. Additional non-limiting examples include:

o Other growth factors with a heparin binding domain such as

o Long (heparin-binding) and short (non-heparin binging) isoforms of PDGF-A generated by alternative splicing (UNIPROT accession numbers P04085-1 and P04085-2), and

o Four splice variants of PlGF containing alternative and truncated forms of heparin-

binding domains (UNIPROT accession numbers P49763-1, P49763-2, P49763-3, P49763-4);

o Other cytokines with a heparin binding domain such as

o Interferon gamma (IFN gamma) containing unstructured C-terminal heparin-binding domain (UNIPROT accession number P01579-1),

o Human TNF-alpha (heparin-binding) and its N-terminally truncated version lacking ability to bind heparin (Kenig, M. et al. 2008. Journal of Chromatography B, 867, pp. 119-125), and

o IL-12, where heparin-binding is attributed to an independently folding C-terminal domain of the p40 subunit that can be removed by molecular cloning (Garnier, P. et al. 2018. Cytokine 110, pp 159-168);

o Growth factors engineered to include a heparin binding domain such as

o a recombinant construct including a short isoform of PDGF-A and a C-terminal HBD from IFN gamma;

o Cytokines engineered to include a heparin binding domain such as

o a recombinant construct including IL-1 and a HBD from a long isoform PDGF-A, and

o a recombinant construct including IL-2 and a HBD from a cell surface Hep/HS-interacting protein (Liu, S., 1997. Proc. Natl. Acad. Sci. USA 94, pp. 1739-1744);

o Other secreted proteins with a heparin binding domain such as

o Heparin/Heparan Sulfate (HP/HS) Interacting Protein (Liu et al. 1997. The Journal of Biological Chemistry 272, pp. 25856-25862; Liu, S., 1997. Proc. Natl. Acad. Sci. USA 94, pp. 1739-1744);

o Other secreted proteins with an engineered heparin binding domain;

o Proteins (including growth factors, cytokines, and secreted proteins) that include a domain that is separately targetable from the domain having the desired biological function, such as

o Four isoforms of human IGF-1 generated by alternative splicing that contain distinct variants of the C-terminal E-peptide that can be proteolytically removed without loss of receptor binding (UNIPROT accession numbers P05019-1, P05019-2, P05019-3, P05019-4), and

o Four isoforms of human FGF-2 generated by alternative initiation and bearing distinct N-terminal domains that do not interfere with receptor binding (UNIPROT accession numbers P09038-1, P09038-2, P09038-3, P09038-4); and

o Proteins (including growth factors, cytokines, and secreted proteins) that are engineered to include a domain (e.g. albumin, FC SUMO, GSO, or other domains that are well known to those skilled in the art) that is separately targetable from the domain having the desired biological function.

[0111] The step (B) is the step of selecting the first selected nucleic acid molecule as an intended nucleic acid molecule.

[0112] According to the method for producing a nucleic acid molecule according to the present invention, it is possible to obtain a nucleic acid molecule that binds to a first biological molecule and does not inhibit the function of the first biological molecule.

Third Example Embodiment

[0113] (1) Biomaterial

The biomaterial according to the present invention includes, as described above, a binding nucleic acid molecule; and a carrier, the binding nucleic acid molecule being bound to the carrier, wherein the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein.

[0114] The carrier is, for example, a polymer. Examples of the polymer include gels and particles. An example of a polymer carrier is a poly(ethylene glycol)-based gel created with a photo-initiated chemistry. Specifically, a monomer solution is prepared in an aqueous buffer containing 10% wt/vol 4600MW poly(ethylene glycol)-diacrylate (PEGDA), 0.05% photoinitiator Irgacure-2959 (I2959), and a binding nucleic acid that has been functionalized with a free thiol or acrylate moiety. Polymerization of the gel is accomplished by shining a 100WHg short-arc lamp (Omnicure (Registered trademark) 1000, EXFO, Mississaugua, Ont., Canada) with the manufacturer-supplied filter for 365 nm exposure on the sample for 10 minutes. During the polymerization reaction, the binding nucleic acid will become covalently incorporated into the polymer. In this system, the presence of ester bonds within each PEGDA monomer makes the resulting polymer susceptible to hydrolytic degradation. However, the rate of degradation would be generally considered too slow for tissue engineering applications. Thus, for the sake of this disclosure, PEGDA hydrogels are considered non-degradable.

The polymer carrier can also be formulated to be hydrolytically degradable. An example of a hydrolytically degradable polymer carrier is a caprolatone-based gel created with a photo-initiated chemistry. Specifically, a monomer solution is prepared in an aqueous buffer containing 6% wt/vol methacrylated hyaluronic acid (MeHA) and methacrylated caprolactone HA (MeCLHA) at a 1:1 ratio, 0.05% photoinitiator Irgacure-2959 (I2959), and a binding nucleic acid that has been functionalized with a free thiol or acrylate moiety. Polymerization of the gel is accomplished by shining a 100WHg short-arc lamp (Omnicure (Registered trademark) 1000, EXFO, Mississaugua, Ont., Canada) with the manufacturer-supplied filter for 365 nm exposure on the sample for 10 minutes. In this system, the presence of caprolactone linkages makes the polymer system susceptible to hydrolytic degradation at a rate that is significantly faster than the PEGDA-based polymers described in the previous section.

The polymer carrier can also be formulated to be enzymatically degradable. An example of an enzymatically degradable polymer carrier is a poly(ethylene glycol)-based gel created with thiol-ene photo-initiated chemistry. Specifically, a monomer solution is prepared in an aqueous buffer containing 6% wt/vol 20K 4-arm poly(ethylene glycol)-norbornene (PEG-NB), 6mM di-cysteine enzymatically degradable crosslinker peptide (KCGPQGIAGQCK; SEQ ID NO: 14), 0.01% photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), and a binding nucleic acid that has been functionalized with a free thiol or norbornene moiety. In this system, cysteine-containing peptides, 4-arm PEG-NB monomers, and the functionalized binding nucleic acid are mixed in a 1:1 stoichiometric ratio with respect to "thiol" and "ene" functional groups. In the presence of the LAP photoinitiator, and upon exposure 385nm light, the thiol group is added to the norbornene, forming a norbornene-thioether linkage. Polymerization of the hydrogels is accomplished by shining a handheld LED flashlight emitting 385nm light over the solution for 1 minute. In this system, crosslinking peptides require the presences of at least two cystienes (R-groups contain "thiol" moieties), allowing them to covalently react with more than one multi-armed "ene"-functionalized PEG. A 4-arm PEG-NB is reacted with a dicysteine peptide crosslinker that is designed to be susceptible to enzymatic degradation by naturally occurring extracellular matrix remodeling enzymes. Specifically, a collagen-based sequence, KCGPQG*IAGQCK (SEQ ID NO: 14), is used for its known susceptibility to cleavage by matrix metaloproteinases (MMPs), where the "*" denotes the site of enzymatic cleavage.

The polymer carrier systems described in this document are provided as examples only and are chosen to illustrate how specific compositional elements can be incorporated into the polymer matrix to impart specific properties to the material carrier. In these examples, distinct degradation properties of the material carrier would be achieved. It is understood that carriers for a binding nucleic acid can be designed to incorporate other mechanisms of degradation, alone or in combination, to create a wide range of materials with distinct degradation profiles.

Materials containing substrates for cellular adhesion and migration can be used to create nucleic acid carriers that also provide a scaffolding function for cells and tissues. The incorporation of polypeptides (e.g. CRGD), full-length proteins (e.g. fibrinogen, collagen, laminin, etc.), or protein fragments thereof, that present binding sites for cell attachment can be used in combination with a binding nucleic acid to create material carriers with additional functionality for biological applications.

Molecular components used to create carriers for a binding nucleic acid are not limited to poly(ethylene glycol)-diacrylate (PEGDA), methacrylated hyaluronic acid (MeHA), methacrylated caprolactone HA (MeCLHA), and poly(ethylene glycol)-norbornene

(PEG-NB) as presented in the examples. Those skilled in the art would understand that a plurality of molecular components can be employed to create material carriers for binding nucleic acids.

Chemistries and reactable groups used to create carriers for a binding nucleic acid are not limited to the photo-initiated chemistries presented in the examples. Those skilled in the art would understand that a plurality of reaction mechanisms utilizing a plurality of reactable chemical functional groups can be employed to create material carriers for binding nucleic acids.

[0115] The method for binding the binding nucleic acid molecule to the carrier is not particularly limited and can be performed by a known method. The binding nucleic acid molecule can be caused to bind to the carrier via an additional sequence.

[0116] The binding nucleic acid molecule is not particularly limited as long as it binds to a predetermined protein and does not inhibit a function of the predetermined protein. The function of the predetermined protein is, for example, a function of binding to a receptor for the protein. Also, the function of the predetermined protein is, for example, a function of regulating activity of the receptor by binding to the receptor. The function of regulating activity of the receptor may be, for example, a function of activating or deactivating the receptor. The activation may be, for example, phosphorylation. The nucleic acid molecule produced by the method for producing a nucleic acid molecule according to the present invention can be used as the binding nucleic acid molecule, for example.

[0117] The predetermined protein is, for example, a growth factor. The growth factor may be, for example, VEGF. When the growth factor is VEGF, the nucleic acid molecule that binds to VEGF of the present invention can be used as the binding nucleic acid molecule, for example.

[0118] The binding nucleic acid molecule may be contained in the biomaterial as a conjugate bound to the predetermined protein or in a state of being unbound to the predetermined protein, for example. In the latter case, for example, by disposing the biomaterial in a living organism, the binding nucleic acid molecule can bind to the predetermined protein in the living organism.

[0119] According to the biomaterial of the present invention, for example, by bringing the biomaterial of the present invention into contact with a living organism, it is possible to exhibit the function of the predetermined protein bound to the binding nucleic acid molecule on the living organism. Examples of the living organism include humans and non-human animals, and examples of the non-human animal include mammals such as monkey, dog, rabbit, cow, horse and the like. The contact is not particularly limited. For example, the biomaterial may be disposed in the living organism or may be taken by the living organism. The site of the living organism in which the biomaterial is

disposed may be any site where it is required to exhibit the function of the predetermined protein, and is not particularly limited, and is, for example, the skin.

[0120] The usage form of the biomaterial of the present invention will be described below in detail. As a first example, the biomaterial of the present invention is a biological scaffold, and the biological scaffold includes the binding nucleic acid molecule of the present invention bound to VEGF and a biodegradable gel bound to the binding nucleic acid molecule. When the biological scaffold is disposed at a wound site of the living organism, the VEGF bound to the binding nucleic acid molecule binds to the VEGF receptor at the wound site to activate the VEGF receptor. This can, for example, promote healing of the wound site.

[0121] As a second example, the biomaterial of the present invention is an oral medicine, and the oral medicine includes the binding nucleic acid molecule bound to the predetermined protein and biodegradable particles bound to the binding nucleic acid molecule. When the oral medicine is taken by the living organism, the biodegradable particles are decomposed in the living organism to release the predetermined protein bound to the binding nucleic acid molecule, thereby exhibiting the function of the predetermined protein. Thus, for example, the timing or the like of exhibiting the function of the predetermined protein in the living organism can be regulated.

[0122] As described above, according to the biomaterial of the present invention, for example, the function of the predetermined protein can be exhibited on the living organism at a freely-selected position and at a freely-selected timing.

[0123] The biomaterial of the present invention can be used, for example, in a treatment method of a living organism. The treatment method of a living organism includes, for example, a step of bringing the biomaterial of the present invention into contact with a living organism. The contact is, for example, as described above.

[0124] (2) Production method for biomaterial

The method for producing a biomaterial of the present invention includes the step of binding a binding nucleic acid molecule to a carrier, wherein the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein. The carrier, the binding nucleic acid molecule, and the predetermined protein are, for example, as described above.

[0125] The step of binding the binding nucleic acid molecule to the carrier may include, for example, the steps of adding an additional sequence to the carrier and binding the binding nucleic acid molecule to the additional sequence. These steps can be performed, for example, using a known method. Thereby, the binding nucleic acid molecule can caused to bind to the carrier via an additional sequence.

[0126] The method for producing a biomaterial of the present invention may further include, for example, the step of binding the predetermined protein to the binding nucleic acid

molecule. The step of binding the predetermined protein to the binding nucleic acid molecule is not particularly limited, and the binding nucleic acid molecule can be brought into contact with the predetermined protein by a known method.

Examples

[0127] Next, examples of the present invention will be described. It is to be noted, however, that the present invention is not limited by the following examples by any means. Commercially available reagents in the examples were used in accordance with their protocols, unless otherwise stated.

Example 1

[0128] The present example examined the binding properties of aptamers of the present invention to VEGF by SPR analysis.

[0129] (1) Aptamers

As aptamers of Example 1, aptamers 1 to 5 consisting of the following polynucleotides were synthesized. The aptamers 2 to 4 were aptamers obtained by truncating the aptamer 1. The aptamer 5 is a polynucleotide consisting of a base sequence having 89.5% identity to the base sequence of the aptamer 1.

[0130] Aptamer 1: VEGF746CaP_R8m1 (SEQ ID NO: 1)
GGATTGCCCGATTCACCGTAAACAGTTCTGGTTCCTTAGTTTAAAGTCAC
GTCTTAGTTTAAGGCATTCTGGAGCGGCATAAC

Aptamer 2: VEGF746CaP_5s31 (SEQ ID NO: 2)
CCTTAGTTTAAAGTCACGTCTTAGTTTAAGG

Aptamer 3: VEGF746CaP_5s49 (SEQ ID NO: 3)
CCGTAAACAGTTCTGGTTCCTTAGTTTAAAGTCACGTCTTAGTTTAAGG

Aptamer 4: VEGF746CaP_5s62 (SEQ ID NO: 4)
TTCACCGTAAACAGTTCTGGTTCCTTAGTTTAAAGTCACGTCTTAGTTTAA
GGCATTCTGGA

Aptamer 5: VEGF746CaP_R8m49 (SEQ ID NO: 7)
GGATTGCCCGATTCACCGTAAACGTTCTTACATTAGTTTAAAGTCACGTCT
TAGTTTAATTAAGCATTCTGGAGCGGCATAAC

[0131] The predicted secondary structures of the aptamers 1 to 5 are shown in FIGs. 1A to 1E, respectively. It is to be noted, however, that the predicted secondary structures are not limited thereto.

[0132] To the 3' end of each of the aptamers, 20-mer polydeoxyadenine (poly(dA)) was added. The thus-obtained poly(dA)-added aptamers were used in SPR to be described below.

[0133] (2) Sample

A VEGF sample was prepared using VEGF165 in the following manner. As shown

in FIG. 2, VEGF165 does not contain the amino acids encoded by exon 6, but does contain the amino acids encoded by exon 7 (the HBD), represented by the following SEQ ID NO: 13.

HBD (SEQ ID NO: 13)

ARQENPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRCDKP RR

[0134] VEGF165 (PeproTech, Cat: 100-20) was dissolved at a concentration of 1 mg/mL in distilled water that had been sterilized. The resultant solution was used as a VEGF sample. In the analysis of the binding properties etc. to be described below, an SB1T buffer was used to dilute the VEGF sample. The composition of the SB1T buffer was as follows: 40 mmol/L HEPES, 125 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, and 0.05% Tween (Registered trademark) 20. The pH of the SB1T buffer was 7.5.

[0135] As control 1, a sample was prepared in the same manner, except that VEGF121 (PeproTech, Cat: 100-20A) was used instead of the VEGF165. As shown in FIG. 2, the amino acid sequence of VEGF121 corresponds to the amino acid sequence of VEGF in which the nucleotides encoding exon 6 and exon 7 are deleted. Further, as control 2, a sample was prepared in the same manner, except that BSA (Sigma, Cat. No:#A7906) was used instead of the VEGF165.

[0136] (3) Analysis of binding properties by SPR

The analysis of the binding properties was carried out using a ProteOn XPR36 (BioRad) in accordance with its instructions for use.

[0137] First, as a sensor chip designed specifically for the ProteOn, a streptavidin-immobilized chip (trade name: ProteOn NLC Sensor Chip, BioRad) was set in the ProteOn XPR36. 5 mmol/L biotinylated poly(dT) was injected to a flow cell of the sensor chip using ultrapure water (DDW), and the binding was allowed to proceed until the signal intensity (RU: Resonance Unit) was saturated. The biotinylated poly(dT) was prepared by biotinylating the 5' end of 20-mer deoxythymidine. Then, each of the poly(dA)-added aptamers (200 nmol/L) was injected to the flow cell of the chip using an SB1T buffer at a flow rate of 25 mL/min for 80 seconds, and the binding was allowed to proceed until the signal intensity was saturated. Subsequently, each of the samples (5 nmol/L) was injected using an SB1T buffer at a flow rate of 50 mL/min for 300 seconds, followed by washing performed by flowing the SB1T buffer under the same conditions. With the time at which the injection was started being 0 seconds, the signal intensity after the sample injection was measured. The SPR was carried out at 25°C.

[0138] The results obtained are shown in FIGs. 3A to 3E. FIGs. 3A to 3E are graphs respectively showing the binding properties of the aptamers 1 to 5 to 5 nmol/L VEGF165. FIG. 3A shows the result obtained regarding the aptamer 1

(VEGF746CaP_R8m1). FIG. 3B shows the result obtained regarding the aptamer 2 (VEGF746CaP_5s31). FIG. 3C shows the result obtained regarding the aptamer 3 (VEGF746CaP_5s49). FIG. 3D shows the result obtained regarding the aptamer 4 (VEGF746CaP_5s62). FIG. 3E shows the result obtained regarding the aptamer 5 (VEGF746CaP_R8m49). The horizontal axis indicates the time (sec) elapsed after the start of the sample injection, and the vertical axis indicates the signal intensity (RU). As can be seen in FIGs. 3A to 3E, the aptamers 1 to 5 exhibited binding properties to the VEGF165. In contrast, in control 1 (VEGF121) and control 2 (BSA), the aptamers 1 to 5 all exhibited a signal intensity of 0 or less, and they all exhibited no binding properties. These results demonstrate that the aptamers 1 to 5 bind to VEGF165 with high specificity, and the binding can be detected by measuring the signal intensity.

[0139] As described above, VEGF165 includes exon 7 (HBD) in the amino acid sequence of VEGF, whereas HBD is deleted in VEGF121. Accordingly, it is considered that the aptamers 1 to 5 bind to exon 7 (HBD) in the amino acid sequence of VEGF. Also, it is considered that the aptamers 1 to 5 can bind not only to VEGF165 but also to, for example, proteins, cytokines, and growth factors having the amino acid sequence corresponding to the HBD. It is to be noted, however, that these considerations are based on speculation, and the present invention is not limited or restricted by this speculation.

Example 2

[0140] The present example examined the binding properties of the aptamers of the present invention to VEGF in the absence of calcium ions and in the presence of calcium ions.

[0141] First, the binding properties of the aptamers to VEGF in the absence of calcium ions were analyzed using the VEGF sample in the same manner as in Example 1, except that the concentration of the VEGF165 in the sample was set to: 0.8, 1.0, 1.2, 1.6, or 2 nmol/L for the aptamer 1; 0.1, 0.2, 0.4, or 0.6 nmol/L for the aptamers 2, 3, and 4; and 0.2, 0.4, 0.6, and 0.8 nmol/L for the aptamer 5.

[0142] The results obtained are shown in FIGs. 4A to 4E. FIGs. 4A to 4E are graphs respectively showing the binding properties of the aptamers 1 to 5 to the VEGF165 in the absence of calcium ions. FIG. 4A shows the result obtained regarding the aptamer 1 (VEGF746CaP_R8m1). FIG. 4B shows the result obtained regarding the aptamer 2 (VEGF746CaP_5s31). FIG. 4C shows the result obtained regarding the aptamer 3 (VEGF746CaP_5s49). FIG. 4D shows the result obtained regarding the aptamer 4 (VEGF746CaP_5s62). FIG. 4E shows the result obtained regarding the aptamer 5 (VEGF746CaP_R8m49). The horizontal axis indicates the time (sec) elapsed after the start of the sample injection, and the vertical axis indicates the signal intensity (RU). As can be seen in FIGs. 4A to 4E, the aptamers 1 to 5 all exhibited binding properties to the VEGF165. Further, the signal intensities exhibited by the aptamers 1 to 5

increased as the concentration of the VEGF165 became higher. These results demonstrate that the concentration of VEGF165 in a sample can be analyzed quantitatively by measuring the signal intensity using the aptamers 1 to 5 of the present invention.

[0143] Further, from the results of the SPR analysis shown in FIGs. 4A to 4E, the kinetic parameter was calculated. As a result, it was found that the dissociation constants (KD) of the aptamers 1 to 5 against the VEGF165 in the absence of calcium ions were 1.71×10^{-17}, 4.73×10^{-21}, 1.07×10^{-10}, 7.35×10^{-15}, and 2.45×10^{-17} mol/L, respectively, and the aptamers 1 to 5 all exhibited excellent binding properties. Also, it was found that the aptamer 2, which is the truncated aptamer of the aptamer 1, exhibited a higher binding force to the VEGF165 than the aptamer 1.

[0144] Next, the binding properties of the aptamers to VEGF in the presence of calcium ions were analyzed using the VEGF sample in the same manner as in Example 1, except that the concentration of the VEGF165 in the sample was set to 0.1, 0.2, 0.4, or 0.6 nmol/L for the aptamers 1 to 4 and 0.2, 0.4, 0.6, and 0.8 nmol/L for the aptamer 5 and an SB1T buffer (Ca^{2+}) was used instead of the SB1T buffer. The composition of the SB1T buffer (Ca^{2+}) was as follows: 40 mmol/L HEPES, 125 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, and 0.05% Tween (Registered trademark) 20. The pH of the SB1T buffer (Ca^{2+}) was 7.5.

[0145] The results obtained are shown in FIGs. 5A to 5E. FIGs. 5A to 5E are graphs respectively showing the binding properties of the aptamers 1 to 5 to the VEGF165 in the presence of calcium ions. FIG. 5A shows the result obtained regarding the aptamer 1 (VEGF746CaP_R8m1). FIG. 5B shows the result obtained regarding the aptamer 2 (VEGF746CaP_5s31). FIG. 5C shows the result obtained regarding the aptamer 3 (VEGF746CaP_5s49). FIG. 5D shows the result obtained regarding the aptamer 4 (VEGF746CaP_5s62). FIG. 5E shows the result obtained regarding the aptamer 5 (VEGF746CaP_R8m49). The horizontal axis indicates the time (sec) elapsed after the start of the sample injection, and the vertical axis indicates the signal intensity (RU). As can be seen in FIGs. 5A to 5E, the aptamers 1 to 5 all exhibited binding properties to the VEGF165 in the presence of calcium ions. Further, the signal intensities exhibited by the aptamers 1 to 5 increased as the concentration of the VEGF165 became higher. These results demonstrate that the aptamers 1 to 5 all bind to VEGF165 also in the presence of calcium ions, for example.

[0146] Further, from the results of the SPR analysis shown in FIGs. 5A to 5E, the kinetic parameter was calculated. As a result, it was found that the dissociation constants (KD) of the aptamers 1 to 5 against the VEGF165 in the presence of calcium ions were 6.02×10^{-17}, 3.66×10^{-17}, 2.17×10^{-21}, 3.32×10^{-16}, and 2.16×10^{-16} mol/L, respectively, and the aptamers 1 to 5 all exhibited excellent binding properties.

Example 3

[0147] The present example examined the binding properties of the aptamers of the present invention to VEGF at 37°C in the absence of calcium ions and in the presence of calcium ions.

[0148] First, the binding properties of the aptamers were analyzed using the VEGF sample in the same manner as in Example 1, except that the concentration of the VEGF165 in the sample was set to 1 nmol/L and the SPR was performed at 37°C.

[0149] The results obtained are shown in FIGs. 6A to 6E. FIGs. 6A to 6E are graphs respectively showing the binding properties of the aptamers 1 to 5 to the VEGF165 in the absence of calcium ions at 37°C. FIG. 6A shows the result obtained regarding the aptamer 1 (VEGF746CaP_R8m1). FIG. 6B shows the result obtained regarding the aptamer 2 (VEGF746CaP_5s31). FIG. 6C shows the result obtained regarding the aptamer 3 (VEGF746CaP_5s49). FIG. 6D shows the result obtained regarding the aptamer 4 (VEGF746CaP_5s62). FIG. 6E shows the result obtained regarding the aptamer 5 (VEGF746CaP_R8m49). The horizontal axis indicates the time (sec) elapsed after the start of the sample injection, and the vertical axis indicates the signal intensity (RU). As can be seen in FIGs. 6A to 6E, the aptamers 1 to 5 all exhibited binding properties to the VEGF165 in the absence of calcium ions at 37°C.

[0150] Next, the binding properties of the aptamers were analyzed using the VEGF sample in the same manner as in Example 2, except that the concentration of the VEGF165 in the sample was set to 1 nmol/L and the SPR was performed at 37°C.

[0151] The results obtained are shown in FIGs. 7A to 7E. FIGs. 7A to 7E are graphs respectively showing the binding properties of the aptamers 1 to 5 to the VEGF165 in the presence of calcium ions at 37°C. FIG. 7A shows the result obtained regarding the aptamer 1 (VEGF746CaP_R8m1). FIG. 7B shows the result obtained regarding the aptamer 2 (VEGF746CaP_5s31). FIG. 7C shows the result obtained regarding the aptamer 3 (VEGF746CaP_5s49). FIG. 7D shows the result obtained regarding the aptamer 4 (VEGF746CaP_5s62). FIG. 7E shows the result obtained regarding the aptamer 5 (VEGF746CaP_R8m49). The horizontal axis indicates the time (sec) elapsed after the start of the sample injection, and the vertical axis indicates the signal intensity (RU). As can be seen in FIGs. 7A to 7E, the aptamers 1 to 5 all exhibited binding properties to the VEGF165 in the presence of calcium ions at 37°C.

[0152] The above results demonstrate that the aptamers 1 to 5 all can bind to VEGF165 when they are used in living organisms, for example.

Example 4

[0153] The present example examined through SPR analysis whether VEGFs bound to the aptamers of the present invention further can bind to VEGF receptors.

[0154] The aptamers 1 to 5 were used as aptamers, and the VEGF sample and a VEGF receptor sample were used as samples. The VEGF receptor sample was prepared in the same manner as the VEGF sample, except that a VEGF receptor (R&D systems, Cat. No: 357-KD-050/CF) was used instead of the VEGF.

[0155] The analysis of binding properties by SPR was carried out in the following manner. First, biotinylated poly(dT) was bound to the sensor chip and each of the poly(dA)-added aptamers was then bound to the chip in the same manner as in Example 1. Subsequently, the 5 nmol/L VEGF sample was injected using an SB1T buffer at a flow rate of 50 μL/min for 240 seconds (first injection), followed by washing performed by flowing the SB1T buffer under the same conditions. Thereafter, the 5 nmol/L VEGF receptor sample was injected using an SB1T buffer at a flow rate of 50 μL/min for 240 seconds (second injection), followed by washing performed by flowing the SB1T buffer under the same conditions. With the time at which the first injection was started being 0 seconds, the signal intensity after the sample injection was measured. The SPR was carried out at 25°C. Also, analysis of binding properties by SPR was carried out in the same manner, except that the first and second injections were performed using the combinations shown in Table 1 below instead of the combination of the VEGF sample and the VEGF receptor sample.

[Table 1]

	First injection	Second injection
Example	5 nM VEGF	5 nM VEGF receptor
Control 1	5 nM VEGF	SB1T buffer
Control 2	5 nM VEGF receptor	SB1T buffer
Control 3	SB1T buffer	5 nM VEGF receptor

[0156] The results obtained are shown in FIGs. 8A to 8E. FIGs. 8A to 8E are graphs respectively showing the binding properties of the aptamers 1 to 5 to the samples. FIG. 8A shows the result obtained regarding the aptamer 1 (VEGF746CaP_R8m1). FIG. 8B shows the result obtained regarding the aptamer 2 (VEGF746CaP_5s31). FIG. 8C shows the result obtained regarding the aptamer 3 (VEGF746CaP_5s49). FIG. 8D shows the result obtained regarding the aptamer 4 (VEGF746CaP_5s62). FIG. 8E shows the result obtained regarding the aptamer 5 (VEGF746CaP_R8m49). The horizontal axis indicates the time (sec) elapsed after the start of the first injection, and the vertical axis indicates the signal intensity (RU). As can be seen in FIGs. 8A to 8E, the aptamers 1 to 5 exhibited increase in signal intensity when the VEGF sample was injected in the first injection. That is, the aptamers 1 to 5 exhibited binding properties to the VEGF165. Further, the aptamers 1 to 5 also exhibited increase in signal intensity

when the VEGF receptor sample was injected in the second injection. That is, the VEGF165 bound to the aptamers 1 to 5 exhibited binding properties to the VEGF receptors.

[0157] In contrast, in the case where the injections were performed using the combination of control 1 shown in Table 1, the aptamers 1 to 5 exhibited increase in signal intensity when the VEGF sample was injected in the first injection. However, when the SB1T buffer was injected in the second injection, increase in signal intensity was not observed. That is, the VEGF165 bound to the aptamers 1 to 5 did not exhibit binding properties to the SB1T buffer.

[0158] In the case where the injections were performed using the combination of control 2 shown in Table 1, the aptamers 1 to 5 did not exhibit increase in signal intensity when the VEGF receptor sample was injected in the first injection. That is, the aptamers 1 to 5 did not exhibit binding properties to VEGF receptors. Further, the aptamers 1 to 5 also did not exhibit increase in signal intensity when the SB1T buffer was injected in the second injection.

[0159] When the injections were performed using the combination of control 3 shown in Table 1, the aptamers 1 to 5 did not exhibit increase in signal intensity when the SB1T buffer was injected in the first injection. That is, the aptamers 1 to 5 did not exhibit binding properties to the SB1T buffer. Further, the aptamers 1 to 5 also did not exhibit increase in signal intensity when the VEGF receptor sample was injected in the second injection.

[0160] The above results demonstrate that VEGFs bound to the aptamers 1 to 5 further bind to VEGF receptors.

Example 5

[0161] The present example examined whether VEGFs bound to the aptamers of the present invention can phosphorylate VEGF receptors.

[0162] Phosphorylation of VEGF receptors was measured using the Phospho-VEGF R2/KDR (Catalog No.: DYC1766, R&D systems) according to the manufacturer's protocol. Antibodies and a substrate solution to be described below were those included in the kit. 24 hours prior to the start of the experiment, a medium (ATCC (Registered trademark), PCS-100-040) containing microvascular endothelial cell growth supplements (ATCC (Registered trademark), PCS-100-030) for HUVEC cells (ATCC (Registered trademark), PCS-100-010) was replaced with a serum-free medium. The VEGF165 (0.01, 0.04, 0.12, 0.37, 1.11, 3.33, 10, or 30 nmol/L) was added to the medium containing the HUVEC cells. The cells were treated with the VEGF165 for 5 minutes. Thereafter the cells were washed with PBS. The amount of phosphorylated VEGF receptor was measured using the Phospho-VEGFR2/KDR

according to the manufacturer's protocol.

[0163] The results obtained are shown in FIG. 9. FIG. 9 is a dose response curve for VEGF receptor phosphorylation as a function of VEGF165 concentration. The horizontal axis indicates the concentration of the VEGF165, and the vertical axis indicates the proportion of phosphorylated VEGF receptors in the total amount of the VEGF receptors (the value obtained by dividing the measured value of the phosphorylated VEGF receptors by the measured value of the total amount of the VEGF receptors). As can be seen in FIG. 9, when the concentration of the VEGF165 was 10 nmol/L or lower, the proportion of the phosphorylated VEGF receptors increased as the concentration of the VEGF165 became higher. On the other hand, when the concentration of the VEGF165 was higher than 10 nmol/L, the proportion of the phosphorylated VEGF receptors decreased as the concentration of the VEGF165 became higher.

[0164] Next, phosphorylation of VEGF receptors was measured in the same manner using the VEGF165 sample, except that the concentration of the VEGF165 in the sample was set to 0, 0.5, 1, or 3 nmol/L. Further, phosphorylation of VEGF receptors was measured in the same manner, except that 1 nmol/L of the aptamer 2 was used in addition to the VEGF165, and the sample was prepared by mixing the VEGF165 sample and the aptamer 2 for 30 minutes. As a control, phosphorylation of VEGF receptors was measured in the same manner, except that the sample was prepared using 1 nmol/L of Pat224 (VEGF aptamer, disclosed in U.S. Patent No. 5,811,533) and 1 nmol/L of Macugen (VEGF receptor inhibitor, Bausch+Lomb).

[0165] The results obtained are shown in FIG. 10. FIG. 10 is a graph showing the phosphorylation of the VEGF receptors by the VEGF165. The horizontal axis indicates the concentration of the VEGF165, and the vertical axis indicates the proportion of phosphorylated VEGF receptors in the total amount of the VEGF receptors. As can be seen in FIG. 10, the proportion of the phosphorylated VEGF receptors increased as the concentration of the VEGF165 became higher. The proportion of the phosphorylated VEGF receptors in the sample containing the aptamer 2 (Cap5s31) was roughly equivalent to that in the sample containing the VEGF165 only. This revealed that the aptamer 2 does not inhibit phosphorylation of VEGF receptors by VEGF165. In contrast, in the sample containing the Macugen, the proportion of the phosphorylated VEGF receptors was much lower than those in the sample containing the VEGF165 only over the entire concentration range of the VEGF165. That is, Macugen, which is a VEGF receptor inhibitor, inhibited the phosphorylation of the VEGF receptors by the VEGF165. Further, in the sample containing the Pat224, the proportion of the phosphorylated VEGF receptors was much lower than that in the sample containing the VEGF165 only, when the concentration of VEGF165 was 1, or 0.5 nmol/L. That is, Pat224, which is the VEGF aptamer, inhibited phosphorylation of the VEGF receptors

by the VEGF165 when the concentration of the VEGF165 was 1 nmol/L or lower.

[0166] Next, the phosphorylation of VEGF receptors was measured using the VEGF165 sample in the same manner, except that: the concentration of the VEGF165 in the sample was set to 1 nmol/L; in addition to the VEGF165, the aptamers 1 and 2 were used as aptamers; and the concentration of each of the aptamers in the sample was set to 7.81, 15.63, 31.25, 62.5, 125, 250, 500, or 1000 nmol/L.

[0167] The results obtained are shown in FIG. 11. FIG. 11 is a graph showing the phosphorylation of the VEGF receptors after the treatment of the cells with the VEGF165 in the presence of the respective aptamers. The horizontal axis indicates the concentration of each aptamer, and the vertical axis indicates the proportion of phosphorylated VEGF receptors in the total amount of the VEGF receptors.

[0168] As indicated in FIG. 11, the proportion of the phosphorylated VEGF receptors was 3.78 in the sample containing the VEGF165 only and 1.16 in the VEGF165-free sample. In the sample containing the aptamer 1, the proportion of the phosphorylated VEGF receptors was roughly equivalent to or higher than that in the sample containing the VEGF165 only over the entire concentration range of the aptamer 1. In the sample containing the aptamer 2, the proportion of the phosphorylated VEGF receptors was roughly equivalent to or higher than that in the sample containing the VEGF165 only over the entire concentration range of the aptamer 2.

[0169] Phosphorylation of VEGF receptors was measured using the VEGF165 sample in the same manner, except that the aptamers 2 and 3 were used as aptamers and the concentration of each of the aptamers in the sample was set to 0.01, 0.06, 0.32, 1.6, 8, 40, 200, or 1000 nmol/L.

[0170] The results obtained are shown in FIG. 12. FIG. 12 is a graph showing the phosphorylation of the VEGF receptors after the treatment of the cells with the VEGF165 in the presence of the respective aptamers. The horizontal axis indicates the concentration of each aptamer, and the vertical axis indicates the proportion of phosphorylated VEGF receptors in the total amount of the VEGF receptors.

[0171] As indicated in FIG. 12, the proportion of the phosphorylated VEGF receptors was 3.07 in the sample containing the VEGF165 only and 1.65 in the VEGF165-free sample. In the sample containing the aptamer 2, the proportion of the phosphorylated VEGF receptors was roughly equivalent to or higher than that in the sample containing the VEGF165 only over the entire concentration range of the aptamer 2. In the sample containing the aptamer 3, the proportion of the phosphorylated VEGF receptors was roughly equivalent to or higher than that in the sample containing the VEGF165 only over the entire concentration range of the aptamer 3.

[0172] The above results demonstrate that VEGF165 can phosphorylate VEGF receptors on cell surfaces under conditions where all VEGF165 is expected to be bound by the

aptamers 1, 2, and 3 respectively.

[0173] While the present invention has been described above with reference to example embodiments, the present invention is by no means limited thereto. Various changes and modifications that may become apparent to those skilled in the art may be made in the configuration and specifics of the present invention without departing from the scope of the present invention.

[0174] This application claims priority from US Patent Application No. 16/668,717 filed on October 30, 2019. The entire subject matter of the US Patent Application is incorporated herein by reference.

Industrial Applicability

[0175] The present invention can provide a method for producing a nucleic acid molecule that can obtain a nucleic acid molecule that binds to a target and does not inhibit a function of the target. The present invention is a very useful tool in the field of pharmaceuticals and the like, for example.

[0176] A part of or the whole of the above-described embodiments can be described as the following supplementary notes. However, the present invention is by no means limited thereto.

(Supplementary Note 1)

A method for producing a nucleic acid molecule that binds to a first biological molecule and does not inhibit a function of the first biological molecule, the method including the steps of:

(A) bringing a candidate nucleic acid molecule into contact with the first biological molecule to select a nucleic acid molecule that has bound to the first biological molecule as a first selected nucleic acid molecule; and

(B) selecting the first selected nucleic acid molecule as an intended nucleic acid molecule.

(Supplementary Note 2)

The method according to supplementary note 1, further including, after the step (A), the step of:

(C) bringing a second biological molecule into contact with a complex of the first selected nucleic acid molecule and the first biological molecule to detect a function of the second biological molecule, wherein

after the step (C), in the step (B), the first selected nucleic acid molecule of the complex with which the function of the second biological molecule is detected is selected as an intended nucleic acid molecule.

(Supplementary Note 3)

The method according to supplementary note 2, wherein

the function of the first biological molecule is a function of binding to the second bi-ological molecule.

(Supplementary Note 4)

The method according to supplementary note 3, wherein

the function of the first biological molecule is a function of regulating activity of the second biological molecule by binding to the second biological molecule.

(Supplementary Note 5)

The method according to any one of supplementary notes 2 to 4, wherein

the first biological molecule is a protein and the second biological molecule is a receptor.

(Supplementary Note 6)

The method according to supplementary note 5, wherein

the protein is a growth factor.

(Supplementary Note 7)

The method according to supplementary note 6, wherein

the growth factor is VEGF.

(Supplementary Note 8)

The method according to any one of supplementary notes 1 to 7, wherein

in the step (A), the candidate nucleic acid molecule is brought into contact with the first biological molecule, which is a target substance, and a non-target substance, and the nucleic acid molecule that has bound to the target substance and has not bound to the non-target substance is selected as the first selected nucleic acid molecule.

(Supplementary Note 9)

The method according to supplementary note 8, wherein

the non-target substance is the second biological molecule to which the first biological molecule binds.

(Supplementary Note 10)

The method according to supplementary note 9, wherein

the non-target substance is a polypeptide including a sequence corresponding to a binding site of an amino acid sequence of the second biological molecule with the first biological molecule.

(Supplementary Note 11)

The method according to supplementary note 8, wherein

the non-target substance is a polypeptide including a sequence corresponding to a binding site of an amino acid sequence of the first biological molecule with the second biological molecule.

(Supplementary Note 12)

The method according to supplementary note 11, wherein

the target substance is a polypeptide that includes a sequence (S_R) corresponding to a binding site of an amino acid sequence of the first biological molecule with the second biological molecule and also includes a freely-selected nucleic acid binding sequence (S_A) that is different from the sequence corresponding to the binding site with the second biological molecule and the non-target substance is a polypeptide that includes the sequence (S_R) and does not include the nucleic acid binding sequence (S_A).

(Supplementary Note 13)

The method according to supplementary note 11 or 12, wherein

the target substance and the non-target substance are proteins in a splicing variant relationship with each other.

(Supplementary Note 14)

The method according to any one of supplementary notes 8 to 13, wherein

the target substance is an intended protein and the non-target substance is a protein other than the intended protein.

(Supplementary Note 15)

The method according to supplementary note 14, wherein

the target substance is an intended growth factor and the non-target substance is a growth factor other than the intended growth factor.

(Supplementary Note 16)

The method according to supplementary note 15, wherein

the nucleic acid binding sequence (S_A) is a sequence including a heparin binding domain (HBD).

(Supplementary Note 17)

The method according to supplementary note 16, wherein

the intended growth factor is VEGF165 and the growth factor other than the intended growth factor is VEGF121.

(Supplementary Note 18)

A nucleic acid molecule that binds to VEGF, the nucleic acid molecule including:

any one of the following polynucleotides (a) and (b):

(a) a polynucleotide that consists of a base sequence of SEQ ID NO: 1 or a partial sequence of the base sequence of SEQ ID NO: 1; and

(b) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), binds to VEGF, and does not inhibit a function of the VEGF.

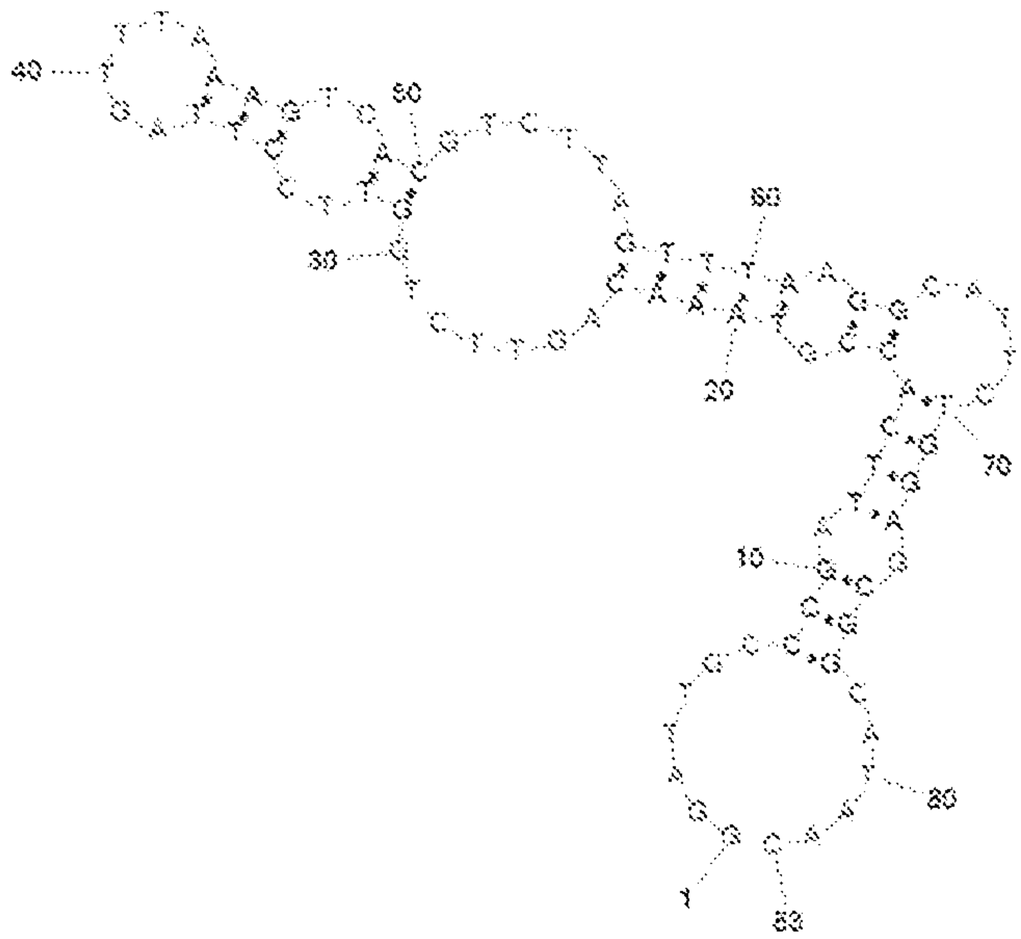
(Supplementary Note 19)

The nucleic acid molecule according to supplementary note 18, wherein

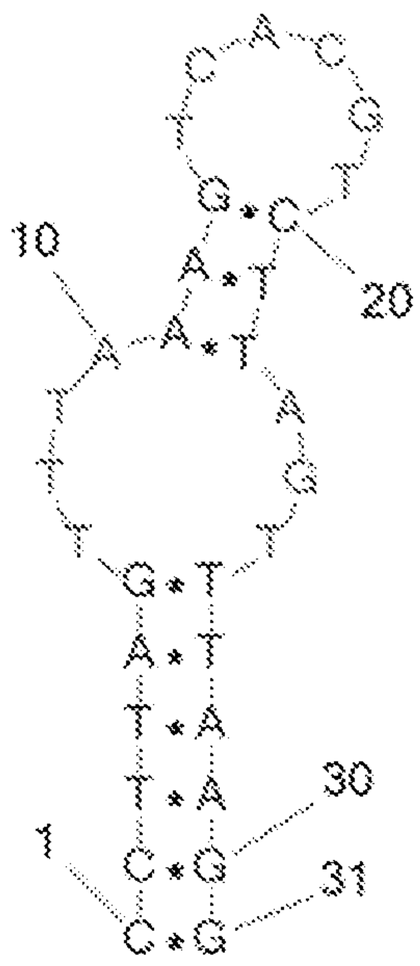
the function of the VEGF is a function of binding to a VEGF receptor.

(Supplementary Note 20)

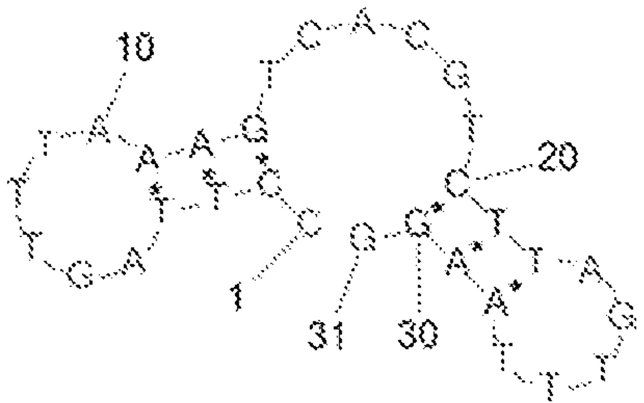
The nucleic acid molecule according to supplementary note 19, wherein
the function of the VEGF is a function of regulating activity of the VEGF receptor by binding to the VEGF receptor.
(Supplementary Note 21)
The nucleic acid molecule according to any one of supplementary notes 18 to 20, wherein
the partial sequence of the base sequence of SEQ ID NO: 1 is at least one base sequence selected from the group consisting of SEQ ID NOs: 2 to 4.
(Supplementary Note 22)
The nucleic acid molecule according to any one of supplementary notes 18 to 20, wherein
the polynucleotide (b) is a polynucleotide consisting of a base sequence of SEQ ID NO: 7.
(Supplementary Note 23)
The nucleic acid molecule according to any one of supplementary notes 18 to 20, wherein
the polynucleotide (b) is the following polynucleotide (b1):
(b1) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), includes a base sequence of any one of SEQ ID NOs: 2 to 4, binds to the VEGF, and does not inhibit the function of the VEGF.
(Supplementary Note 24)
The nucleic acid molecule according to any one of supplementary notes 18 to 20, wherein
the polynucleotide (b) is the following polynucleotide (b2):
(b2) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), can form a secondary structure represented by any one of the following formulae (1) to (5), binds to the VEGF, and does not inhibit the function of the VEGF.



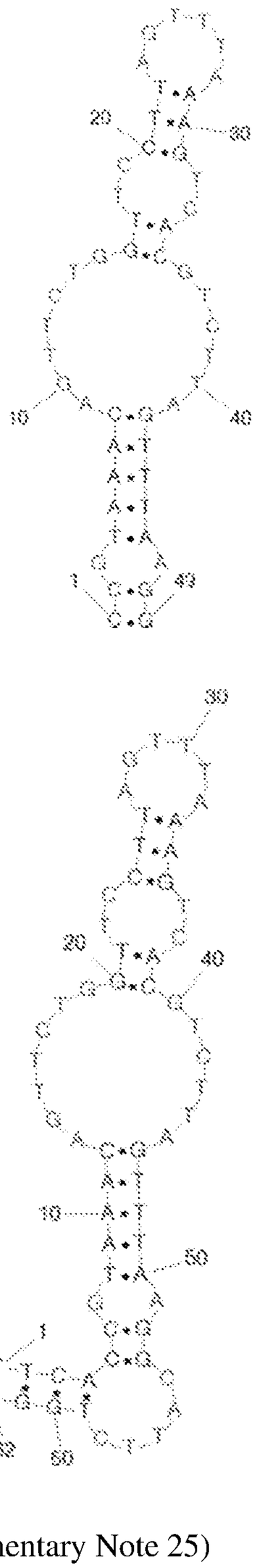
... (1)



... (2)



... (3)



... (4)

... (5)

(Supplementary Note 25)

The nucleic acid molecule according to any one of supplementary notes 18 to 20, wherein

the polynucleotide (b) is the following polynucleotide (b3):

(b3) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), includes a base sequence of at least one of

SEQ ID NOs: 5 and 6, binds to the VEGF, and does not inhibit the function of the VEGF.

(Supplementary Note 26)

The nucleic acid molecule according to any one of supplementary notes 18 to 20, wherein

the polynucleotide is DNA.

(Supplementary Note 27)

A biomaterial including:

a binding nucleic acid molecule; and

a carrier,

the binding nucleic acid molecule being bound to the carrier,

wherein the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein.

(Supplementary Note 28)

The biomaterial according to supplementary note 27, wherein

the function of the predetermined protein is a function of binding to a receptor for the protein.

(Supplementary Note 29)

The biomaterial according to supplementary note 28, wherein

the function of the predetermined protein is a function of regulating activity of the receptor by binding to the receptor.

(Supplementary Note 30)

The biomaterial according to any one of supplementary notes 27 to 29, wherein

the predetermined protein is a growth factor.

(Supplementary Note 31)

The biomaterial according to supplementary note 30, wherein

the growth factor is VEGF.

(Supplementary Note 32)

The biomaterial according to supplementary note 31, wherein

the binding nucleic acid molecule is the binding nucleic acid molecule according to any one of supplementary notes 18 to 26.

(Supplementary Note 33)

The biomaterial according to any one of supplementary notes 27 to 32, wherein

the binding nucleic acid molecule is bound to the carrier via an additional sequence.

(Supplementary Note 34)

The biomaterial according to any one of supplementary notes 27 to 33, wherein

the carrier is a polymer.

(Supplementary Note 35)

The biomaterial according to supplementary note 34, wherein
the polymer is a gel.
(Supplementary Note 36)
The biomaterial according to any one of supplementary notes 27 to 35, which is a biological scaffold.
(Supplementary Note 37)
A method for producing a biomaterial, the method including the step of:
binding a binding nucleic acid molecule to a carrier,
wherein the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein.
(Supplementary Note 38)
The method according to supplementary note 37, further including the step of:
binding the predetermined protein to the binding nucleic acid molecule.
(Supplementary Note 39)
The method according to supplementary note 37 or 38, wherein
the function of the predetermined protein is a function of binding to a receptor for the protein.
(Supplementary Note 40)
The method according to supplementary note 39, wherein
the function of the predetermined protein is a function of regulating activity of the receptor by binding to the receptor.
(Supplementary Note 41)
The method according to any one of supplementary notes 37 to 40, wherein
the predetermined protein is a growth factor.
(Supplementary Note 42)
The method according to supplementary note 41, wherein
the growth factor is VEGF.
(Supplementary Note 43)
The method according to supplementary note 42, wherein
the binding nucleic acid molecule is the binding nucleic acid molecule according to any one of supplementary notes 18 to 26.
(Supplementary Note 44)
The method according to any one of supplementary notes 37 to 43, wherein
the binding nucleic acid molecule is caused to bind to the carrier via an additional sequence.
(Supplementary Note 45)
The method according to any one of supplementary notes 37 to 44, wherein
the carrier is a biodegradable polymer.

(Supplementary Note 46)

The method according to supplementary note 45, wherein

the biodegradable polymer is a gel.

(Supplementary Note 47)

The method according to any one of supplementary notes 37 to 46, wherein

the biomaterial is a biological scaffold.

Claims

[Claim 1] A method for producing a nucleic acid molecule that binds to a first biological molecule and does not inhibit a function of the first biological molecule, the method comprising the steps of:
(A) bringing a candidate nucleic acid molecule into contact with the first biological molecule to select a nucleic acid molecule that has bound to the first biological molecule as a first selected nucleic acid molecule; and
(B) selecting the first selected nucleic acid molecule as an intended nucleic acid molecule.

[Claim 2] The method according to claim 1, further comprising, after the step (A), the step of:
(C) bringing a second biological molecule into contact with a complex of the first selected nucleic acid molecule and the first biological molecule to detect a function of the second biological molecule,
wherein
after the step (C), in the step (B), the first selected nucleic acid molecule of the complex with which the function of the second biological molecule is detected is selected as an intended nucleic acid molecule.

[Claim 3] The method according to claim 2, wherein
the function of the first biological molecule is a function of binding to the second biological molecule.

[Claim 4] The method according to claim 3, wherein
the function of the first biological molecule is a function of regulating activity of the second biological molecule by binding to the second biological molecule.

[Claim 5] The method according to any one of claims 2 to 4, wherein
the first biological molecule is a protein and the second biological molecule is a receptor.

[Claim 6] The method according to claim 5, wherein
the protein is a growth factor.

[Claim 7] The method according to claim 6, wherein
the growth factor is VEGF.

[Claim 8] The method according to any one of claims 1 to 7, wherein
in the step (A), the candidate nucleic acid molecule is brought into contact with the first biological molecule, which is a target substance,

and a non-target substance, and the nucleic acid molecule that has bound to the target substance and has not bound to the non-target substance is selected as the first selected nucleic acid molecule.

[Claim 9] The method according to claim 8, wherein
the non-target substance is the second biological molecule to which the first biological molecule binds.

[Claim 10] The method according to claim 9, wherein
the non-target substance is a polypeptide including a sequence corresponding to a binding site of an amino acid sequence of the second biological molecule with the first biological molecule.

[Claim 11] The method according to claim 8, wherein
the non-target substance is a polypeptide including a sequence corresponding to a binding site of an amino acid sequence of the first biological molecule with the second biological molecule.

[Claim 12] The method according to claim 11, wherein
the target substance is a polypeptide that includes a sequence (S_R) corresponding to a binding site of an amino acid sequence of the first biological molecule with the second biological molecule and also includes a freely-selected nucleic acid binding sequence (S_A) that is different from the sequence corresponding to the binding site with the second biological molecule and the non-target substance is a polypeptide that includes the sequence (S_R) and does not include the nucleic acid binding sequence (S_A).

[Claim 13] The method according to claim 11 or 12, wherein
the target substance and the non-target substance are proteins in a splicing variant relationship with each other.

[Claim 14] The method according to any one of claims 8 to 13, wherein
the target substance is an intended protein and the non-target substance is a protein other than the intended protein.

[Claim 15] The method according to claim 14, wherein
the target substance is an intended growth factor and the non-target substance is a growth factor other than the intended growth factor.

[Claim 16] The method according to claim 15, wherein
the nucleic acid binding sequence (S_A) is a sequence including a heparin binding domain (HBD).

[Claim 17] The method according to claim 16, wherein
the intended growth factor is VEGF165 and the growth factor other than the intended growth factor is VEGF121.

[Claim 18] A nucleic acid molecule that binds to VEGF, the nucleic acid molecule comprising:
any one of the following polynucleotides (a) and (b):
(a) a polynucleotide that consists of a base sequence of SEQ ID NO: 1 or a partial sequence of the base sequence of SEQ ID NO: 1; and
(b) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), binds to VEGF, and does not inhibit a function of the VEGF.

[Claim 19] The nucleic acid molecule according to claim 18, wherein
the function of the VEGF is a function of binding to a VEGF receptor.

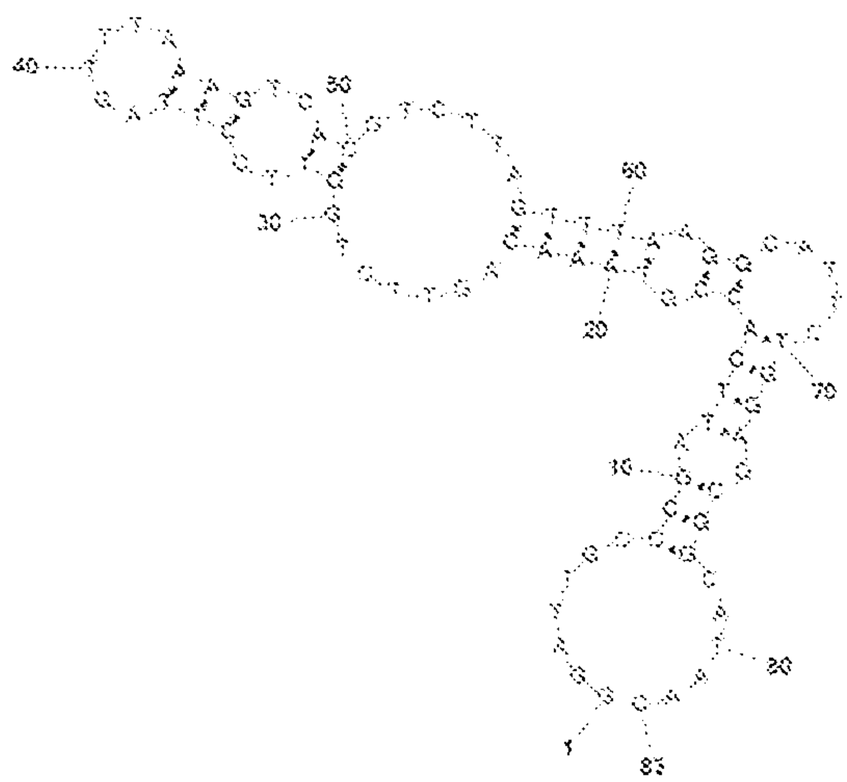
[Claim 20] The nucleic acid molecule according to claim 19, wherein
the function of the VEGF is a function of regulating activity of the VEGF receptor by binding to the VEGF receptor.

[Claim 21] The nucleic acid molecule according to any one of claims 18 to 20, wherein
the partial sequence of the base sequence of SEQ ID NO: 1 is at least one base sequence selected from the group consisting of SEQ ID NOs: 2 to 4.

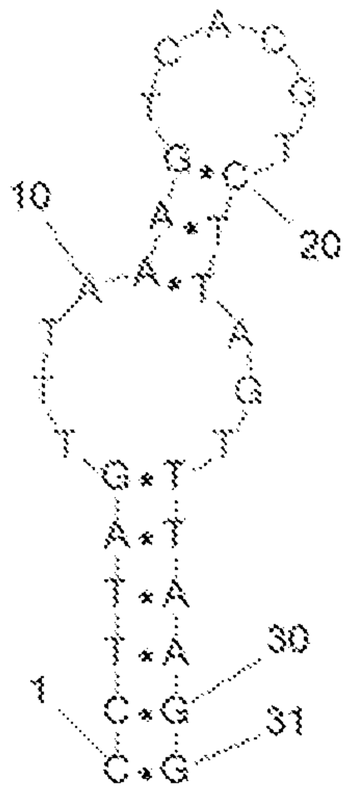
[Claim 22] The nucleic acid molecule according to any one of claims 18 to 20, wherein
the polynucleotide (b) is a polynucleotide consisting of a base sequence of SEQ ID NO: 7.

[Claim 23] The nucleic acid molecule according to any one of claims 18 to 20, wherein
the polynucleotide (b) is the following polynucleotide (b1):
(b1) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), includes a base sequence of any one of SEQ ID NOs: 2 to 4, binds to the VEGF, and does not inhibit the function of the VEGF.

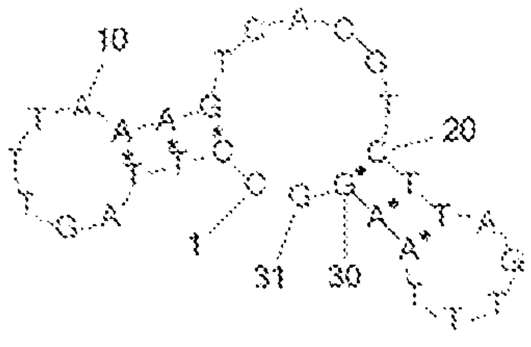
[Claim 24] The nucleic acid molecule according to any one of claims 18 to 20, wherein
the polynucleotide (b) is the following polynucleotide (b2):
(b2) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), can form a secondary structure represented by any one of the following formulae (1) to (5), binds to the VEGF, and does not inhibit the function of the VEGF.



... (1)



... (2)



... (3)



... (4)

... (5)

[Claim 25] The nucleic acid molecule according to any one of claims 18 to 20, wherein
the polynucleotide (b) is the following polynucleotide (b3):
(b3) a polynucleotide that consists of a base sequence having at least 80% identity to the base sequence of the polynucleotide (a), includes a base sequence of at least one of SEQ ID NOs: 5 and 6, binds to the VEGF, and does not inhibit the function of the VEGF.

[Claim 26] The nucleic acid molecule according to any one of claims 18 to 20, wherein
the polynucleotide is DNA.

[Claim 27] A biomaterial comprising:
a binding nucleic acid molecule; and

a carrier,
the binding nucleic acid molecule being bound to the carrier,
wherein the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein.

[Claim 28] The biomaterial according to claim 27, wherein
the function of the predetermined protein is a function of binding to a receptor for the protein.

[Claim 29] The biomaterial according to claim 28, wherein
the function of the predetermined protein is a function of regulating activity of the receptor by binding to the receptor.

[Claim 30] The biomaterial according to any one of claims 27 to 29, wherein
the predetermined protein is a growth factor.

[Claim 31] The biomaterial according to claim 30, wherein
the growth factor is VEGF.

[Claim 32] The biomaterial according to claim 31, wherein
the binding nucleic acid molecule is the binding nucleic acid molecule according to any one of claims 18 to 26.

[Claim 33] The biomaterial according to any one of claims 27 to 32, wherein
the binding nucleic acid molecule is bound to the carrier via an additional sequence.

[Claim 34] The biomaterial according to any one of claims 27 to 33, wherein
the carrier is a polymer.

[Claim 35] The biomaterial according to claim 34, wherein
the polymer is a gel.

[Claim 36] The biomaterial according to any one of claims 27 to 35, which is a biological scaffold.

[Claim 37] A method for producing a biomaterial, the method comprising the step of:
binding a binding nucleic acid molecule to a carrier,
wherein the binding nucleic acid molecule binds to a predetermined protein and does not inhibit a function of the predetermined protein.

[Claim 38] The method according to claim 37, further comprising the step of:
binding the predetermined protein to the binding nucleic acid molecule.

[Claim 39] The method according to claim 37 or 38, wherein
the function of the predetermined protein is a function of binding to a receptor for the protein.

[Claim 40] The method according to claim 39, wherein
the function of the predetermined protein is a function of regulating

activity of the receptor by binding to the receptor.

[Claim 41] The method according to any one of claims 37 to 40, wherein the predetermined protein is a growth factor.

[Claim 42] The method according to claim 41, wherein the growth factor is VEGF.

[Claim 43] The method according to claim 42, wherein the binding nucleic acid molecule is the binding nucleic acid molecule according to any one of claims 18 to 26.

[Claim 44] The method according to any one of claims 37 to 43, wherein the binding nucleic acid molecule is caused to bind to the carrier via an additional sequence.

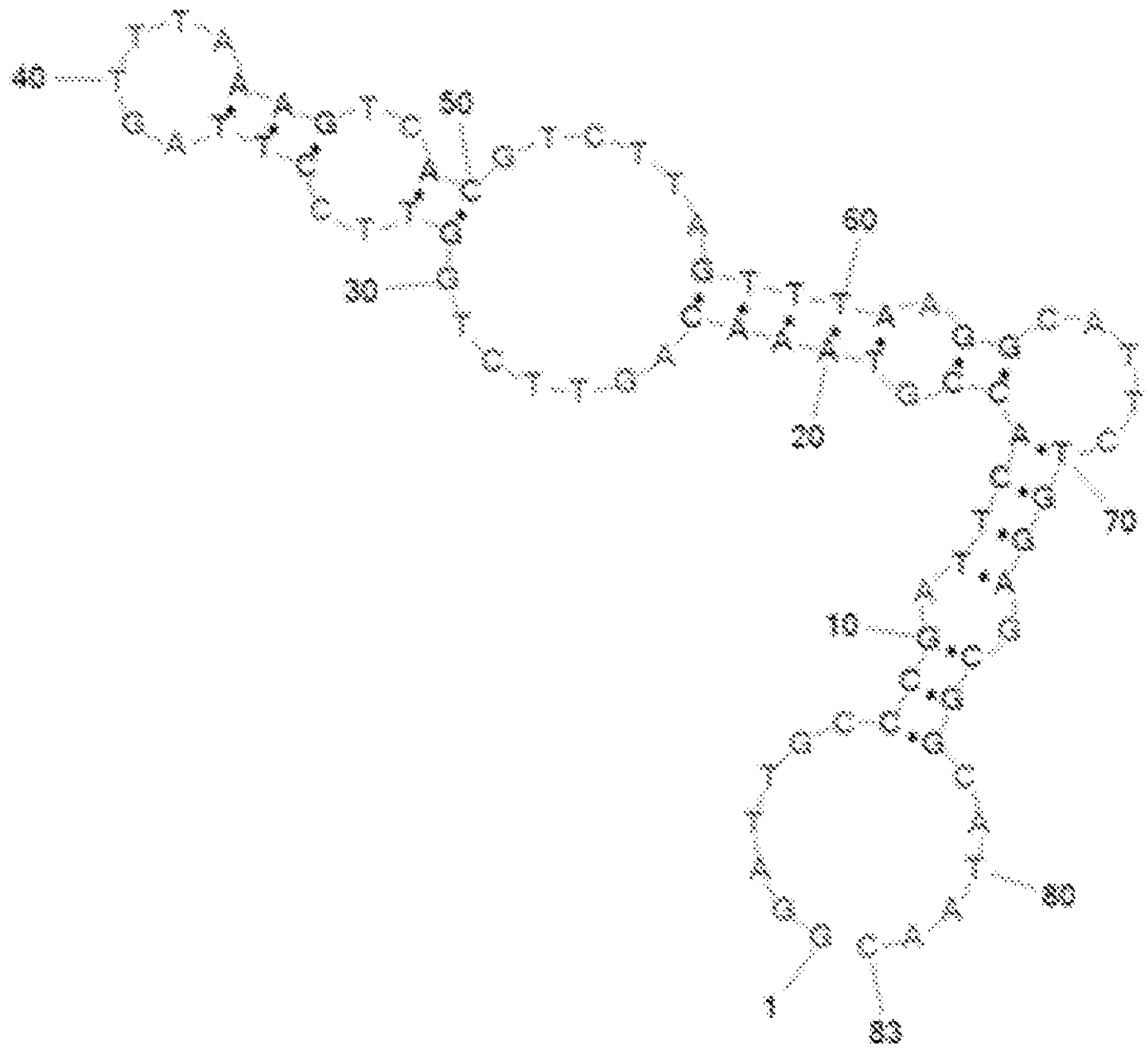
[Claim 45] The method according to any one of claims 37 to 44, wherein the carrier is a biodegradable polymer.

[Claim 46] The method according to claim 45, wherein the biodegradable polymer is a gel.

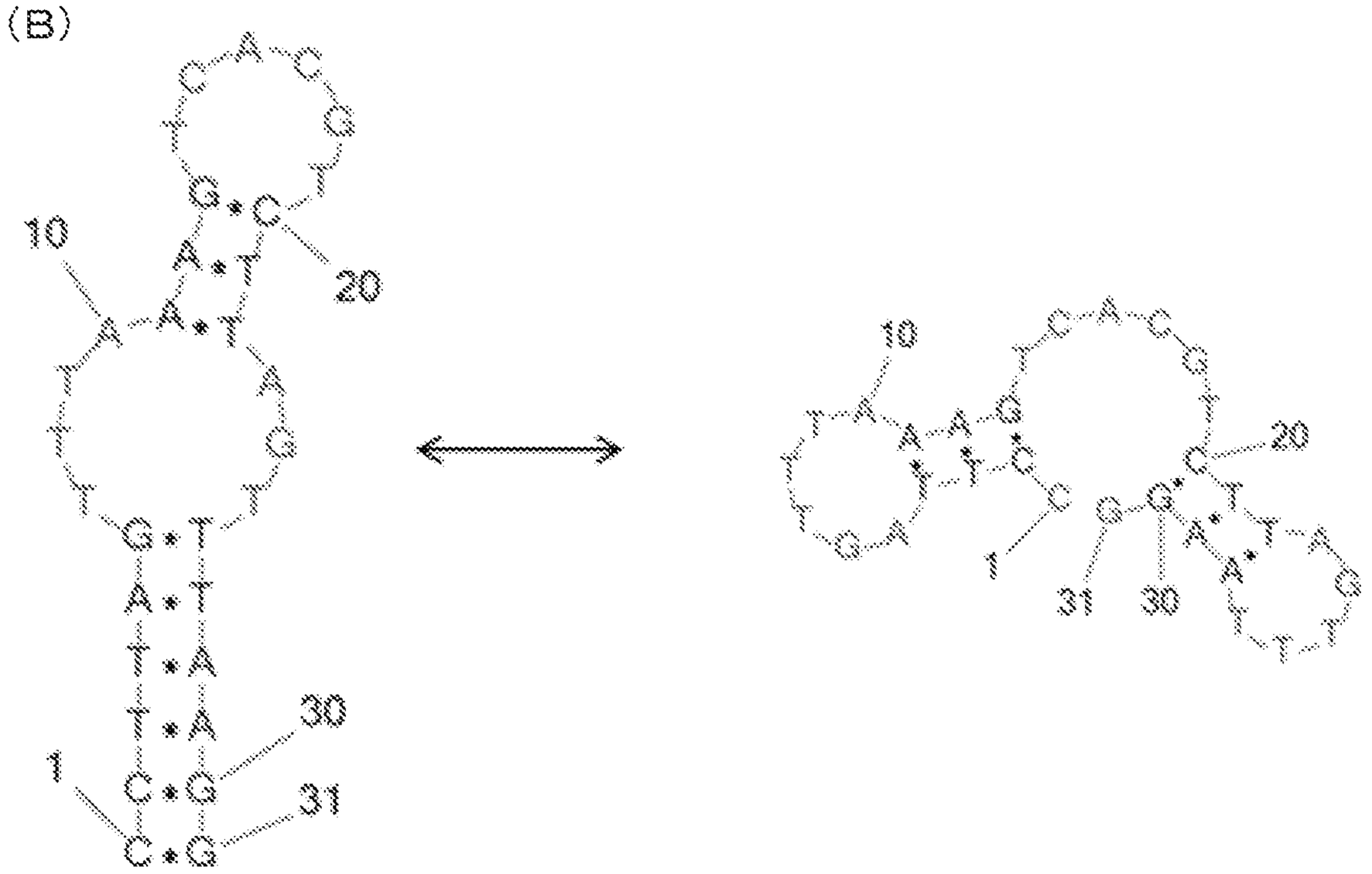
[Claim 47] The method according to any one of claims 37 to 46, wherein the biomaterial is a biological scaffold.

[Fig. 1A-1B]

(A)

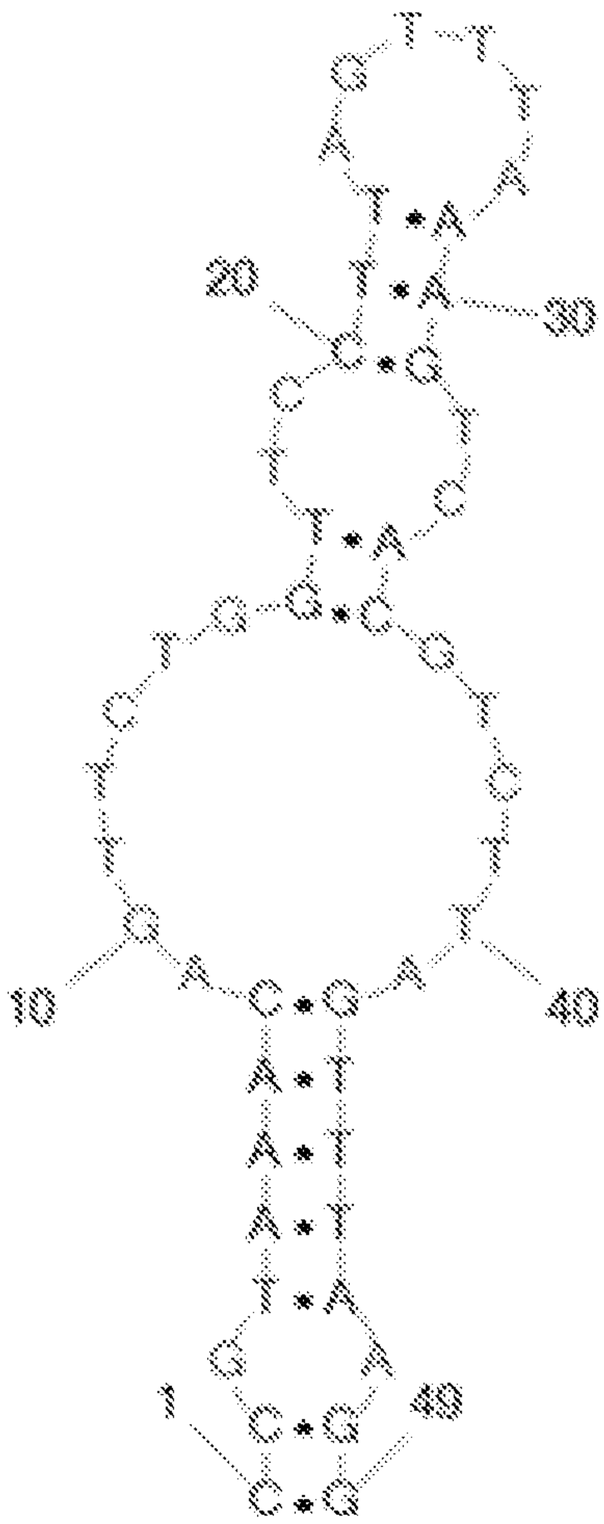


(B)

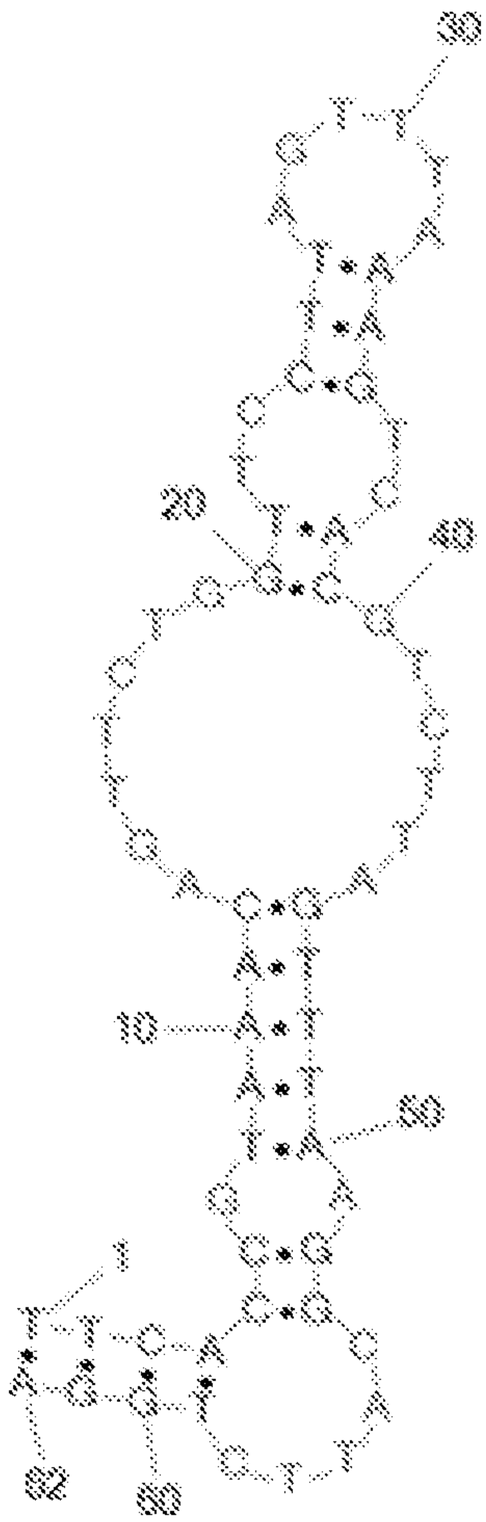


[Fig. 1C-1E]

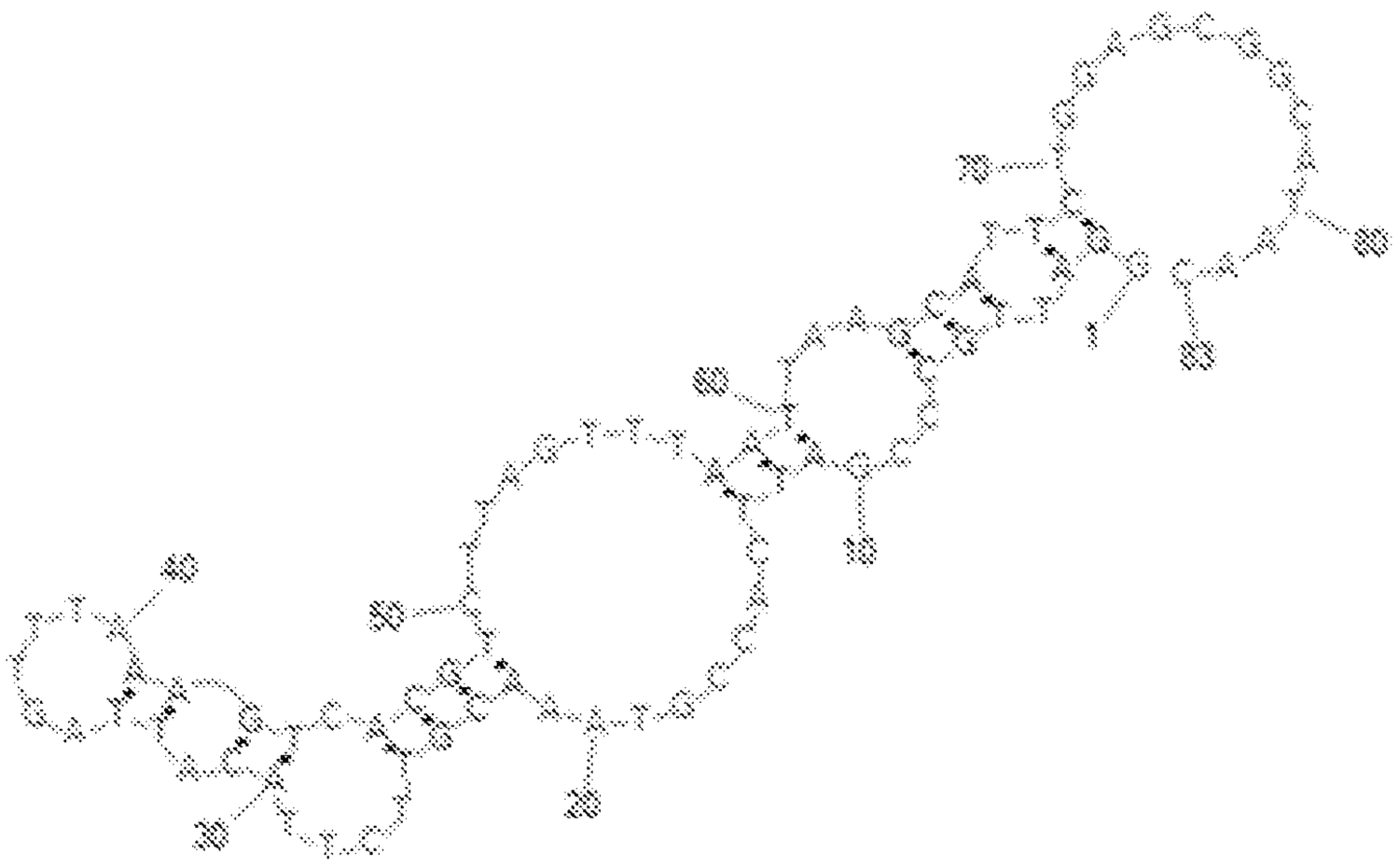
(C)



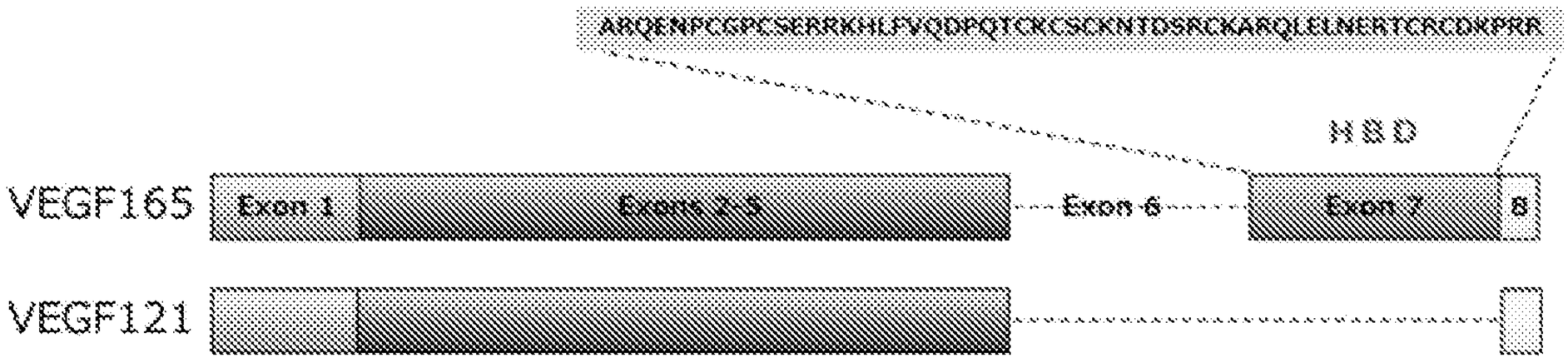
(D)



(E)

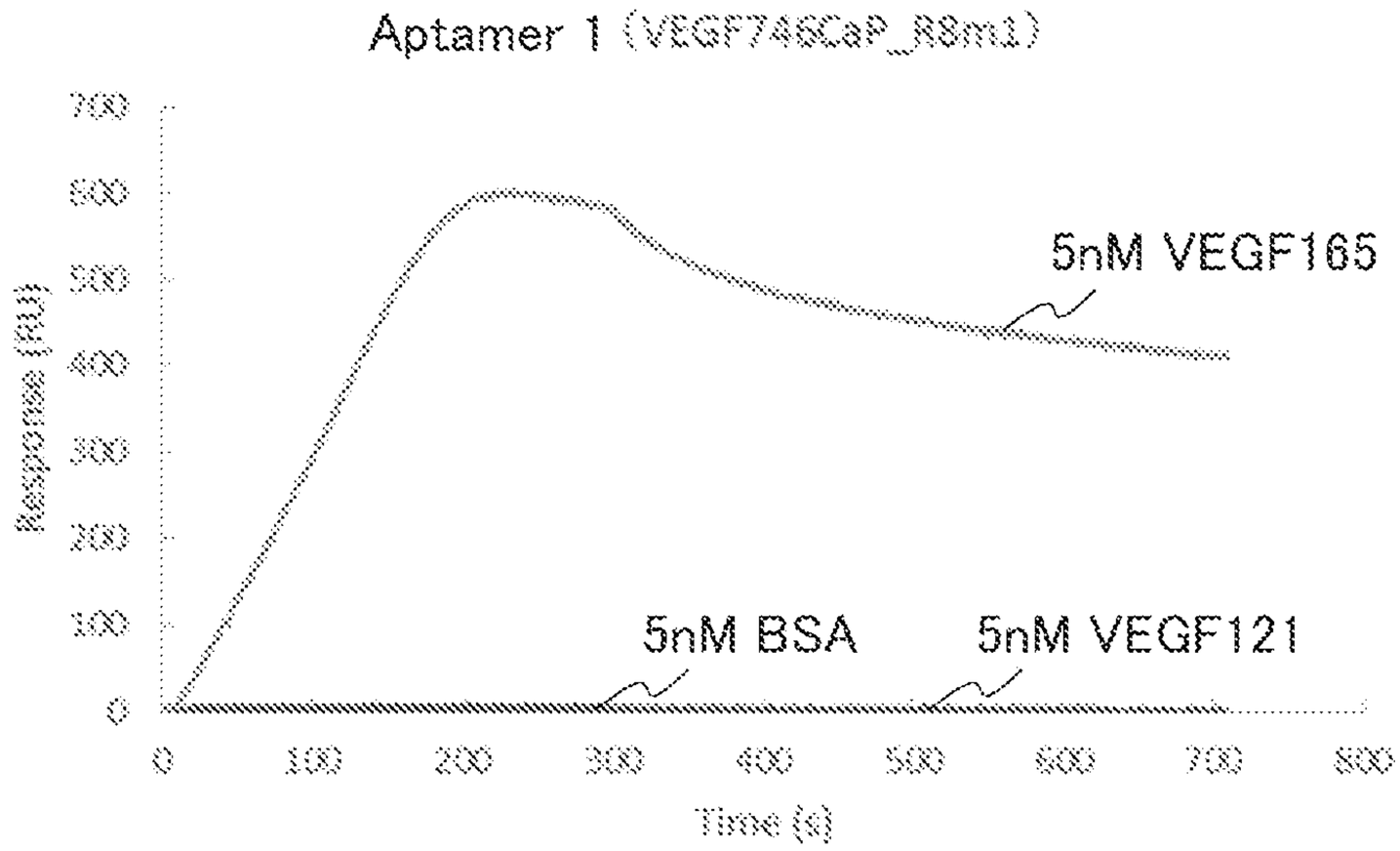


[Fig. 2]

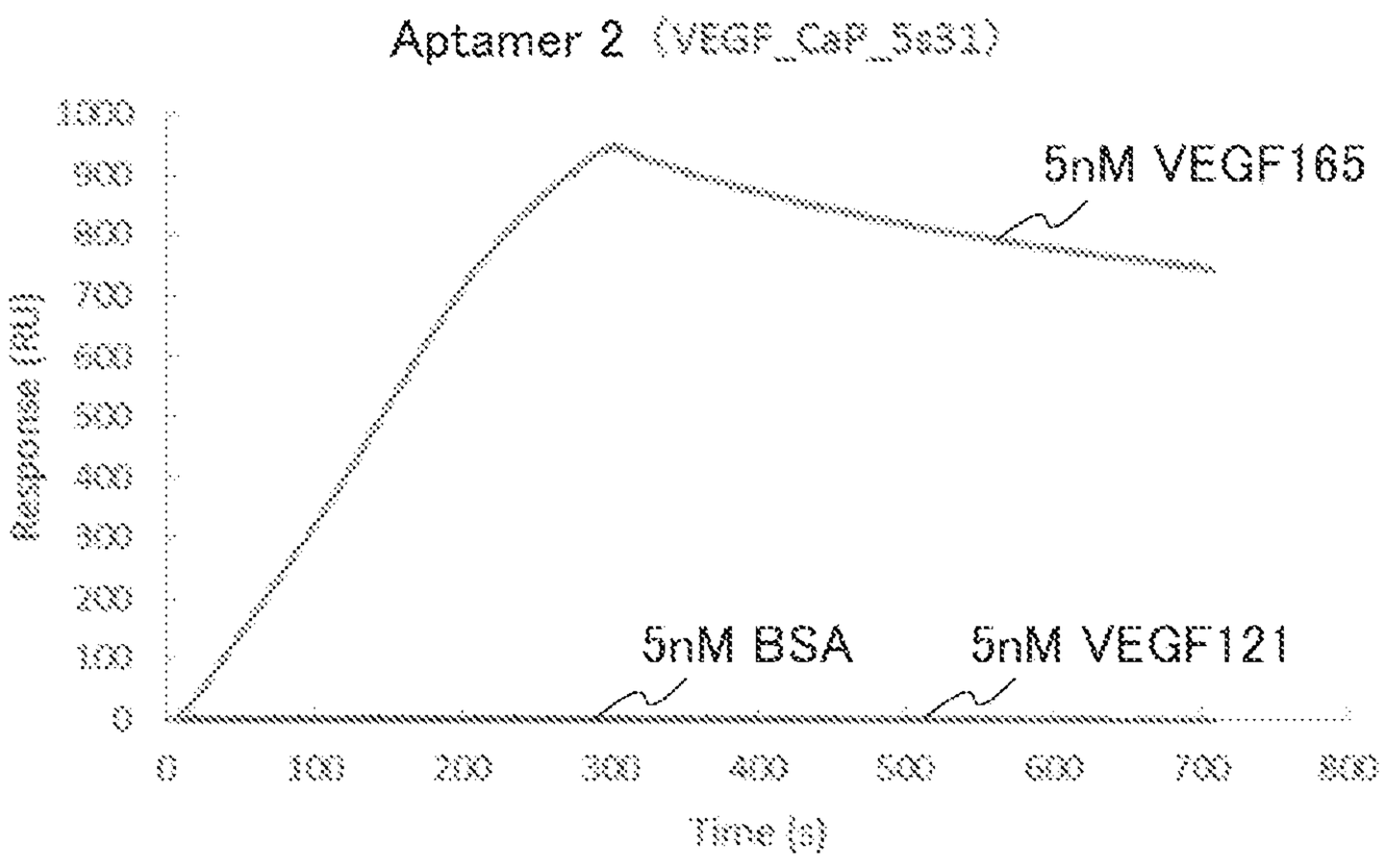


[Fig. 3A-3B]

(A)

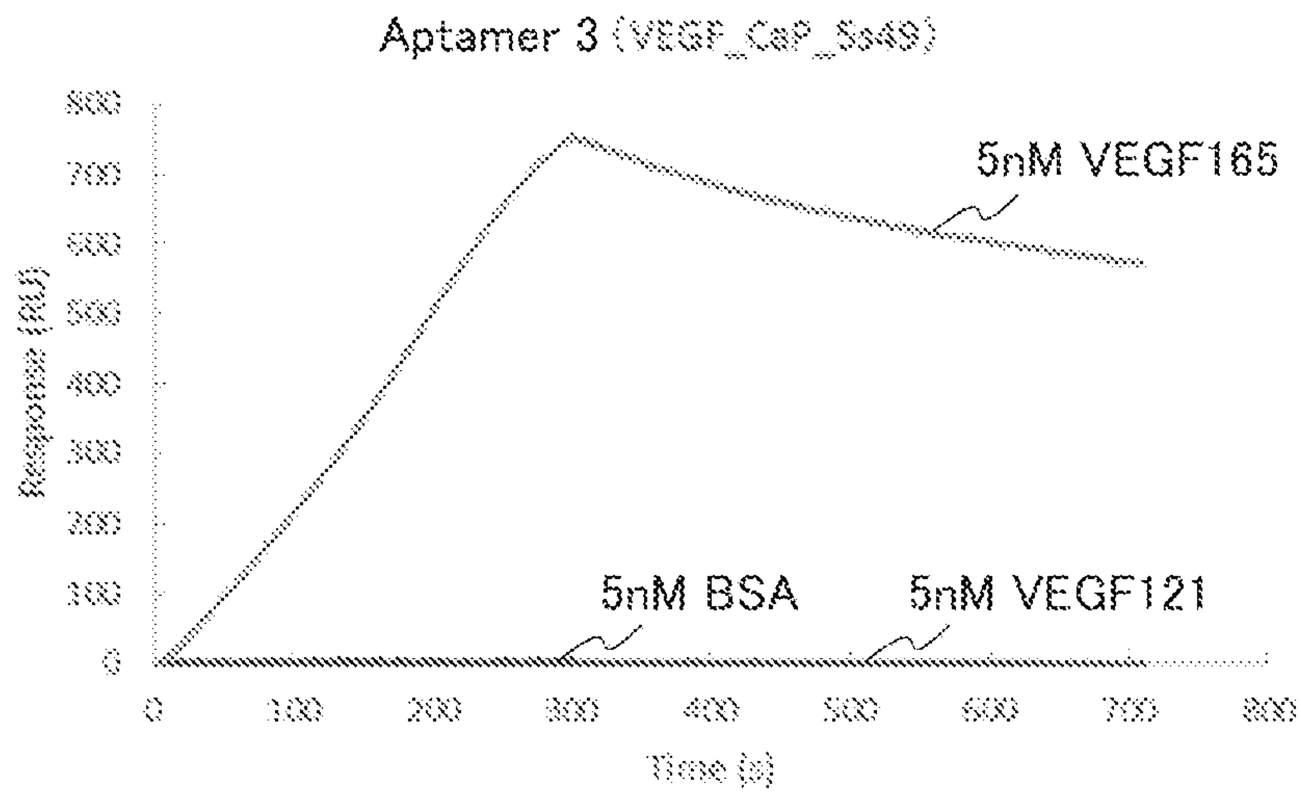


(B)

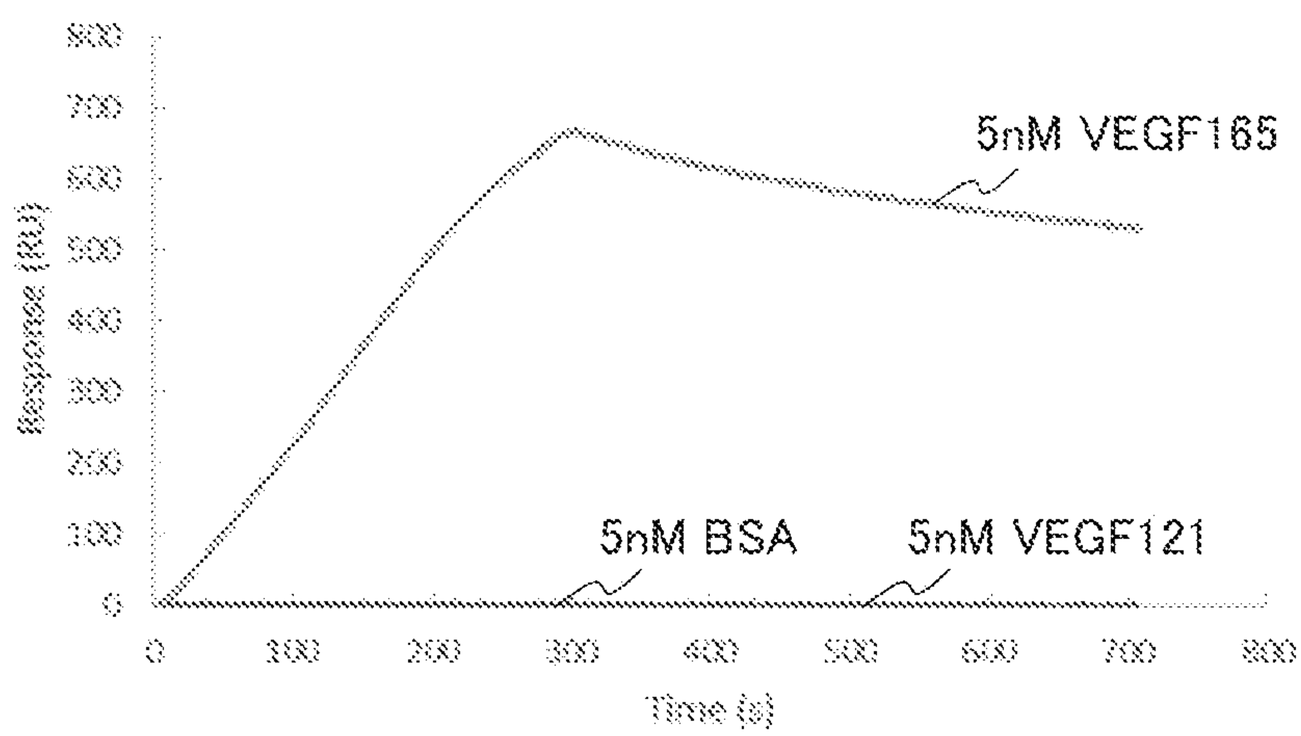


[Fig. 3C-3E]

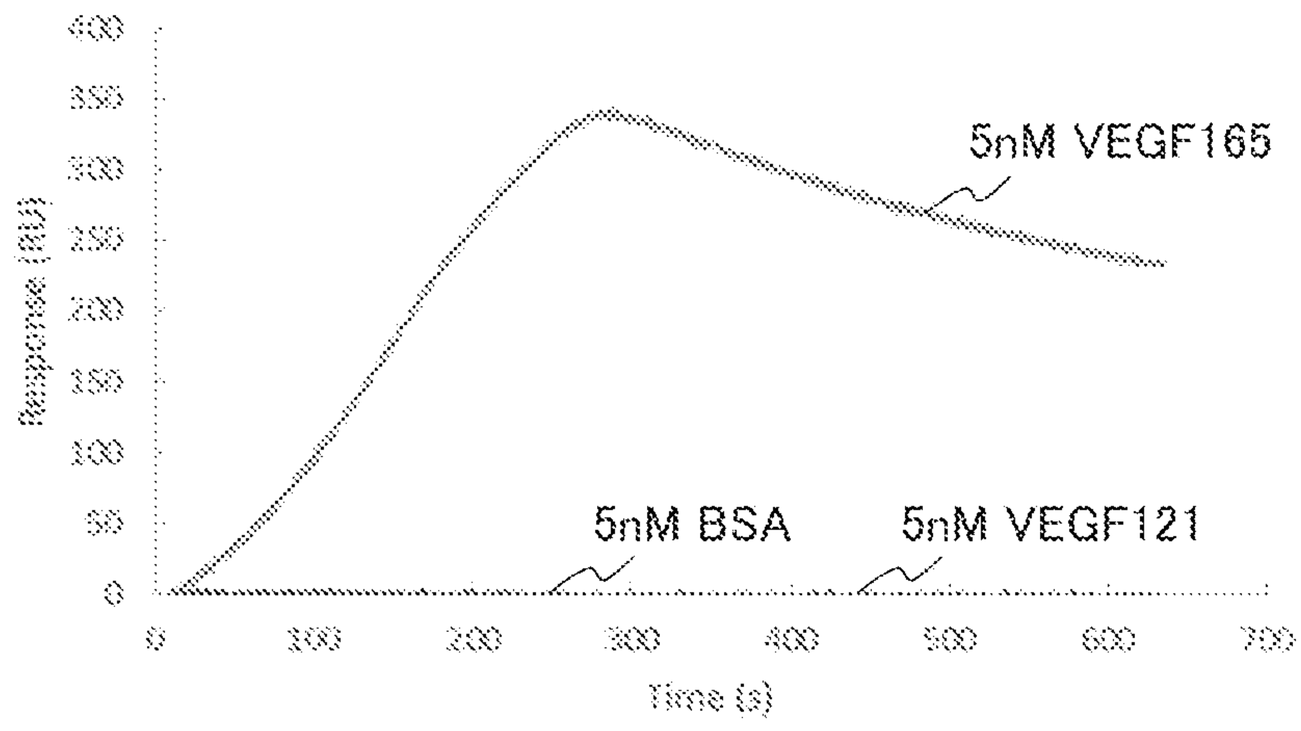
(C)


Aptamer 3 (VEGF_CaP_Ss49)
5nM VEGF165
5nM BSA
5nM VEGF121
Response (RU)
Time (s)

(D)

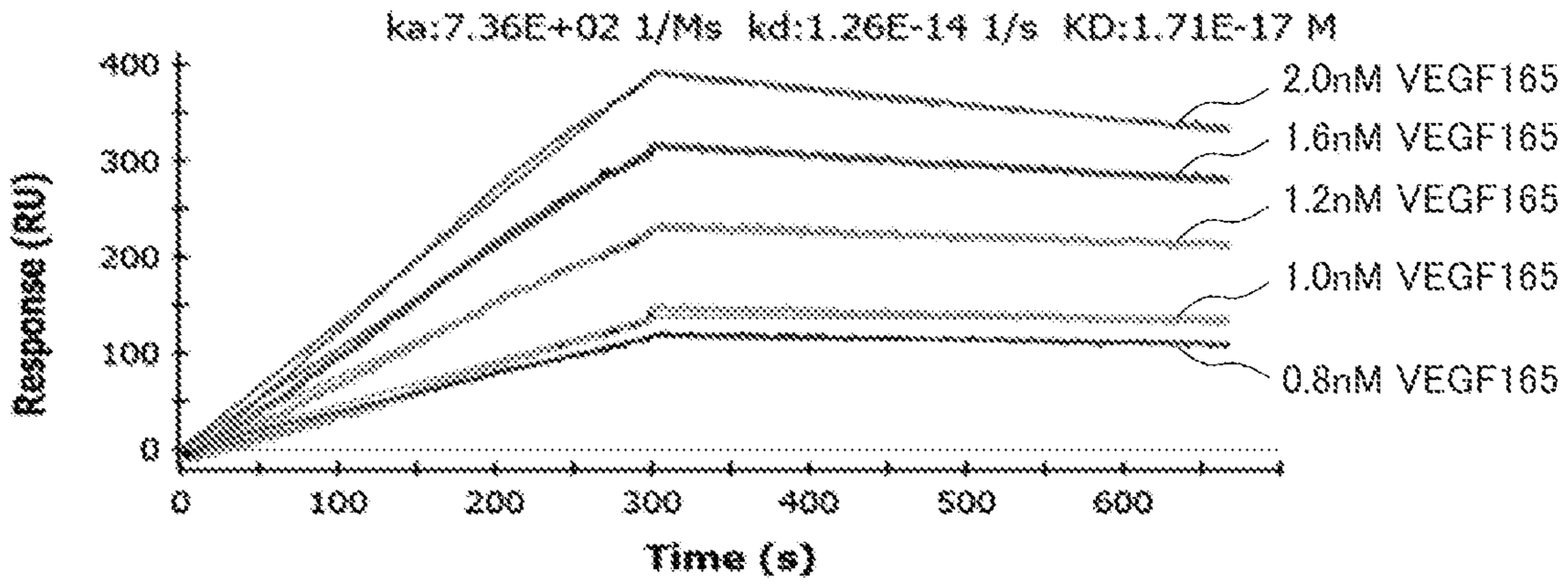

Aptamer 4 (VEGF_CaP_Ss62)
5nM VEGF165
5nM BSA
5nM VEGF121
Response (RU)
Time (s)

(E)

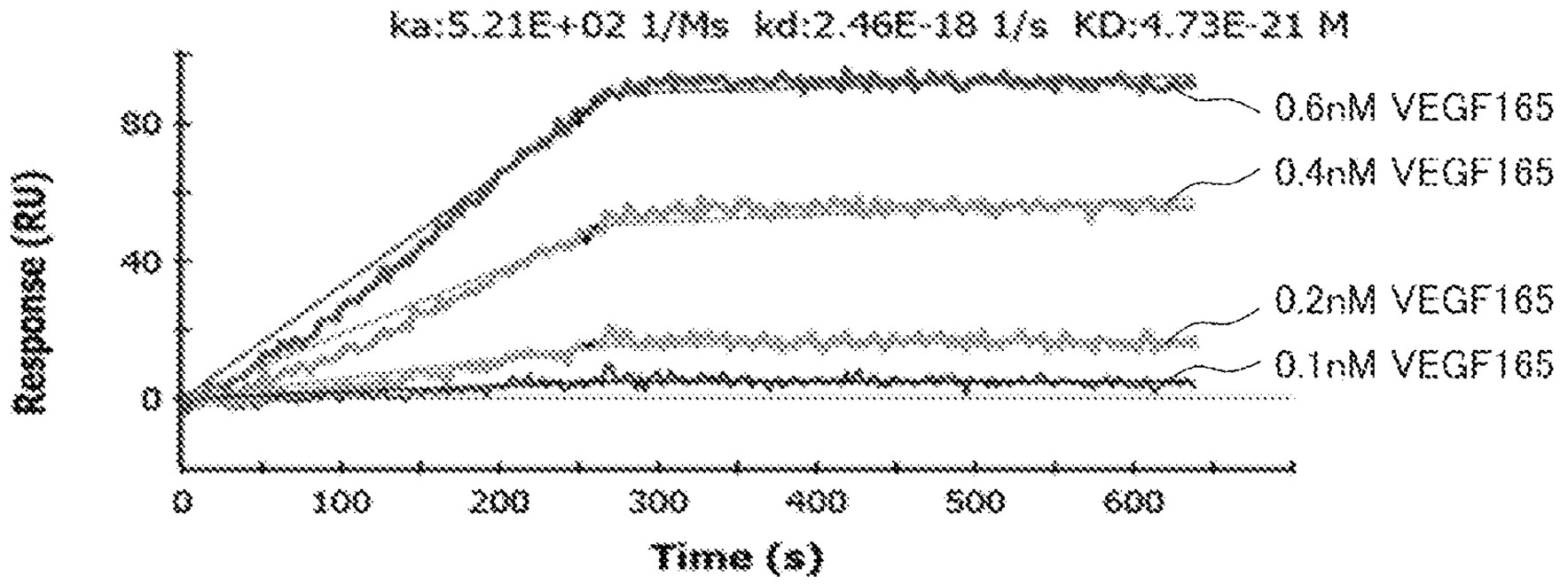

Aptamer 5 (VEGF746CaP_RSm49)
5nM VEGF165
5nM BSA
5nM VEGF121
Response (RU)
Time (s)

[Fig. 4A-4C]

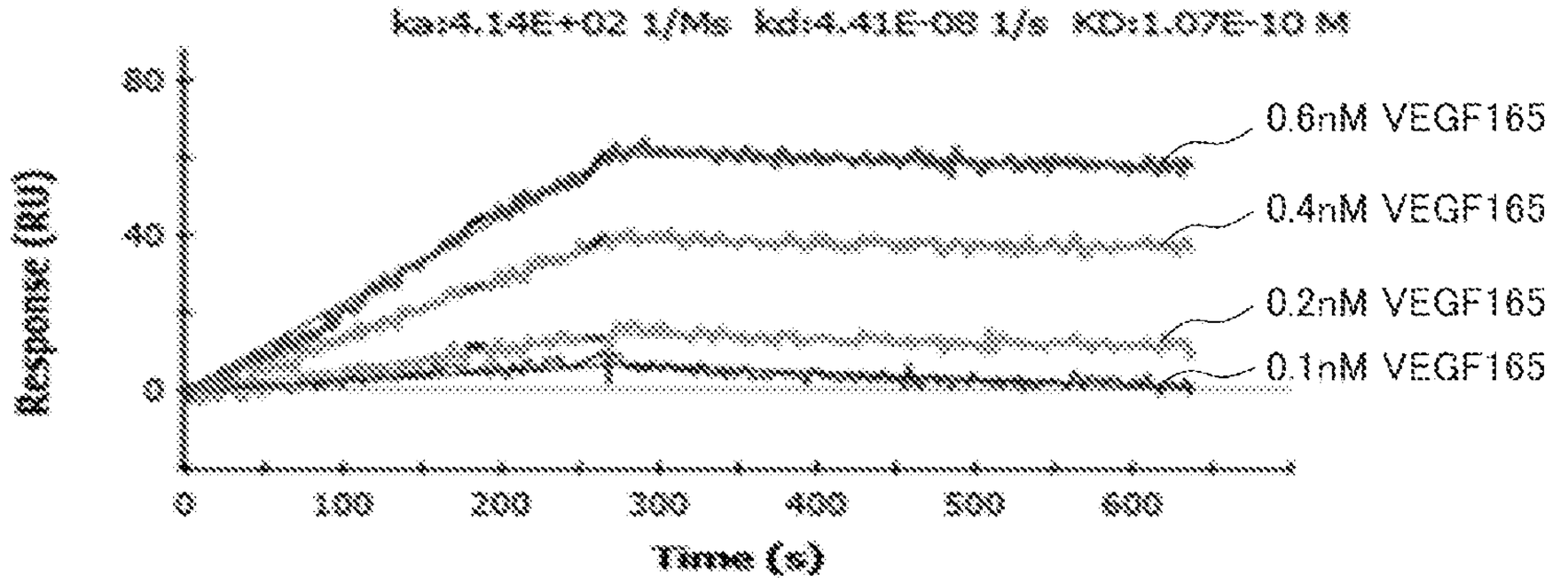
(A)


ka:7.36E+02 1/Ms kd:1.26E-14 1/s KD:1.71E-17 M
2.0nM VEGF165
1.6nM VEGF165
1.2nM VEGF165
1.0nM VEGF165
0.8nM VEGF165
Response (RU)
400
300
200
100
0
0
100
200
300
400
500
600
Time (s)

(B)

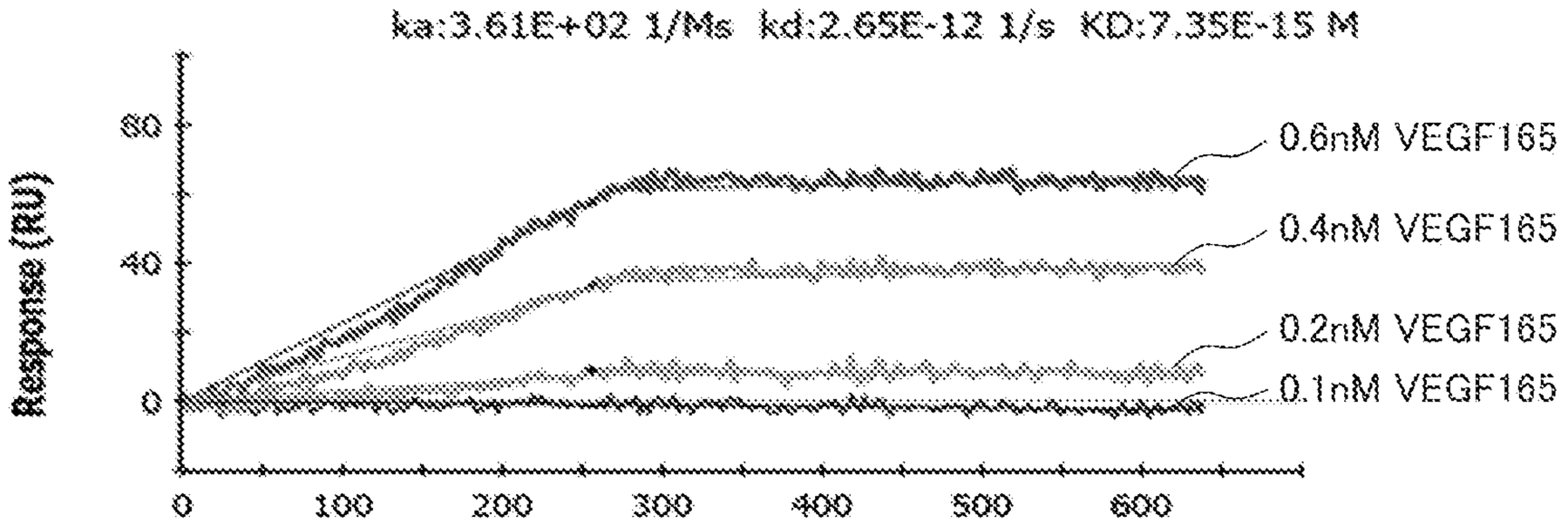

ka:5.21E+02 1/Ms kd:2.46E-18 1/s KD:4.73E-21 M
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
0.1nM VEGF165
Response (RU)
80
40
0
0
100
200
300
400
500
600
Time (s)

(C)

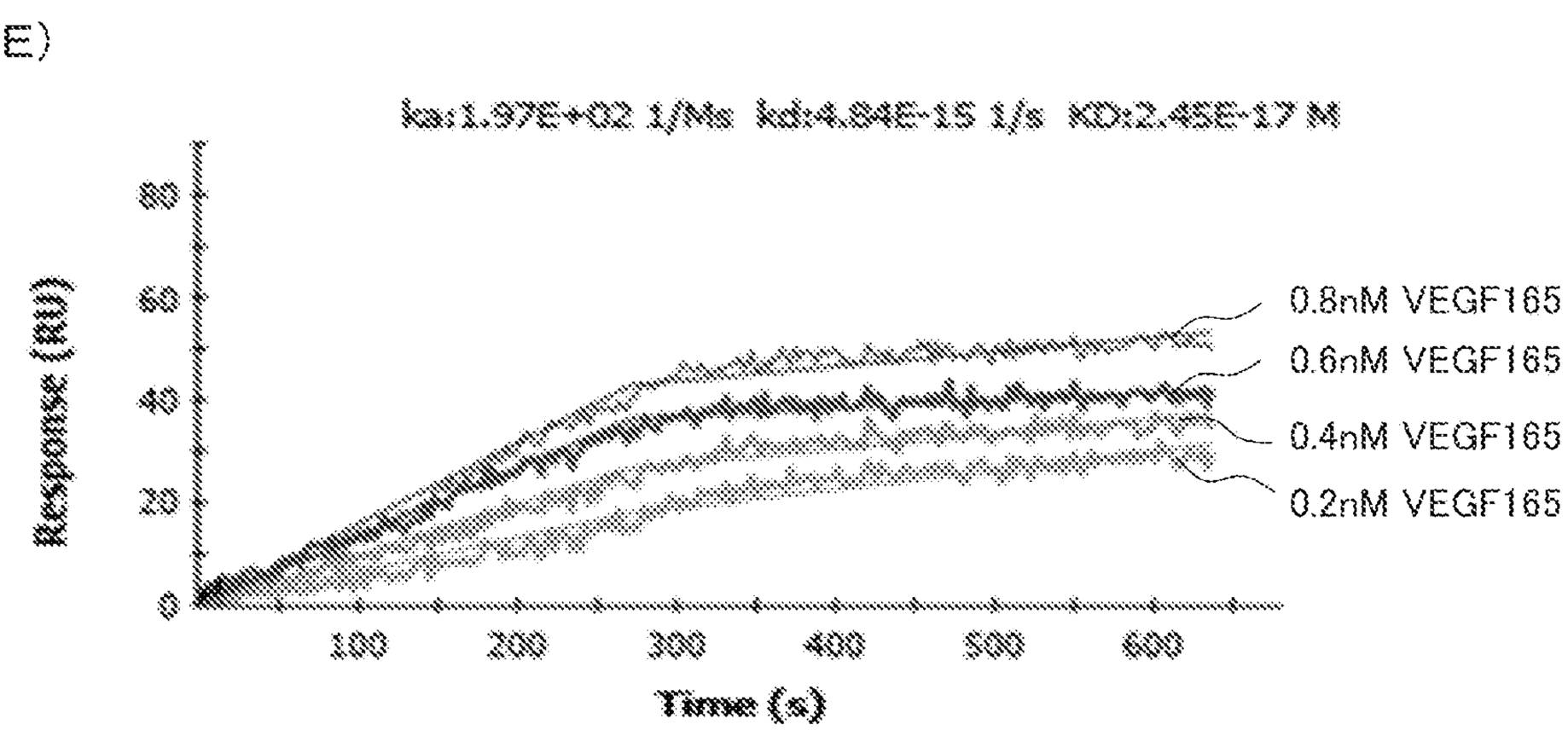

ka:4.14E+02 1/Ms kd:4.41E-08 1/s KD:1.07E-10 M
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
0.1nM VEGF165
Response (RU)
80
40
0
0
100
200
300
400
500
600
Time (s)

[Fig. 4D-4E]

(D)

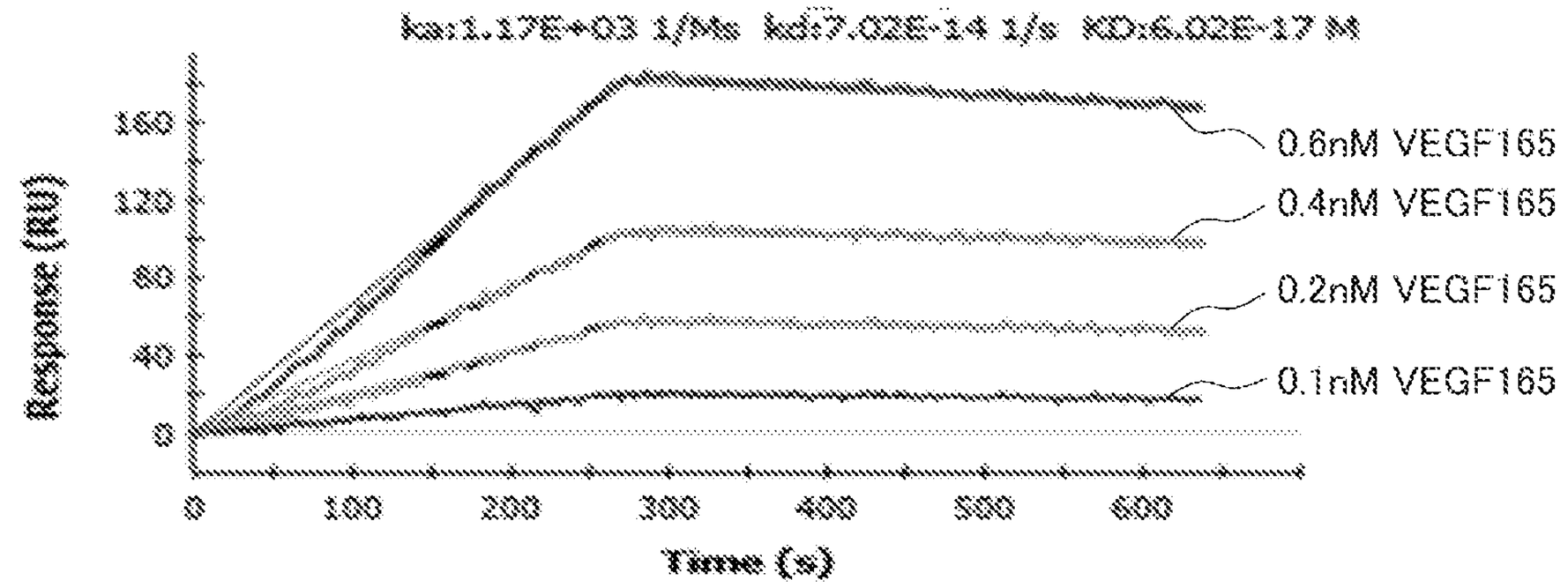

ka:3.61E+02 1/Ms kd:2.65E-12 1/s KD:7.35E-15 M
Response (RU)
80
40
0
0
100
200
300
400
500
600
Time (s)
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
0.1nM VEGF165

(E)

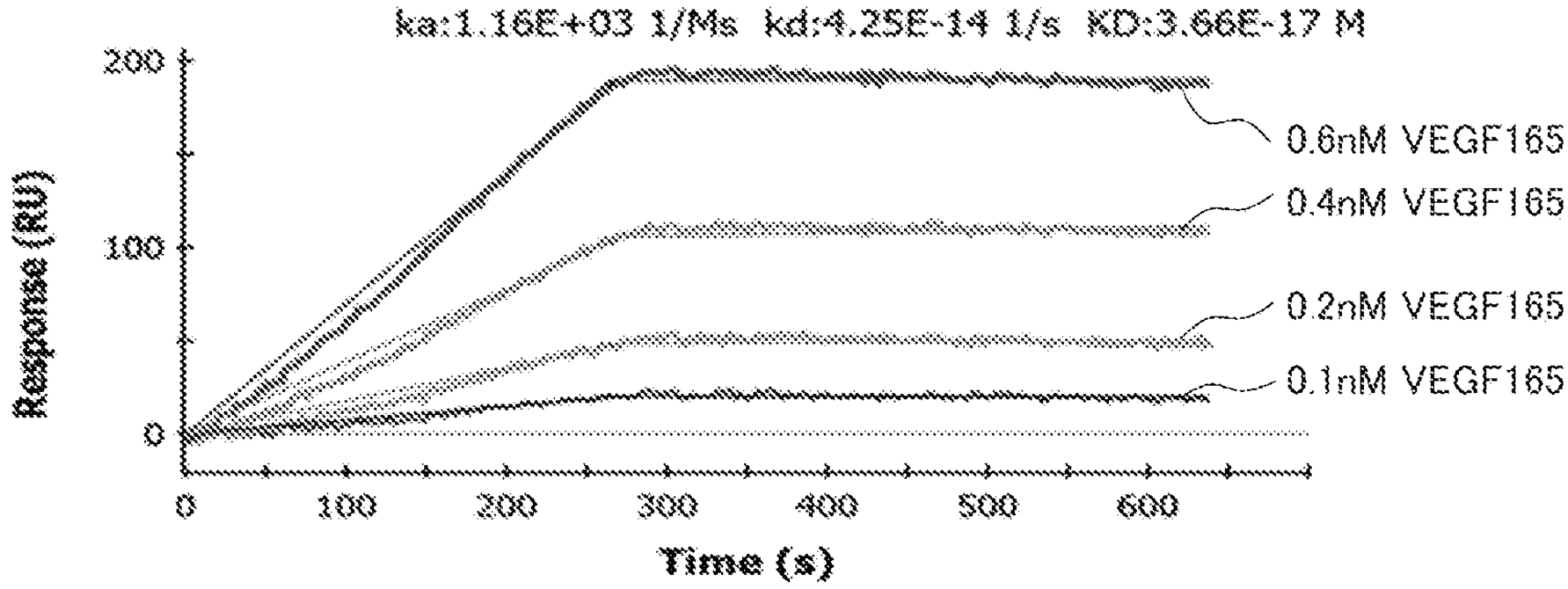

ka:1.97E+02 1/Ms kd:4.84E-15 1/s KD:2.45E-17 M
Response (RU)
80
60
40
20
0
100
200
300
400
500
600
Time (s)
0.8nM VEGF165
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165

[Fig. 5A-5C]

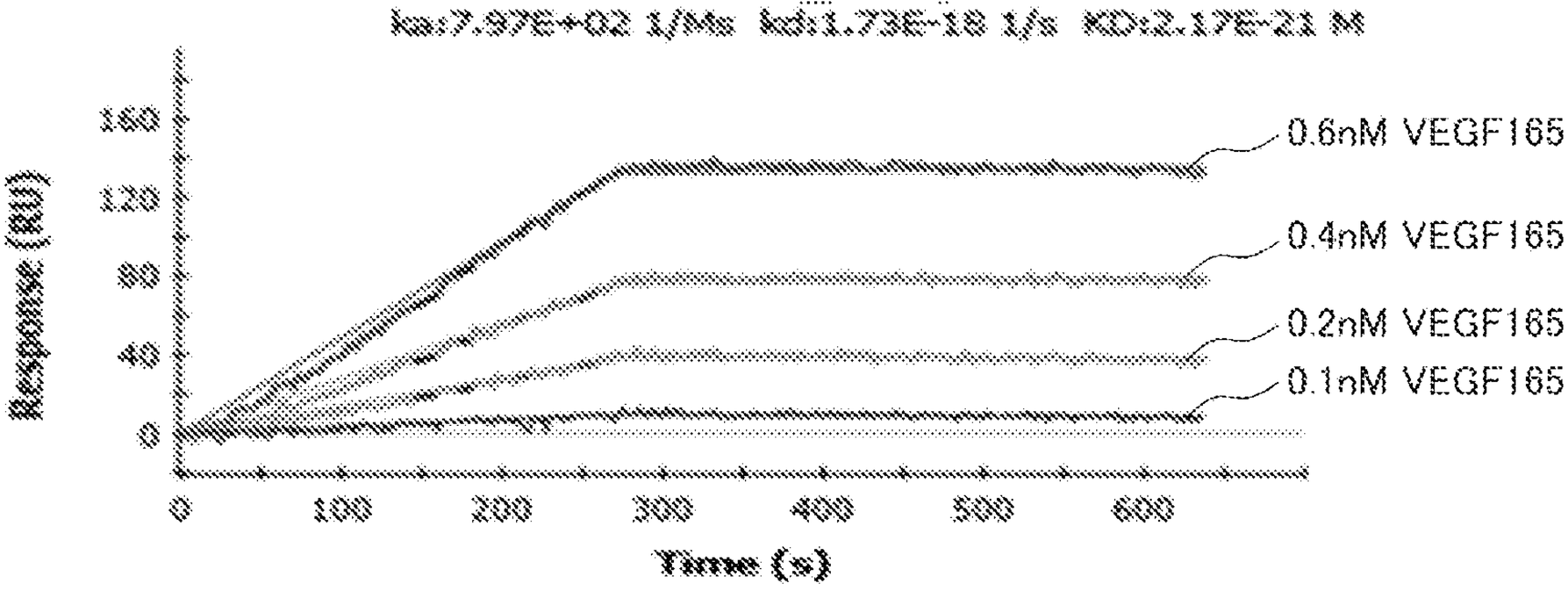
(A)


ka:1.17E+03 1/Ms kd:7.02E-14 1/s KD:6.02E-17 M
Response (RU)
160
120
80
40
0
0
100
200
300
400
500
600
Time (s)
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
0.1nM VEGF165

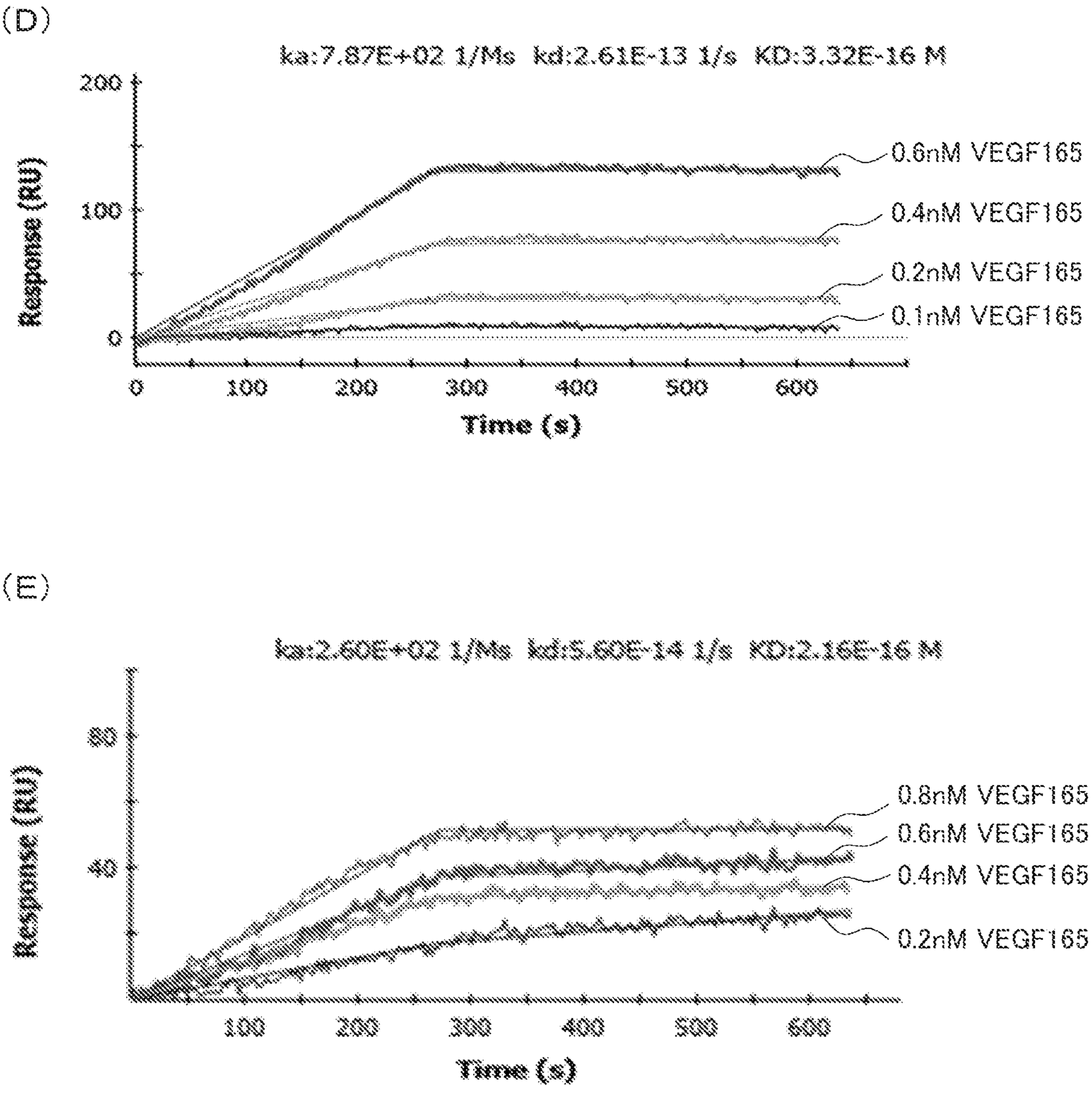
(B)


ka:1.16E+03 1/Ms kd:4.25E-14 1/s KD:3.66E-17 M
Response (RU)
200
100
0
0
100
200
300
400
500
600
Time (s)
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
0.1nM VEGF165

(C)

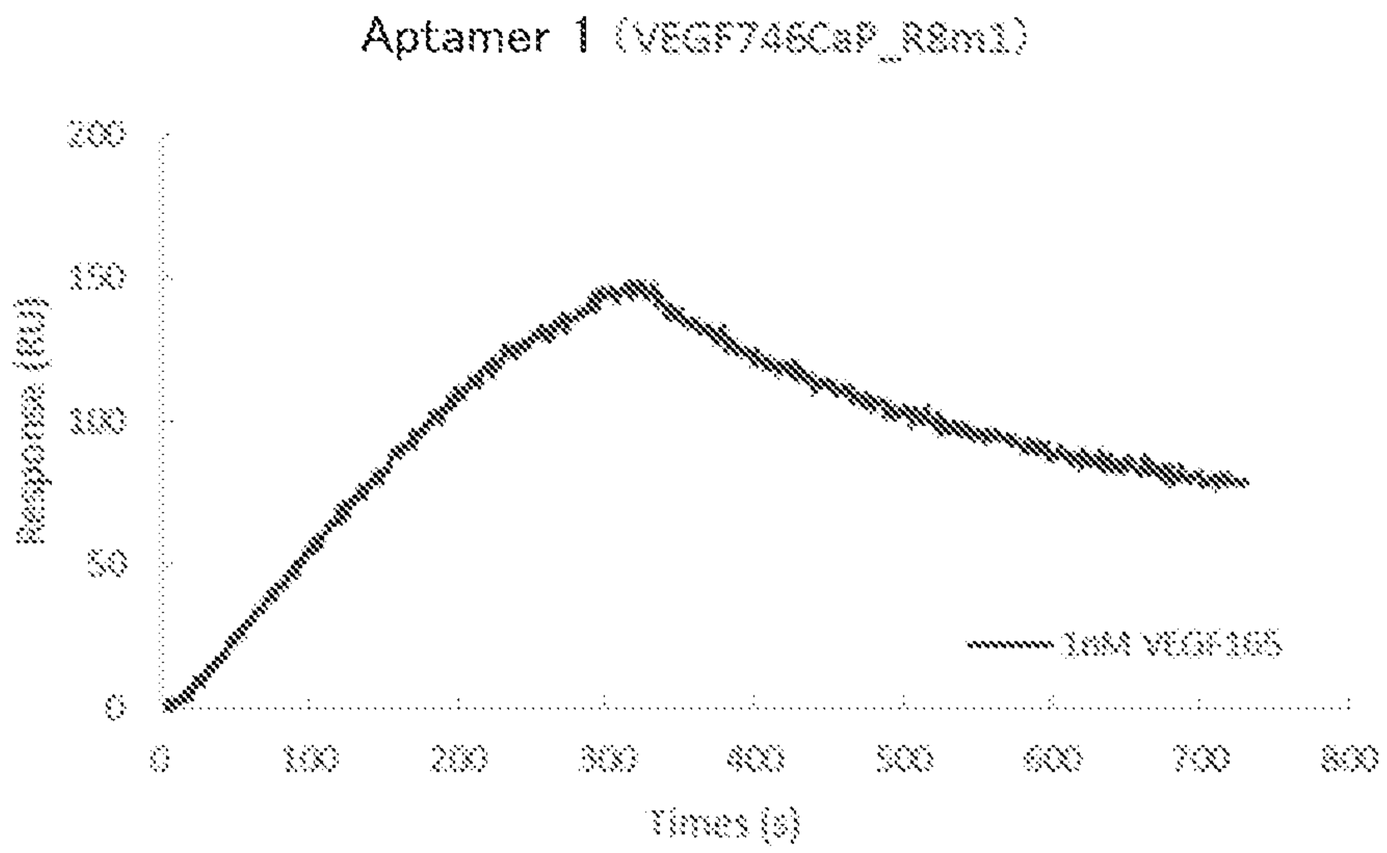

ka:7.97E+02 1/Ms kd:1.73E-18 1/s KD:2.17E-21 M
Response (RU)
160
120
80
40
0
0
100
200
300
400
500
600
Time (s)
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
0.1nM VEGF165

[Fig. 5D-5E]

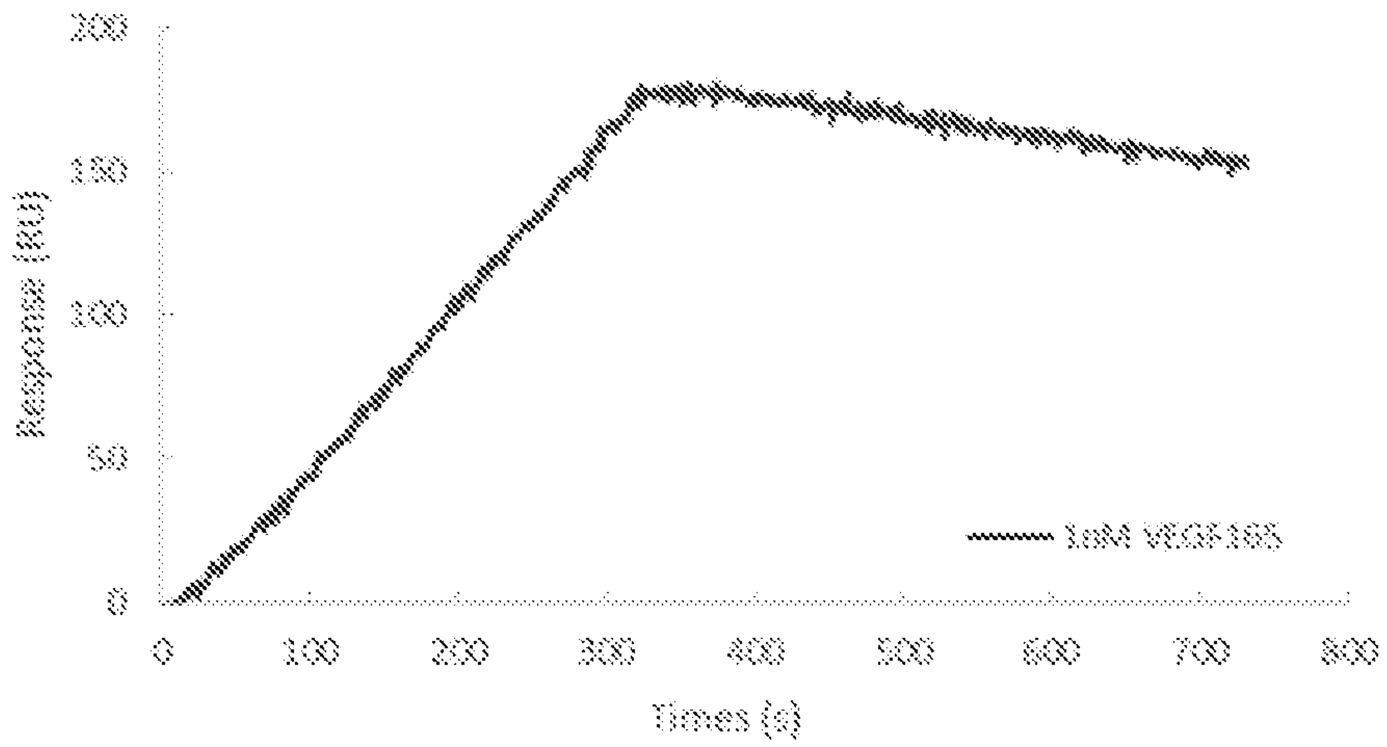

(D)
ka:7.87E+02 1/Ms kd:2.61E-13 1/s KD:3.32E-16 M
Response (RU)
200
100
0
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
0.1nM VEGF165
0
100
200
300
400
500
600
Time (s)
(E)
ka:2.60E+02 1/Ms kd:5.60E-14 1/s KD:2.16E-16 M
Response (RU)
80
40
0.8nM VEGF165
0.6nM VEGF165
0.4nM VEGF165
0.2nM VEGF165
100
200
300
400
500
600
Time (s)

[Fig. 6A-6B]

(A)

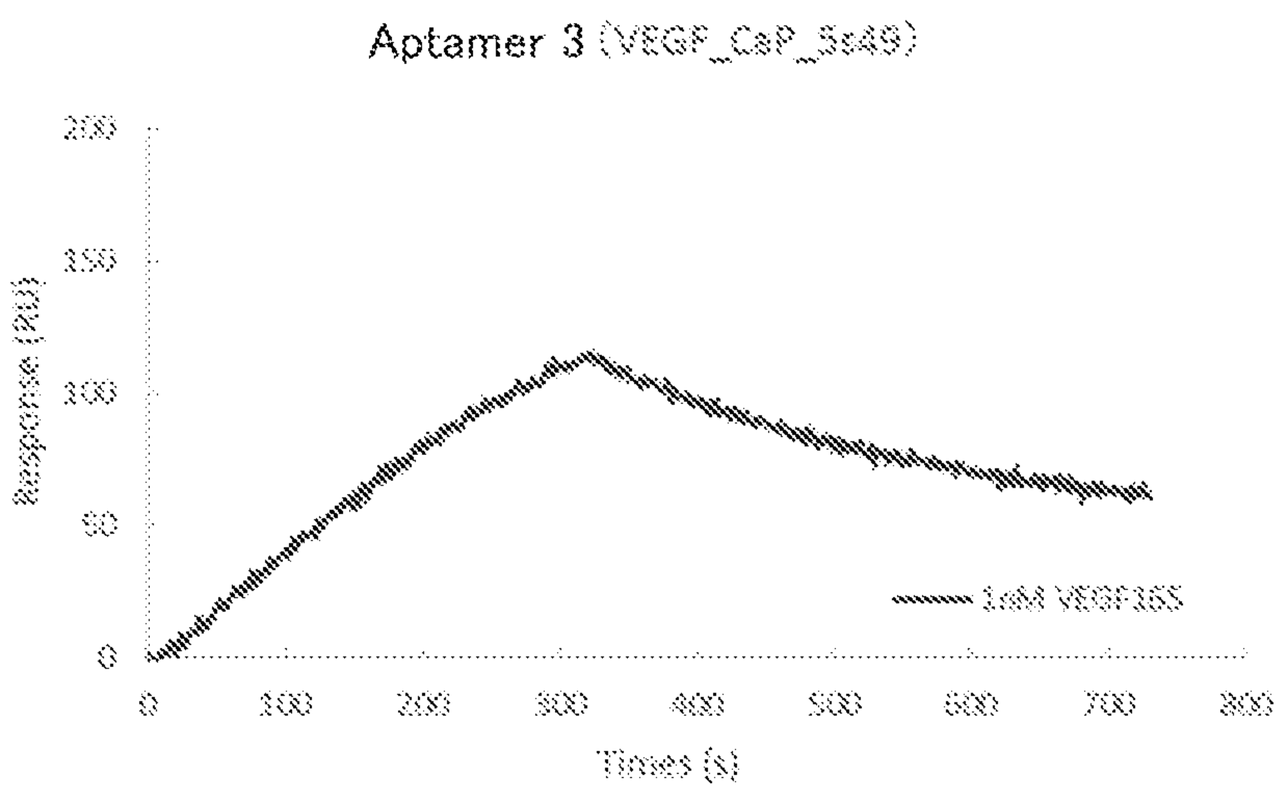

Aptamer 1 (VEGF746CaP_R8m1)
Response (RU)
200
150
100
50
0
1nM VEGF165
0
100
200
300
400
500
600
700
800
Times (s)

(B)

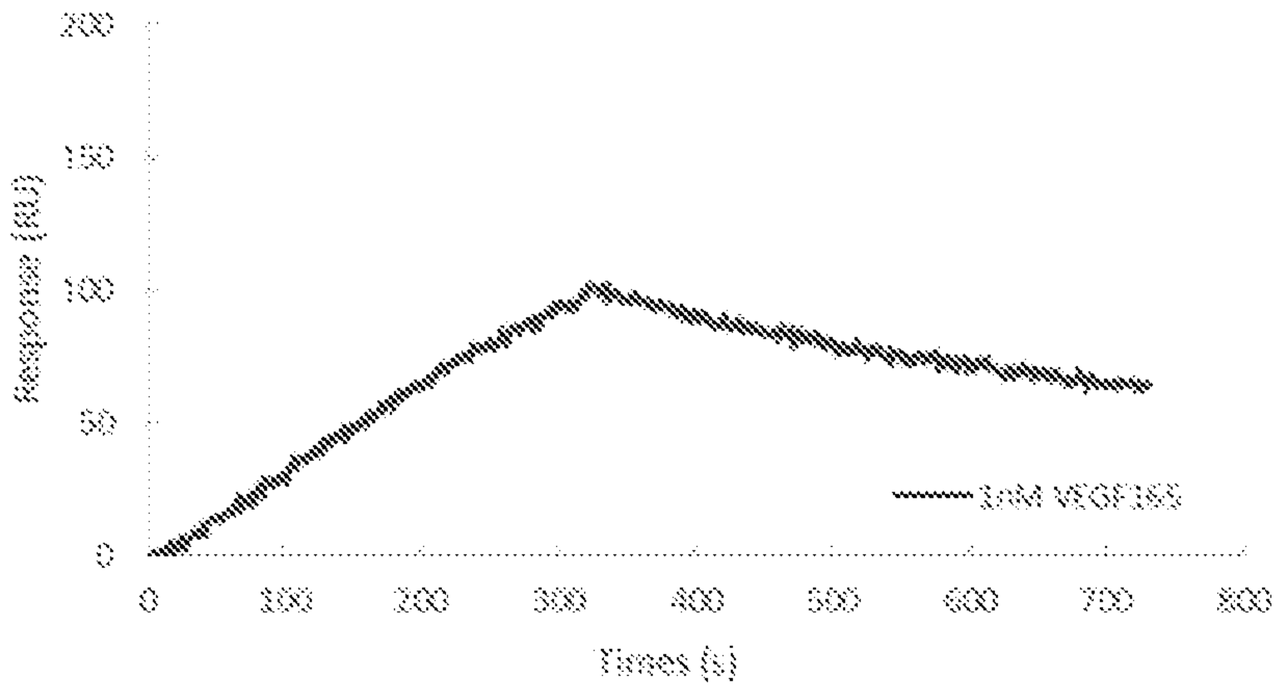

Aptamer 2 (VEGF_CaP_Sc31)
Response (RU)
200
150
100
50
0
1nM VEGF165
0
100
200
300
400
500
600
700
800
Times (s)

[Fig. 6C-6E]

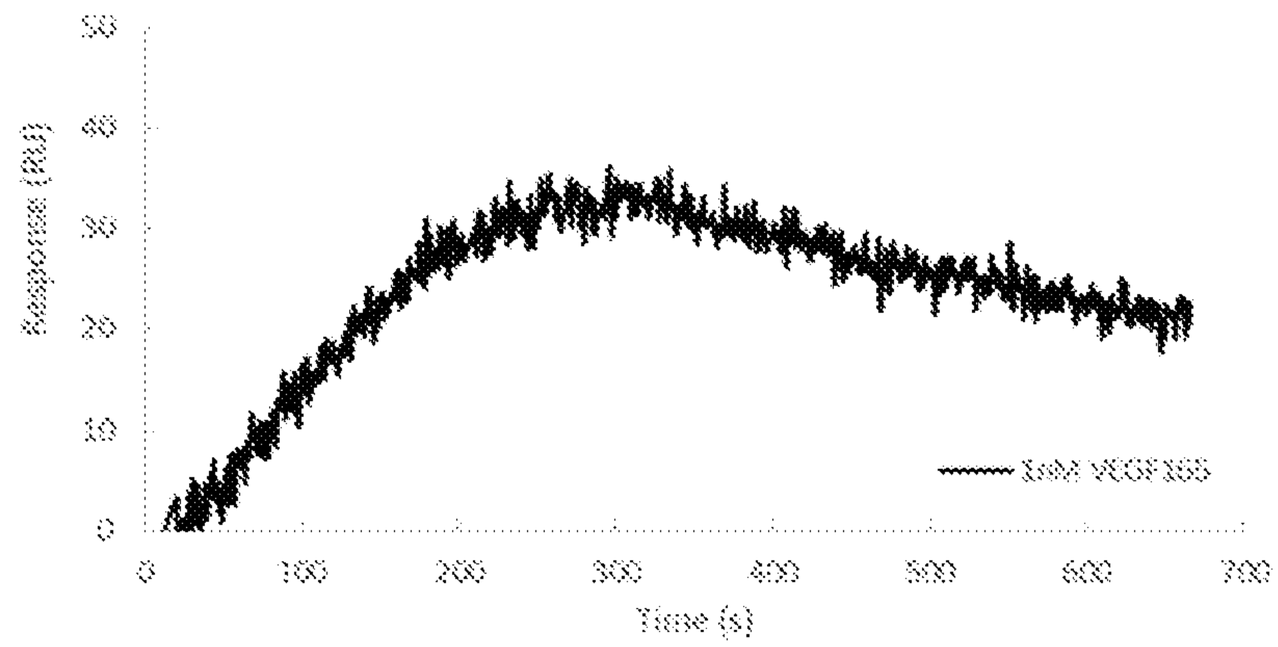
(C)


Aptamer 3 (VEGF_CaP_Ss49)
Response (RU)
200
150
100
50
0
0
100
200
300
400
500
600
700
800
Times (s)
1nM VEGF165

(D)

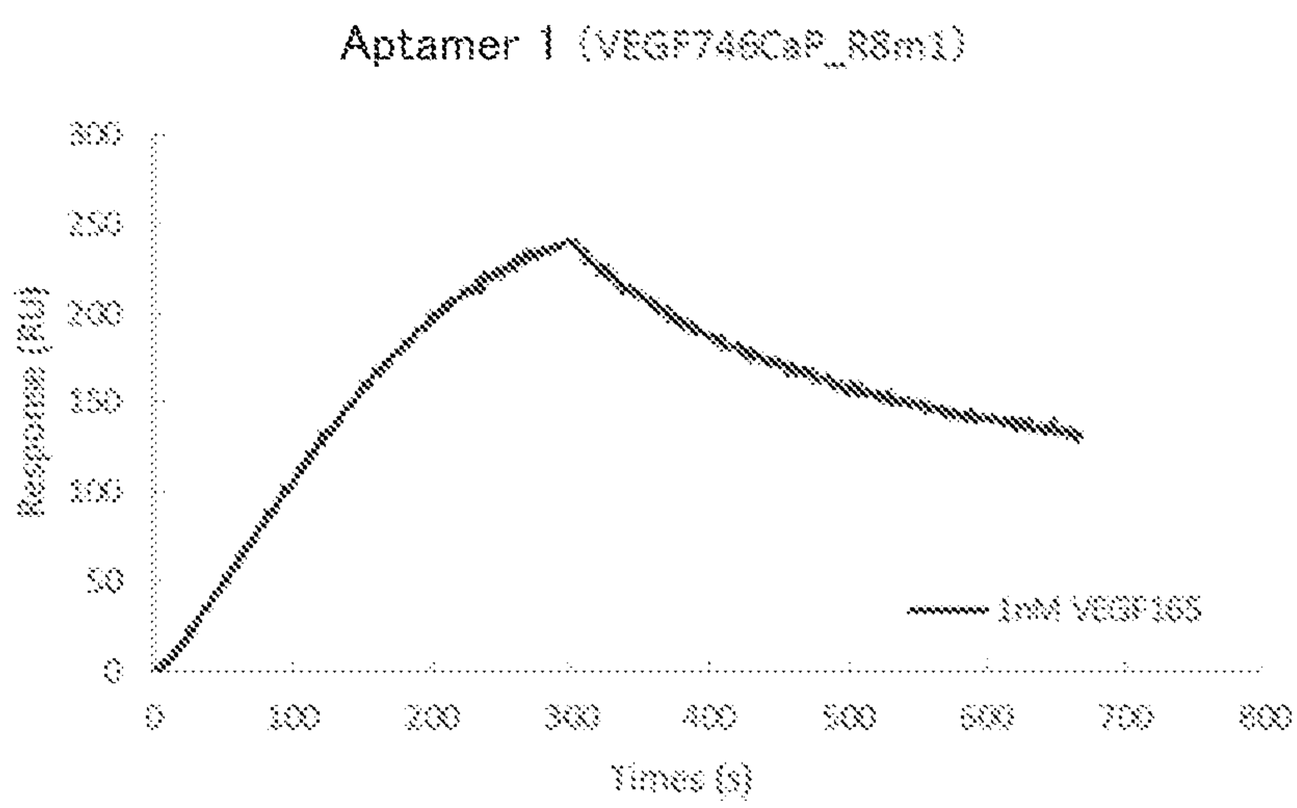

Aptamer 4 (VEGF_CaP_Ss62)
Response (RU)
200
150
100
50
0
0
100
200
300
400
500
600
700
800
Times (s)
3nM VEGF165

(E)

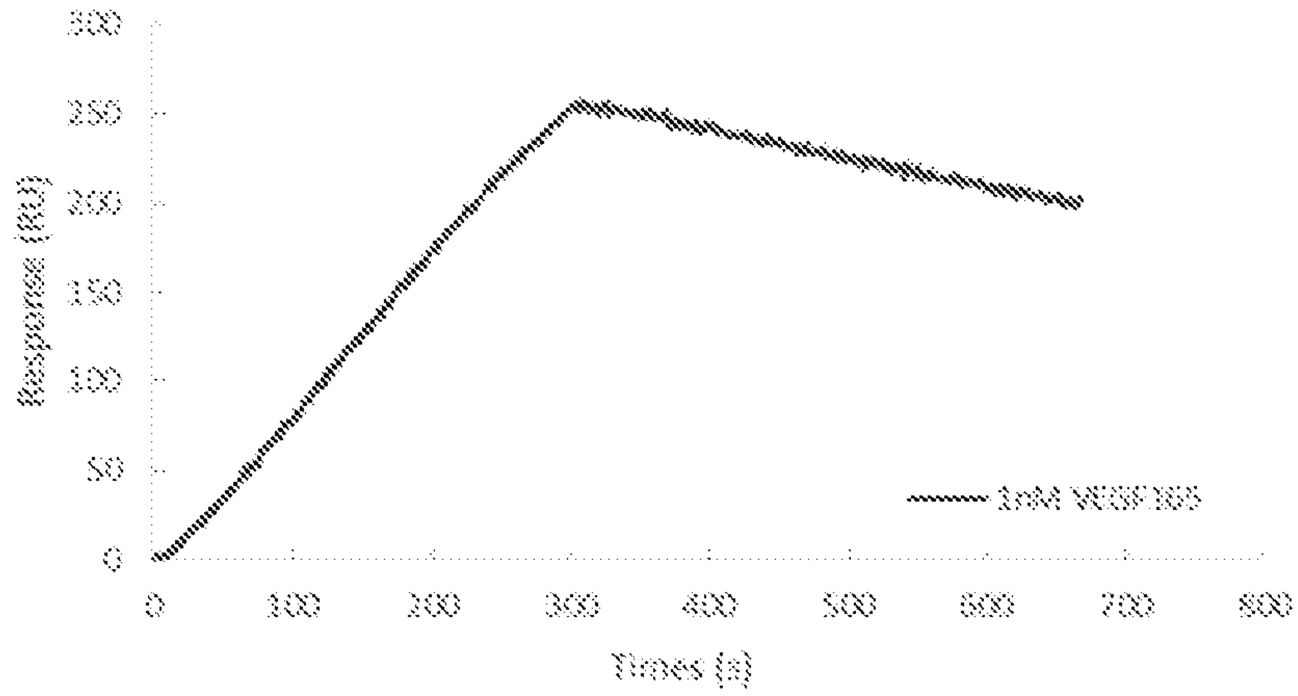

Aptamer 5 (VEGF746CaP_R8m49)
Response (RU)
50
40
30
20
10
0
0
100
200
300
400
500
600
700
Time (s)
1nM VEGF165

[Fig. 7A-7C]

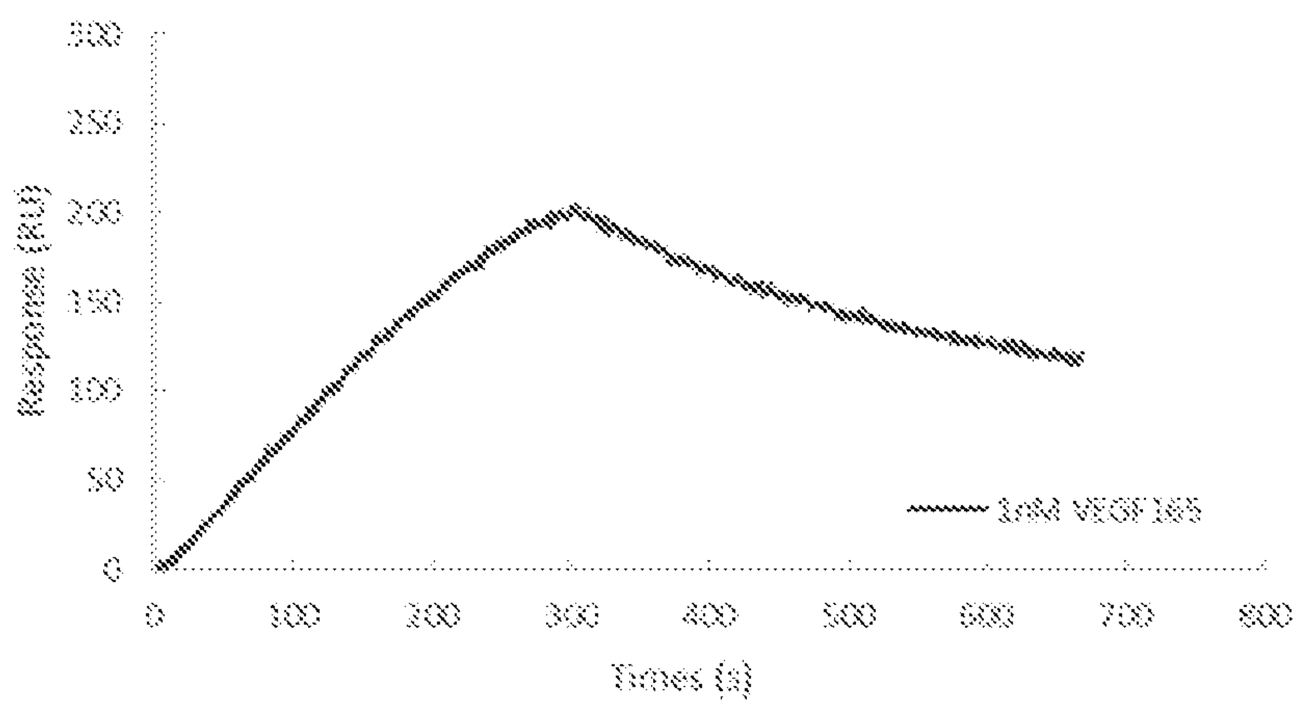
(A)


Aptamer 1 (VEGF746CaP_88m1)
Response (RU)
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
Times (s)
1nM VEGF165

(B)

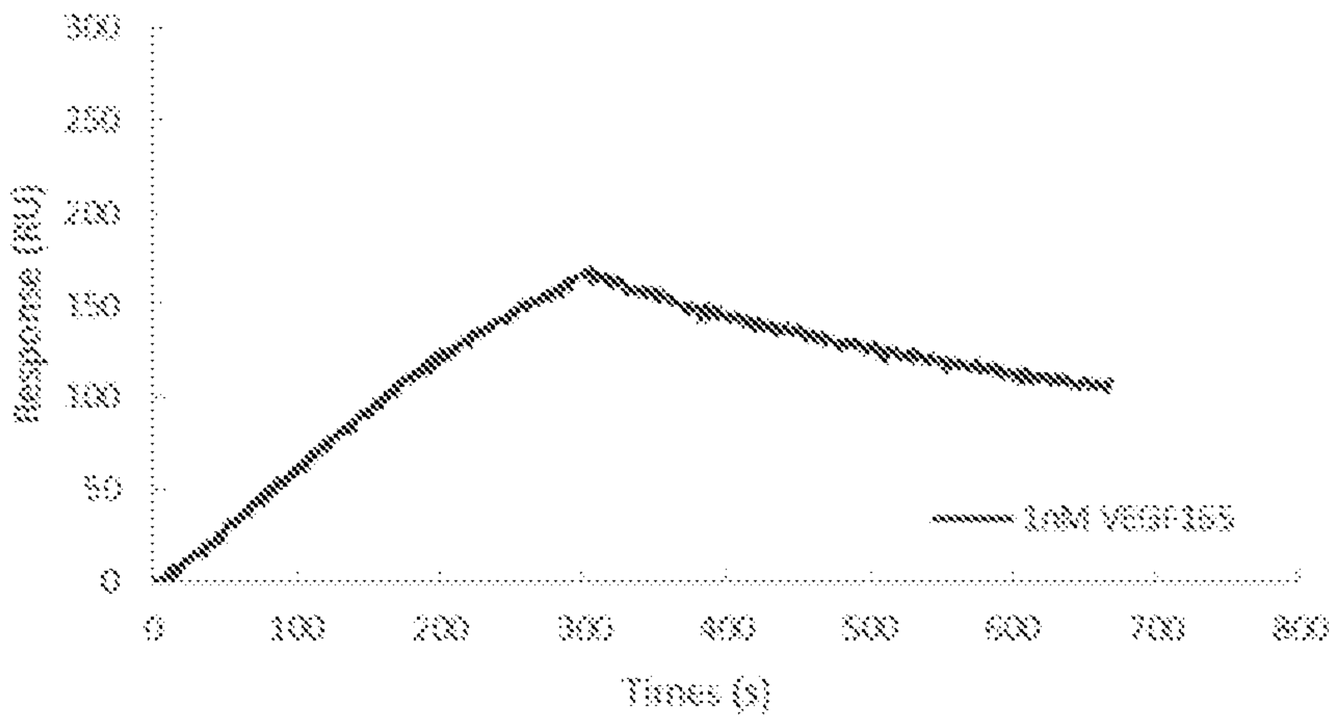

Aptamer 2 (VEGF_CaP_5s31)
Response (RU)
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
Times (s)
1nM VEGF165

(C)

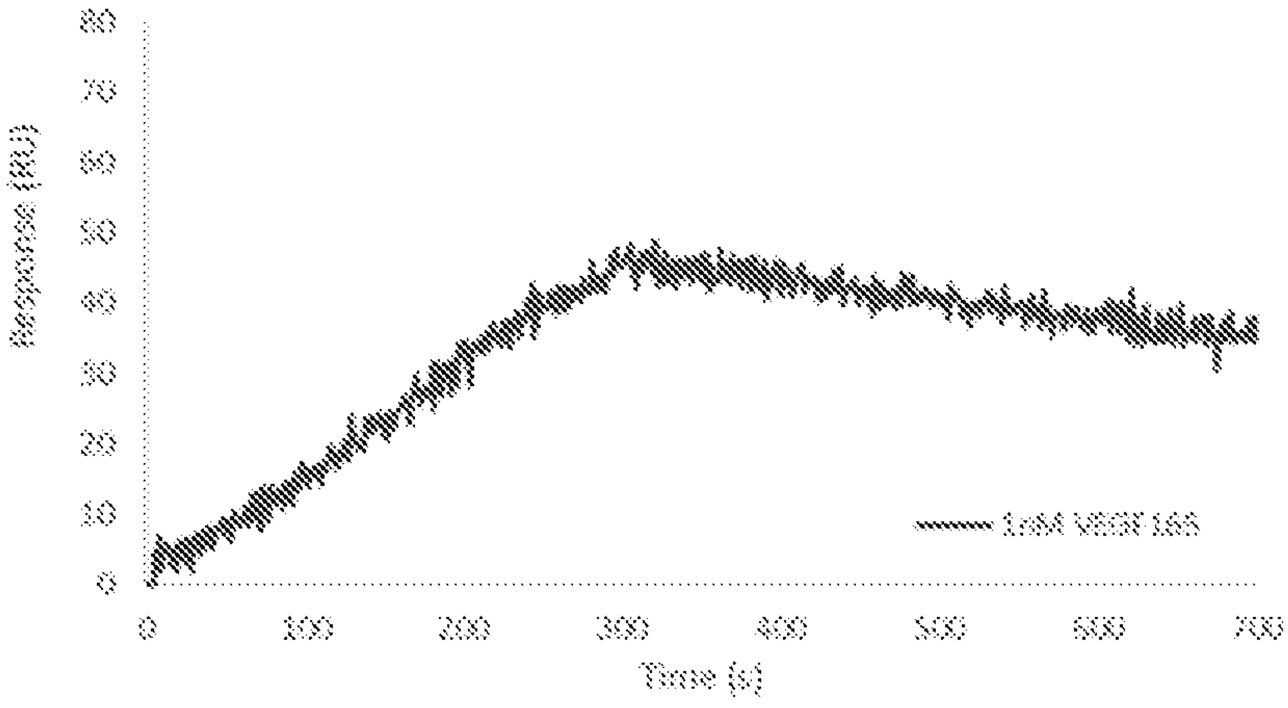

Aptamer 3 (VEGF_CaP_5s49)
Response (RU)
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
Times (s)
1nM VEGF165

[Fig. 7D-7E]

(D)

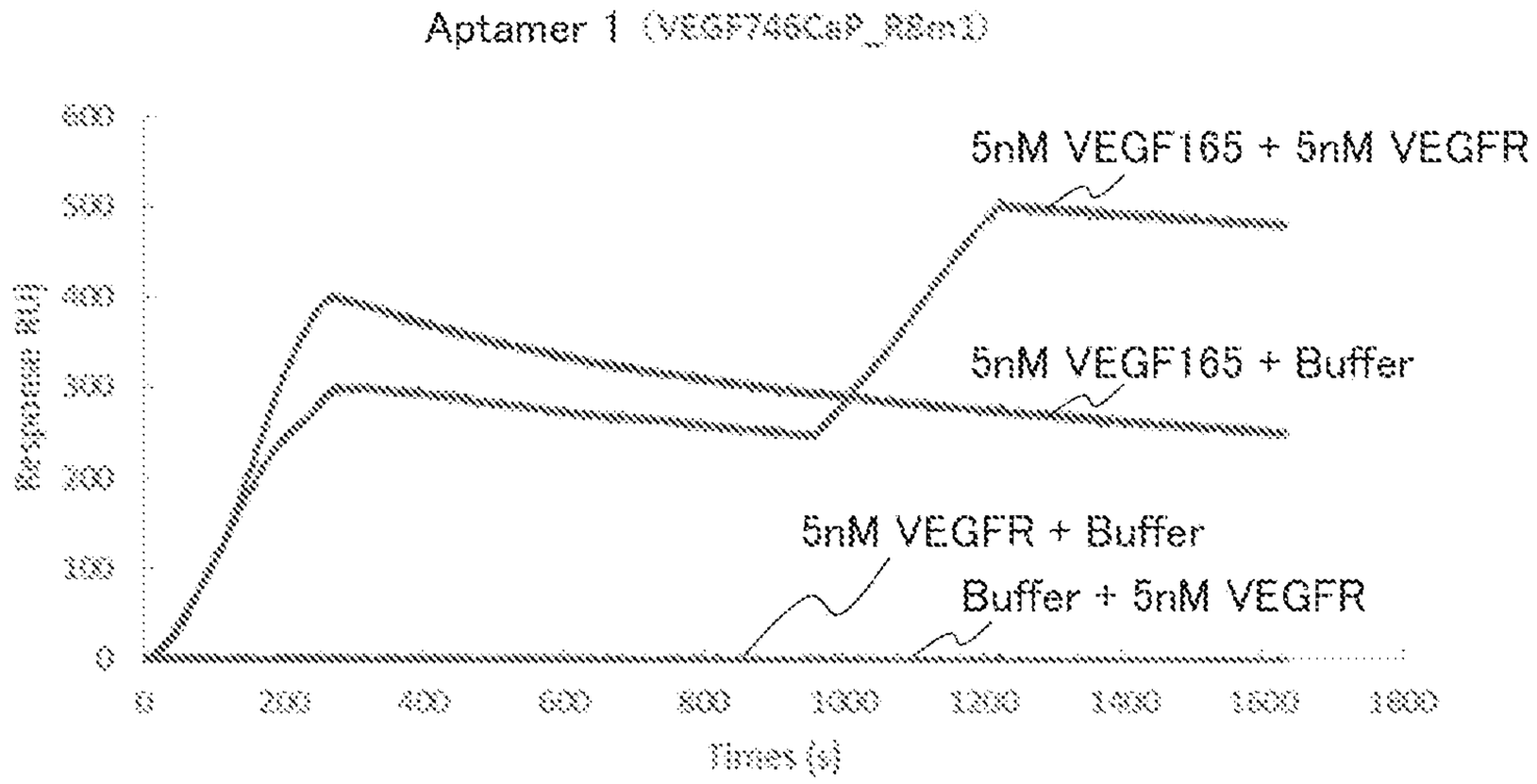

Aptamer 4 (VEGF_CaP_Ss62)
300
250
200
150
100
50
0
0
100
200
300
400
500
600
700
800
1nM VEGF165
Times (s)

(E)

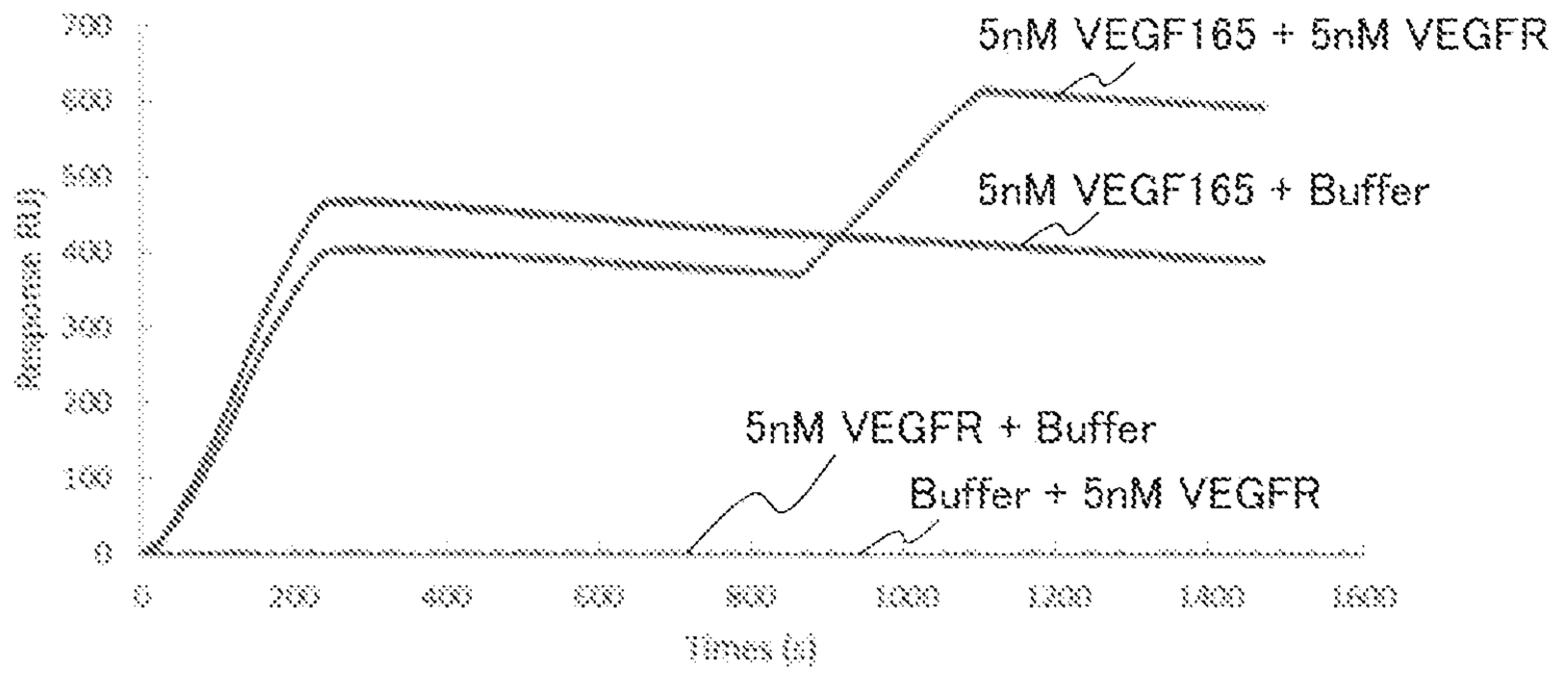

Aptamer 5 (VEGF746CaP_R8m49)
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
1nM VEGF 165
Time (s)

[Fig. 8A-8C]

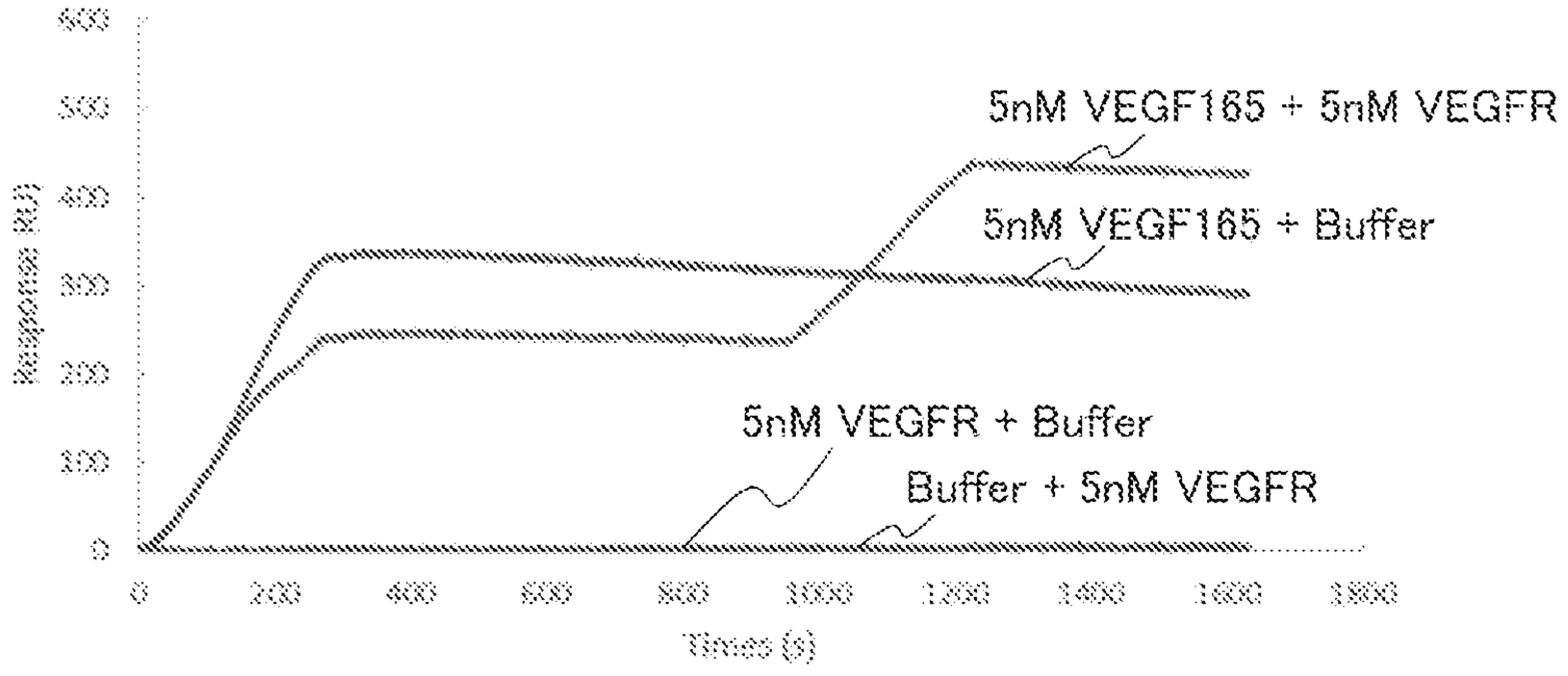
(A)


Aptamer 1 (VEGF746CaP_R8m1)
5nM VEGF165 + 5nM VEGFR
5nM VEGF165 + Buffer
5nM VEGFR + Buffer
Buffer + 5nM VEGFR
Response (RU)
Times (s)

(B)

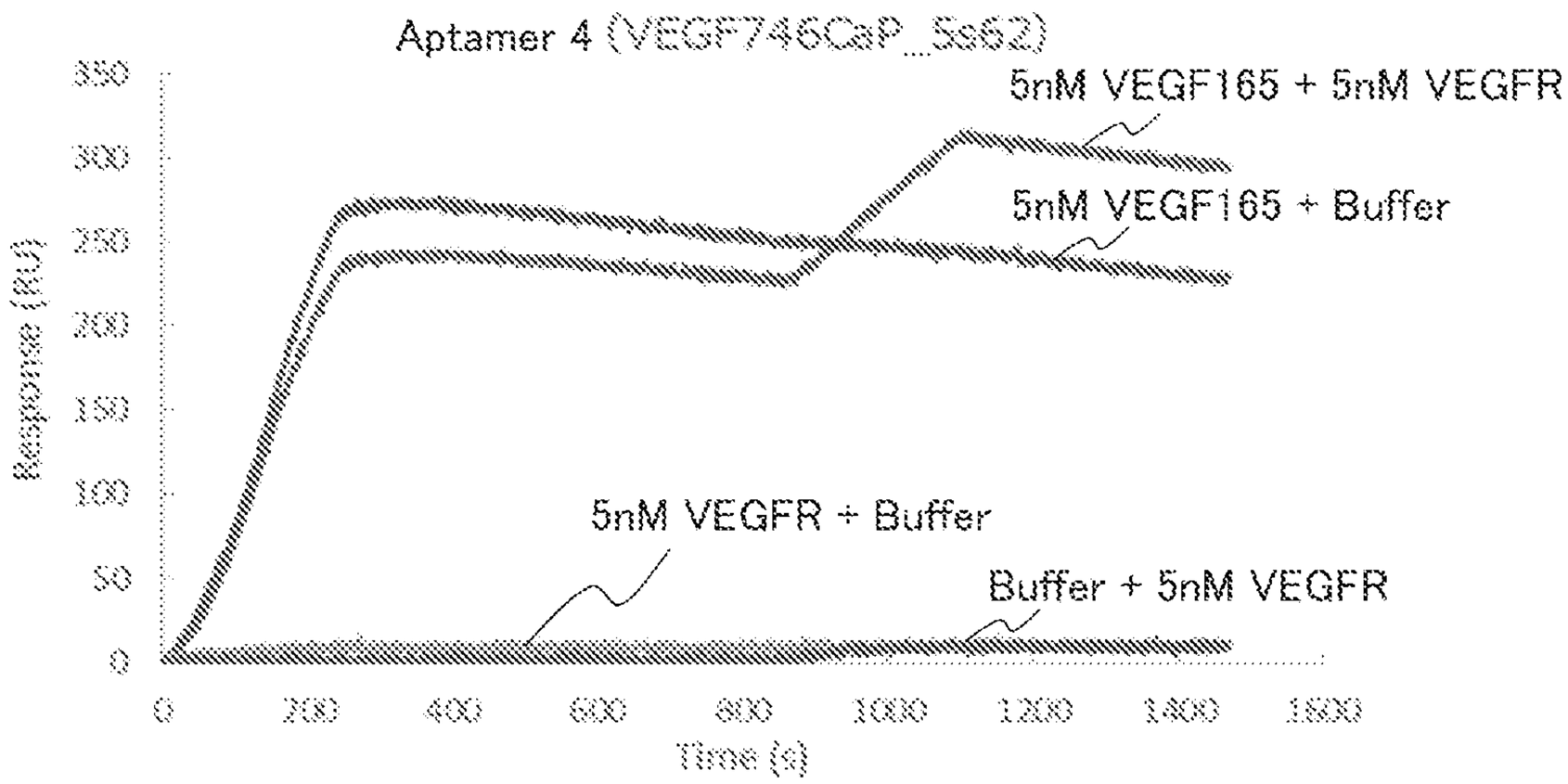

Aptamer 2 (VEGF746CaP_5s31)
5nM VEGF165 + 5nM VEGFR
5nM VEGF165 + Buffer
5nM VEGFR + Buffer
Buffer + 5nM VEGFR
Response (RU)
Times (s)

(C)

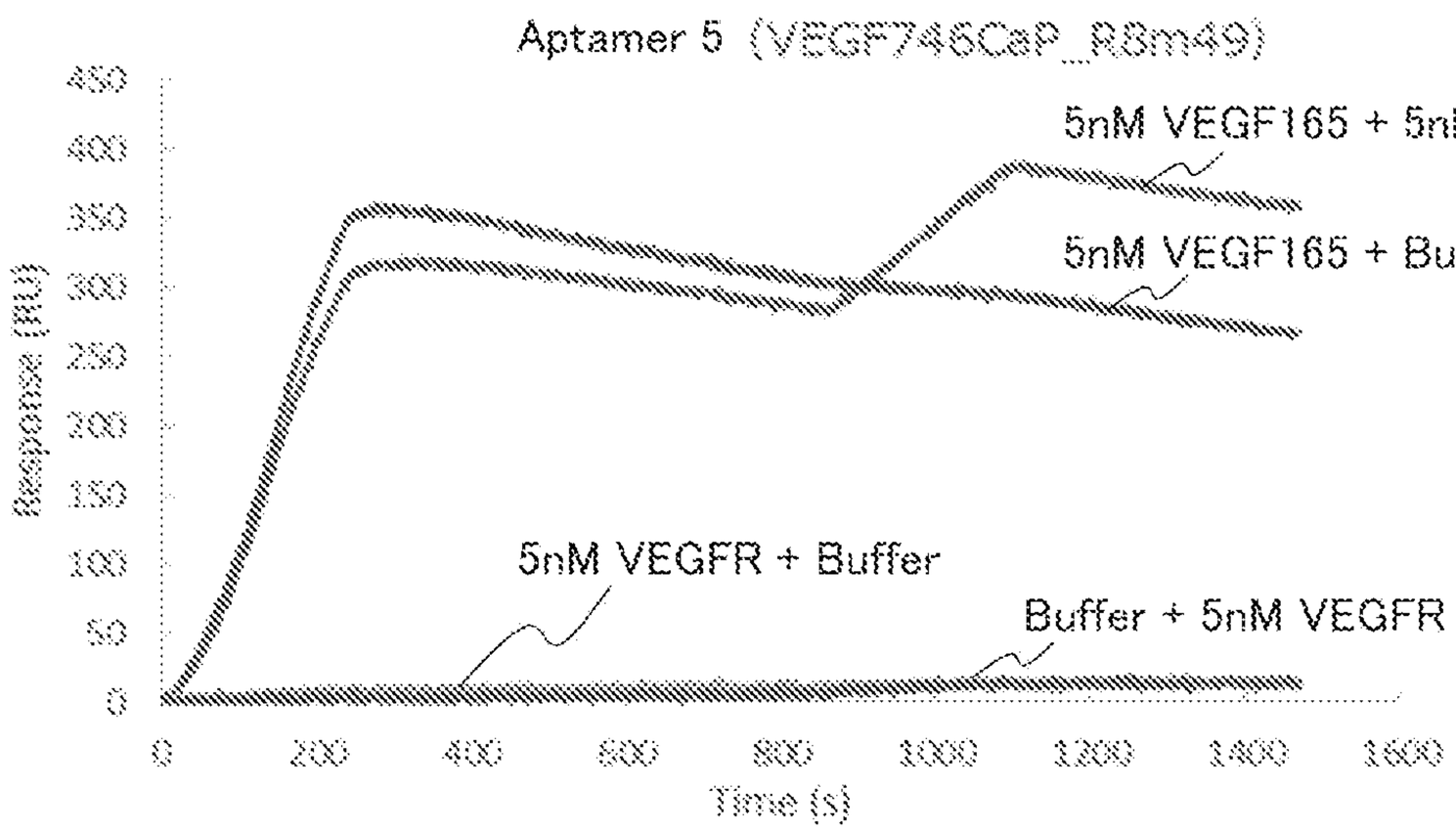

Aptamer 3 (VEGF_CaP_5s48)
5nM VEGF165 + 5nM VEGFR
5nM VEGF165 + Buffer
5nM VEGFR + Buffer
Buffer + 5nM VEGFR
Response (RU)
Times (s)

[Fig. 8D-8E]

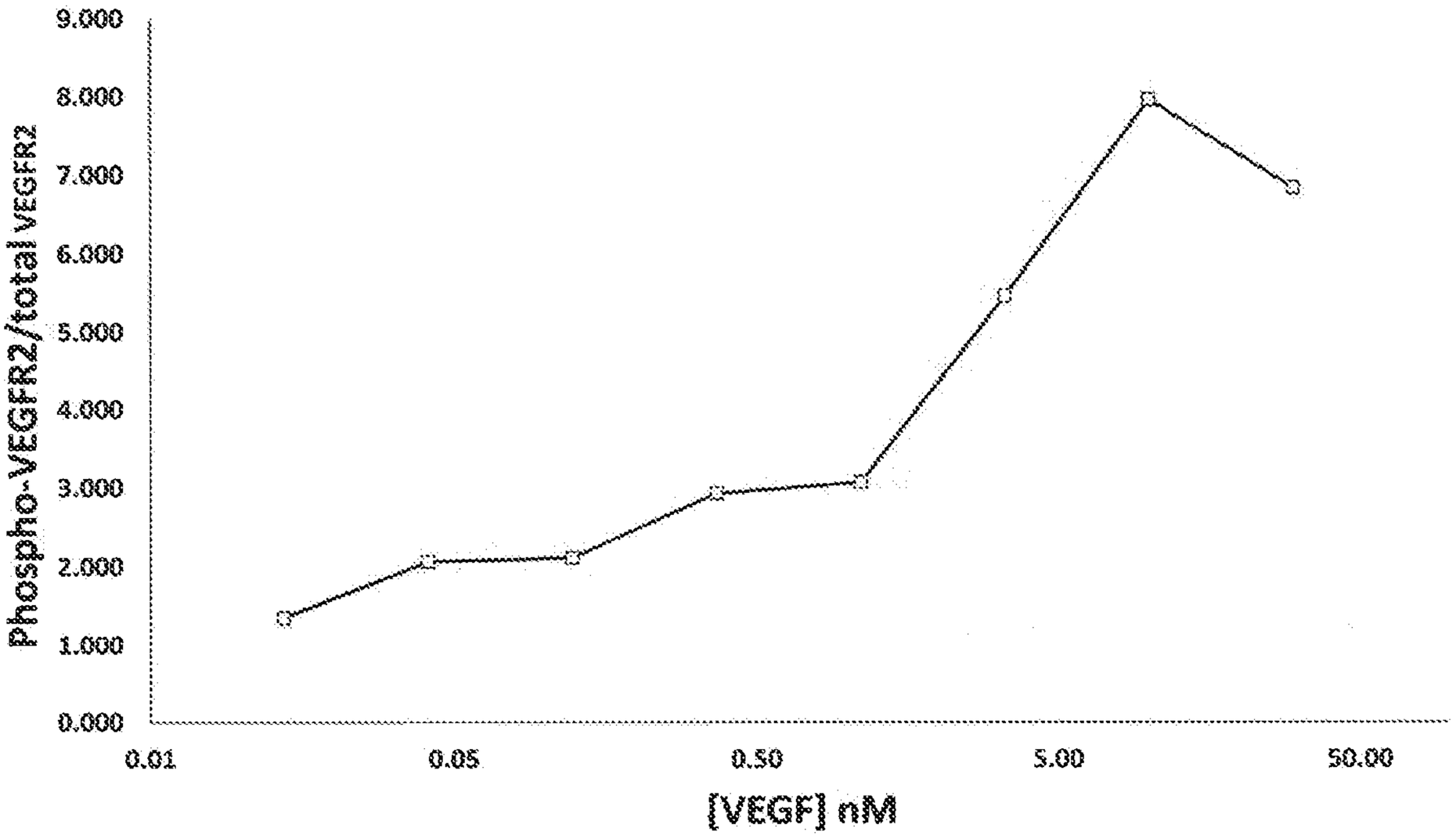
(D)


Aptamer 4 (VEGF746CaP_Ss62)
5nM VEGF165 + 5nM VEGFR
5nM VEGF165 + Buffer
5nM VEGFR + Buffer
Buffer + 5nM VEGFR
Response (RU)
Time (s)
0
50
100
150
200
250
300
350
0
200
400
600
800
1000
1200
1400
1800

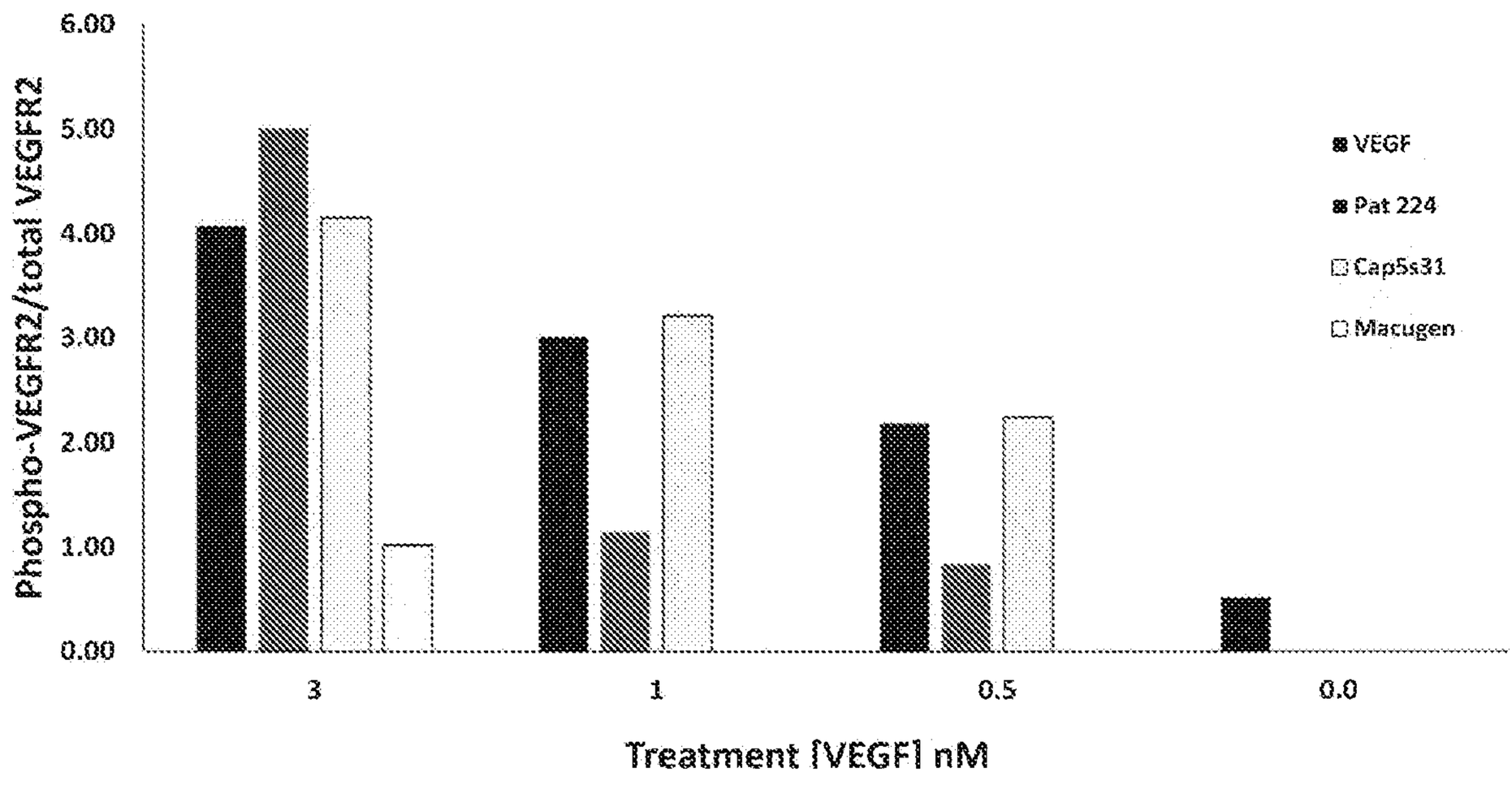
(E)


Aptamer 5 (VEGF746CaP_R8m49)
5nM VEGF165 + 5nM VEGFR
5nM VEGF165 + Buffer
5nM VEGFR + Buffer
Buffer + 5nM VEGFR
Response (RU)
Time (s)
0
50
100
150
200
250
300
350
400
450
0
200
400
600
800
1000
1200
1400
1600

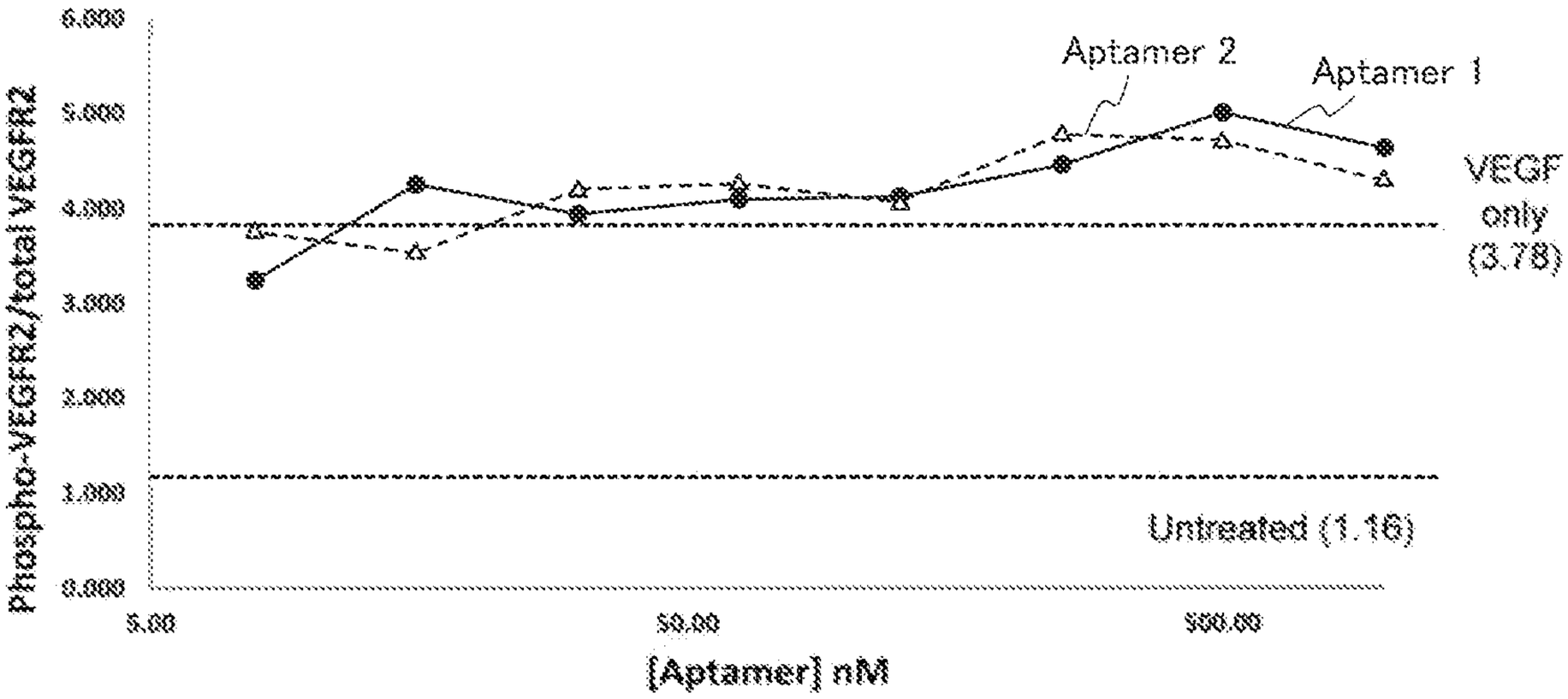
[Fig. 9]


Phospho-VEGFR2/total VEGFR2
9.000
8.000
7.000
6.000
5.000
4.000
3.000
2.000
1.000
0.000
0.01
0.05
0.50
5.00
50.00
[VEGF] nM

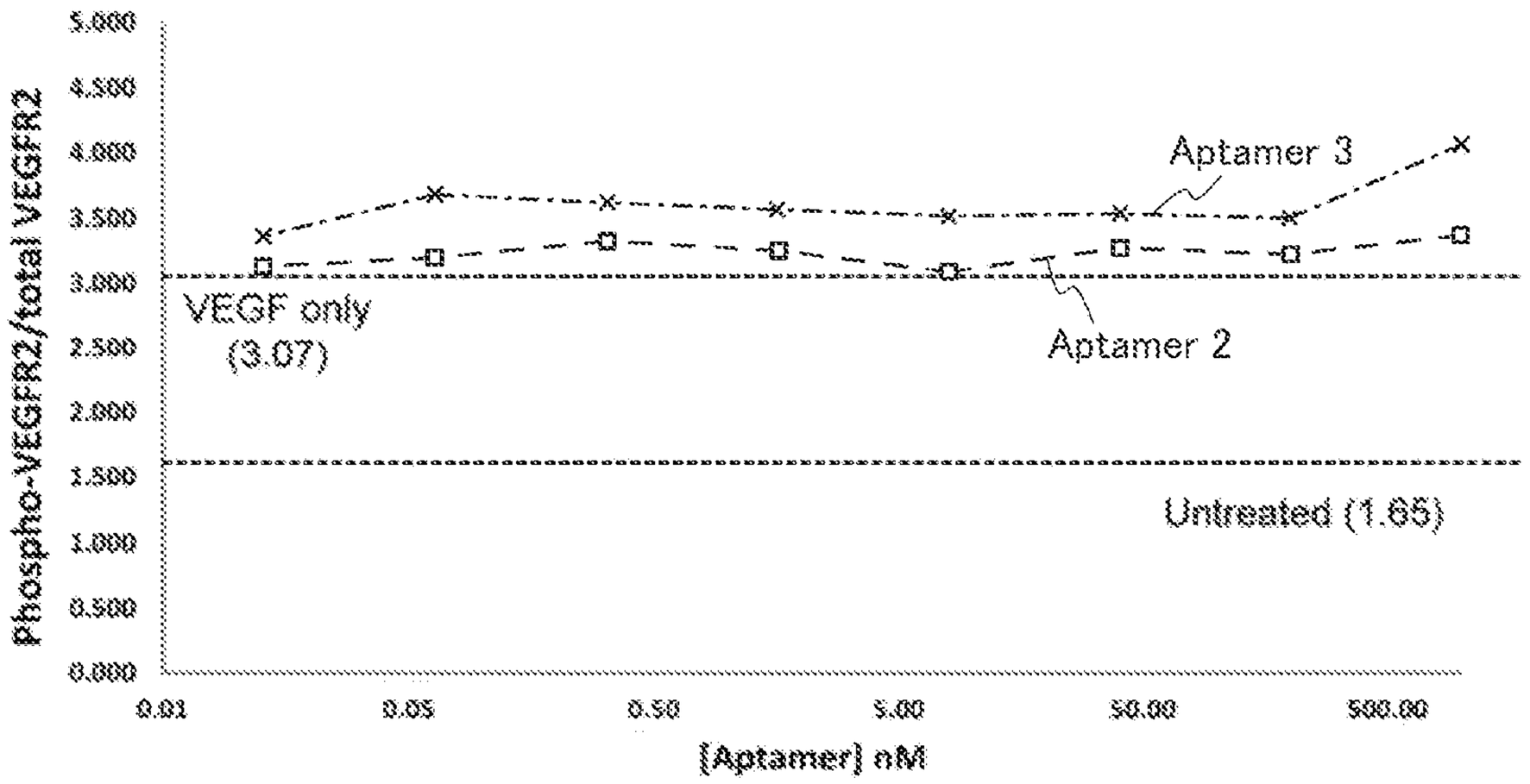
[Fig. 10]


Phospho-VEGFR2/total VEGFR2
6.00
5.00
4.00
3.00
2.00
1.00
0.00
VEGF
Pat 224
Cap5s31
Macugen
3
1
0.5
0.0
Treatment [VEGF] nM

[Fig. 11]



[Fig. 12]



[Fig. 13]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2020/018142

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/115(2010.01)i
FI: C12N15/115 Z

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)

C12N15/115

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

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2020
Registered utility model specifications of Japan 1996-2020
Published registered utility model applications of Japan 1994-2020

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

JSTPlus/JMEDPlus/JST7580 (JDreamIII); CAplus/MEDLINE/EMBASE/BIOSIS/WPIDS (STN); GenBank/EMBL/DDBJ/GeneSeq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORISHITA Y., Performance Report 2016 (project number 15J10771), KAKENHI-PROJECT [online], uploaded on 2018.01.16 [retrieved on 2020.07.14], KAKEN, which retrieved from the internet: <URL: https://kaken.nii.ac.jp/report/KAKENHI-PROJECT-15J10771/15J107712016jisseki/ > columns of abstract, achievement	1-26
Y		27-47
X	MORISHITA Y., e al., 39th annual meeting abstract of Japan Society for Biomaterials, 2017.11.13, vol. 39, p. 157 (1P-103-I) whole abstract	1-26
Y		27-47
Y	WO 2014/128289 A1 (LUMETTI, Simone) 28 August 2014 (2014-08-28) Abstract, claims 1-19, page 19 line 26 - page 20 line 2 (Family: none)	27-47

☑ 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
"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
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Date of the actual completion of the international search	Date of mailing of the international search report
16 July 2020	**28 July 2020**
Name and mailing address of the ISA/JP **Japan Patent Office** **3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo** **100-8915, Japan**	Authorized officer **NAKANO, Ai 4B 3758** Telephone No. **+81-3-3581-1101 Ext. 3448**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2020/018142

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ENAM S. F., et al., Enrichment of Endogenous Fractalkine and Anti-Inflammatory Cells via Aptamer-Functionalized Hydrogels, Biomaterials, 2017.07.10, vol. 142, pp. 52-61 Abstract, fig. 1	27-47
A	LEE J-H., et al., A Therapeutic Aptamer Inhibits Angiogenesis by Specifically Targeting the Heparin Binding Domain of VEGF165, Proceedings of the National Academy of Sciences of the United States of America, 2005.12.27, vol. 102, no. 52, pp. 18902-18907 Abstract	1-47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2020/018142

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: