loops exposed on the surface. The loops are defined as the amino acid regions which flank the six conserved cysteine residues and are highly variable in nature. Furthermore, the unique arrangement of the disulfide bonds renders cystine-knot peptides highly stable to thermal, proteolytic and chemical degradation.

[0006] Thus, there is a need to develop small, stable, artificial antibody-like molecules for a variety of therapeutic and diagnostic applications, such as ocular diseases and disorders. The present invention meets this and other needs.

BRIEF SUMMARY

[0006a] In one aspect of the present disclosure, there is provided a non-naturally occurring cystine knot peptide (CKP) that binds to vascular endothelial growth factor A (VEGF-A), wherein the CKP comprises the cystine scaffold structure:

$$Z_1C_1L1C_2L2C_3L3C_4L4C_5L5C_6Z_2$$

wherein:

Z_1 and Z_2 are any amino acid;

L1 is Loop 1 and has a structure selected from the group consisting of: $X_1X_2X_3X_4X_5X_6$ (SEQ ID NO: 2), $X_1X_2X_3X_4X_5X_6X_7$ (SEQ ID NO: 3), $X_1X_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO: 4), $X_1X_2X_3X_4X_5X_6X_7X_8X_9$ (SEQ ID NO: 5), and $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 6), wherein each of X_1 - X_{10} is any amino acid;

L2 is Loop 2 and has the structure: $X_1X_2X_3X_4X_5$ (SEQ ID NO: 7), wherein each of $X_1 - X_5$ is any amino acid or an unnatural amino acid;

L3 is Loop 3 and has the structure: $X_1X_2X_3$ wherein each of $X_1 - X_3$ is any amino acid or an unnatural amino acid;

L4 is Loop 4 and has the structure: X_1, wherein X_1 is any amino acid or an unnatural amino acid;

L5 is Loop 5 and has the structure: $X_1X_2X_3X_4X_5$ (SEQ ID NO: 7), wherein each of $X_1 - X_5$ is any amino acid or an unnatural amino acid;

wherein the unnatural amino acid is selected from the group consisting of L-propargylglycine-PEG_6-, L-sulfotyrosine, L-norleucine, L-1-naphthylalanine, L-2-naphthylalanine, L-2-chlorotryptophan, L-3-fluorotyrosine, L-4-fluorophenylalanine, gamma-benzyl-L-proline, gamma-(4-fluoro-benzyl)-L-proline, 4-OH-L-proline, 4-fluoro-L-

proline, 4-[4-(trifluoromethyl)-benzyl]-L-proline, 3,4-difluoro-L-phenylalanine, 3,4-dichloro-L-phenylalanine, 4-chloro-L-phenylalanine, 3-F,4-Cl-L-phenylalanine, 2-pyridone(NH para)-L-alanine, pyridone(NH meta)-L-alanine, 3-(1-N-methyl indole)-L-alanine, 3-(1-N-ethyl indole)-L-alanine, 3-(1-N-isopropyl indole)-L-alanine, 3-(5-aza-indole)-L-alanine, 4-methyl-L-phenylalanine, 2-naphthyl-L-alanine, L-4,4'-biphenylalanine, 3-(3-quinolinyl)-L-alanine, 3-(2-quinolinyl)-L-alanine, 3-(2-quinoxalinyl)-L-alanine, 4-methyl-2-pyridyl-alanine, 4-ethyl-2-pyridyl-L-alanine, benzothiazole-L-alanine, benzothiophene-L-alanine, 3-isoquinolinyl-L-alanine, t-butyl-L-alanine (also known as L-Nepentyl glycine), 3-cyclobutyl-L-alanine, cyclopentyl-L-alanine, 5,5,5-Trifluoro-L-leucine, t-butyl-L-glycine (also known as L-tert-Leucine), L-cyclopentylglycine, L-cyclobutylglycine, 3,4-hydroxy-L-phenylalanine, 3,4-fluoro-L-phenylalanine, 3-fluoro,4-OH-L-phenylalanine, 2-chloro-L-tyrosine, 2-methyl-L-tyrosine, 2-ethyl-L-tyrosine, 4-(naphthalen-1-ol)-L-alanine, D-serine, L-beta-homoserine, L-beta-alanine, N-alpha-methyl glycine, glycine amide, glycine ester of glycerol, glycine ester of glycol, glycine ester of oxetane-3-yl, and glycine morpholine amide;

wherein the CKP binds to VEGF-A with an affinity of 500 pM or better.

[0006b] In another aspect of the present disclosure, there is provided a non-naturally occurring cystine knot peptide (CKP) comprising an amino acid selected from the group consisting of: GCNIMLPFWGCGRDFECMEQCICQYYQSCG (SEQ ID NO: 113), GCNIMLPFWGCGRDFECVYRCICQYYQSCG (SEQ ID NO: 114), GCDVMQPYWGCGPDIDCFVRCLCHWYNSCG (SEQ ID NO: 139), GCDVMQPYWGCGPDIDCLSNCICHWYNSCG (SEQ ID NO: 140), GCNIMLPYWGCGRDFECMEQCICQYYQSCG (SEQ ID NO: 142), GCNIXLPFWGCGRDFECMSDCICQYYQSCG (SEQ ID NO: 144), wherein X is norleucine (Nle), GCNIXLPFWGCGRDFECVSQCICQYYQSCG (SEQ ID NO: 145), wherein X is norleucine (Nle), GCNIXLPYWGCGRDFECMEQCICQYYQSCG (SEQ ID NO: 146), wherein X is norleucine (Nle), and GCDVXQPYWGCGPDIDCLSNCICHWYNSCG (SEQ ID NO: 224), wherein X is norleucine.

[0006c] In yet another aspect of the present disclosure, there is provided a non-naturally occurring cystine knot peptide (CKP) comprising an amino acid selected from the group consisting of: GCDVX$_1$QPYWGCGPDI-D/E-CLS-N/K/X$_2$-CICHWYNSCG (SEQ ID NO: 534), GCDVX$_1$QPYWGCGPDI-N/K/X$_2$-CLS-D/E-CICHWYNSCG (SEQ ID NO: 535), GCNIX$_1$LPYWGCGRDF-D/E-CME-N/K/X$_2$.CICQYYQSCG (SEQ ID NO: 538), GCNIX$_1$LPYWGCGRDF-N/K/X$_2$-CME-D/E.CICQYYQSCG (SEQ ID NO: 539), GCNIX$_1$LPFWGCGRDF-D/E-CVS-N/K/X$_2$-CICQYYQSCG (SEQ ID NO: 540), and GCNIX$_1$LPFWGCGRDF-N/K/X$_2$-CVS-D/E-CICQYYQSCG (SEQ ID NO: 541), wherein X$_1$ is norleucine and X$_2$ is ornithine.

[0006d] In a further aspect of the present disclosure, there is provided a non-naturally occurring cystine knot peptide (CKP) that binds to VEGF-A, wherein the CKP comprises the cystine scaffold structure:

$$Z_1C_1L1C_2L2C_3L3C_4L4C_5L5C_6Z_2$$

wherein:

Z_1 and Z_2 are any amino acid, more than one amino acid, or an unnatural amino acid;

L1 is Loop 1 and has a structure selected from the group consisting of: $X_1X_2X_3X_4X_5X_6X_7X_8$, $X_1X_2X_3X_4X_5X_6X_7X_8X_9$, and $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, wherein each of X_1 - X_{10} is any amino acid or an unnatural amino acid;

L2 is Loop 2 and has the structure: $X_1X_2X_3X_4X_5$, wherein each of $X_1 - X_5$ is any amino acid or an unnatural amino acid;

L3 is Loop 3 and has the structure: $X_1X_2X_3$ wherein each of $X_1 - X_3$ is any amino acid or an unnatural amino acid;

L4 is Loop 4 and has the structure: X_1, wherein X_1 is any amino acid or an unnatural amino acid; L5 is Loop 5 and has the structure: $X_1X_2X_3X_4X_5$, wherein each of $X_1 - X_5$ is any amino acid or an unnatural amino acid;

wherein the unnatural amino acid selected from the group consisting of L-propargylglycine-PEG$_6$-, L-sulfotyrosine, L-norleucine, L-1-naphthylalanine, L-2-naphthylalanine, L-2-chlorotryptophan, L-3-fluorotyrosine, L-4-fluorophenylalanine,

gamma-benzyl-L-proline, gamma-(4-fluoro-benzyl)-L-proline, 4-OH-L-proline, 4-fluoro-L-proline, 4-[4-(trifluoromethyl)-benzyl]-L-proline, 3,4-difluoro-L-phenylalanine, 3,4-dichloro-L-phenylalanine, 4-chloro-L-phenylalanine, 3-F,4-Cl-L-phenylalanine, 2-pyridone(NH para)-L-alanine, pyridone(NH meta)-L-alanine, 3-(1-N-methyl indole)-L-alanine, 3-(1-N-ethyl indole)-L-alanine, 3-(1-N-isopropyl indole)-L-alanine, 3-(5-aza-indole)-L-alanine, 4-methyl-L-phenylalanine, 2-naphthyl-L-alanine, L-4,4'-biphenylalanine, 3-(3-quinolinyl)-L-alanine, 3-(2-quinolinyl)-L-alanine, 3-(2-quinoxalinyl)-L-alanine, 4-methyl-2-pyridyl-alanine, 4-ethyl-2-pyridyl-L-alanine, benzothiazole-L-alanine, benzothiophene-L-alanine, 3-isoquinolinyl-L-alanine, t-butyl-L-alanine (also known as L-Nepentyl glycine), 3-cyclobutyl-L-alanine, cyclopentyl-L-alanine, 5,5,5-Trifluoro-L-leucine, t-butyl-L-glycine (also known as L-tert-Leucine), L-cyclopentylglycine, L-cyclobutylglycine, 3,4-hydroxy-L-phenylalanine, 3,4-fluoro-L-phenylalanine, 3-fluoro,4-OH-L-phenylalanine, 2-chloro-L-tyrosine, 2-methyl-L-tyrosine, 2-ethyl-L-tyrosine, 4-(naphthalen-1-ol)-L-alanine, D-serine, L-beta-homoserine, L-beta-alanine, N-alpha-methyl glycine, glycine amide, glycine ester of glycerol, glycine ester of glycol, glycine ester of oxetane-3-yl, and glycine morpholine amide, and

wherein the CKP has an altered disulfide bond connectivity with reference to a wild-type *Ecballium elaterium* trypsin inhibitor EETI-II protein having the amino acid sequence set forth in SEQ ID NO: 1; wherein the altered disulfide bond connectivity is C1-C4, C2-C3 and C5-C6; and wherein the CKP has a percent alpha helix content of at least 20%.

[0006e] In another aspect of the present disclosure, there is provided a non-naturally occurring CKP that competes with the antibody G6.31 for binding to VEGF-A.

[0006f] In yet another aspect of the present disclosure, there is provided a non-naturally occurring CKP that competes with the CKP disclosed herein for binding to VEGF-A.

[0006g] In a further aspect of the present disclosure, there is provided a non-naturally occurring CKP that binds to an epitope on VEGF-A comprising at least one of the amino acid residues selected from the group consisting of: V14, V15, F17, D19,Y21, Q22, Y25, I46, K48, N62, D63, L66, M81, I83, K84, P85, H86, G88, Q89, I91, C104, R105, and P106.

[0006h] In another aspect of the present disclosure, there is provided an isolated nucleic acid encoding the CKP disclosed herein.

[0006i] In yet another aspect of the present disclosure, there is provided an expression vector encoding the nucleic acid molecule disclosed herein.

[0006j] In a further aspect of the present disclosure, there is provided a cell comprising the expression vector disclosed herein.

[0006k] In another aspect of the present disclosure, there is provided a method of producing the CKP disclosed herein comprising culturing the cell disclosed herein and recovering the CKP from the cell culture.

[0006l] In yet another aspect of the present disclosure, there is provided a method of producing the CKP disclosed herein, comprising chemically synthesizing the CKP.

[0006m] In a further aspect of the present disclosure, there is provided a composition comprising the CKP disclosed herein and a pharmaceutically acceptable carrier.

[0006n] In another aspect of the present disclosure, there is provided a composition comprising the CKP disclosed herein, for use in treating an ocular disease characterized by angiogenesis and/or vascular permeability or leakage in a subject.

[0006o] In yet another aspect of the present disclosure, there is provided a use of a composition comprising the CKP disclosed herein in the manufacture of a medicament for treating an ocular disease characterized by angiogenesis and/or vascular permeability or leakage in a subject.

[0006p] In a further aspect of the present disclosure, there is provided a formulation comprising the CKP disclosed herein and PLGA.

[0006q] In another aspect of the present disclosure, there is provided a non-naturally occurring cystine knot peptide (CKP) that binds to human low density lipoprotein receptor-related protein 6 (LRP6), wherein the CKP comprises the cystine scaffold structure:

$$Z_1C_1L1C_2L2C_3L3C_4L4C_5L5C_6Z_2;$$

wherein:

Z_1 and Z_2 are any amino acid;

L1 is Loop 1 and has a structure selected from the group consisting of: $X_1X_2X_3X_4X_5X_6$, $X_1X_2X_3X_4X_5X_6X_7$, $X_1X_2X_3X_4X_5X_6X_7X_8$, $X_1X_2X_3X_4X_5X_6X_7X_8X_9$, and $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, wherein each of X_1 - X_{10} is any amino acid;

L2 is Loop 2 and has the structure: $X_1X_2X_3X_4X_5$, wherein each of $X_1 - X_5$ is any amino acid;

L3 is Loop 3 and has the structure: $X_1X_2X_3$ wherein each of $X_1 - X_3$ is any amino acid;

L4 is Loop 4 and has the structure: X_1, wherein X_1 is any amino acid; and

L5 is Loop 5 and has the structure: $X_1X_2X_3X_4X_5$, wherein each of $X_1 - X_5$ is any amino acid.

[0007] In certain embodiments, provided herein is a non-naturally occurring cystine knot peptide (CKP) that binds to vascular endothelial growth factor A (VEGF-A), wherein the CKP comprises the cystine scaffold structure:

$$Z_1C_1L1C_2L2C_3L3C_4L4C_5L5C_6Z_2$$

wherein:

Z_1 and Z_2 are any amino acid;

L1 is Loop 1 and has a structure selected from the group consisting of: $X_1X_2X_3X_4X_5X_6$ (SEQ ID NO: 2), $X_1X_2X_3X_4X_5X_6X_7$ (SEQ ID NO: 3), $X_1X_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO: 4), $X_1X_2X_3X_4X_5X_6X_7X_8X_9$ (SEQ ID NO: 5), and $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$ (SEQ ID NO: 6), wherein each of X_1 - X_{10} is any amino acid;

L2 is Loop 2 and has the structure: $X_1X_2X_3X_4X_5$ (SEQ ID NO: 7), wherein each of $X_1 - X_5$ is any amino acid or an unnatural amino acid;

L3 is Loop 3 and has the structure: $X_1X_2X_3$, wherein each of $X_1 - X_3$ is any amino acid or an unnatural amino acid;

L4 is Loop 4 and has the structure: X_1, wherein X_1 is any amino acid or an unnatural amino acid;

L5 is Loop 5 and has the structure: $X_1X_2X_3X_4X_5$ (SEQ ID NO: 7), wherein each of $X_1 - X_5$ is any amino acid or an unnatural amino acid;

wherein the unnatural amino acid is selected from the group consisting of L-propargylglycine-PEG_6-, L-sulfotyrosine, L-norleucine, L-1-naphthylalanine, L-2-naphthylalanine, L-2-chlorotryptophan, L-3-fluorotyrosine, and L-4-fluorophenylalanine; and

wherein the CKP binds to VEGF-A with an affinity of 500 pM or better.

[0008] In certain embodiments according to (or as applied to) any of the embodiments above, the non-naturally occurring (CKP) that binds to VEGF-A has an altered disulfide bond

104. The CKP of any one of claims 1-103 conjugated to a therapeutic agent.

105. The CKP of any one of claims 1-103 conjugated to a label.

106. The CKP of claim 105, wherein the label is selected from the group consisting of a radioisotope, a fluorescent dye, and an enzyme.

107. An isolated nucleic acid encoding the CKP of any one of claims 1-103.

108. An expression vector encoding the nucleic acid molecule of claim 107.

109. A cell comprising the expression vector of claim 108.

110. A method of producing the CKP of any one of claims 1-103 comprising culturing the cell of claim 109 and recovering the CKP from the cell culture.

111. A method of producing the CKP of any one of claims 1-103, comprising chemically synthesizing the CKP.

112. A composition comprising the CKP of any one of claims 1-104 and a pharmaceutically acceptable carrier.

113. A composition comprising the CKP of any one of claims 1-104, for use in treating an ocular disease characterized by angiogenesis and/or vascular permeability or leakage in a subject.

114. The composition for use according to claim 113, wherein the ocular disease is an intraocular neovascular disease selected from the group consisting of proliferative retinopathies, choroidal neovascularization (CNV), age-related macular degeneration (AMD), diabetic and other ischemia-related retinopathies, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, retinal vein occlusion (RVO), including Central Retinal Vein Occlusion (CRVO) and branched retinal vein occlusion (BRVO), corneal neovascularization, retinal neovascularization, and retinopathy of prematurity (ROP).

115. The composition for use according to claim 113 or 114, wherein the composition is to be administered to the subject via an implantable device.

116. The composition for use according to claim 115, wherein the implantable device selected from the group consisting of: an ocular insert, a slow-release depot, an ocular plug/reservoir, an non-biodegradable ocular implant or a biodegradable ocular implant.

117. Use of a composition comprising the CKP of any one of claims 1-104 in the manufacture of a medicament for treating an ocular disease characterized by angiogenesis and/or vascular permeability or leakage in a subject.

118. The use according to claim 117, wherein the ocular disease is an intraocular neovascular disease selected from the group consisting of proliferative retinopathies, choroidal neovascularization (CNV), age-related macular degeneration (AMD), diabetic and

other ischemia-related retinopathies, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, retinal vein occlusion (RVO), including Central Retinal Vein Occlusion (CRVO) and branched retinal vein occlusion (BRVO), corneal neovascularization, retinal neovascularization, and retinopathy of prematurity (ROP).

119. The use according to claim 117 or 118, wherein the medicament is to be administered to the subject via an implantable device.

120. The use according to claim 119, wherein the implantable device selected from the group consisting of: an ocular insert, a slow-release depot, an ocular plug/reservoir, an non-biodegradable ocular implant or a biodegradable ocular implant.

121. The CKP of any one of claims 1-104, wherein the CKP is formulated for long acting delivery.

122. A formulation comprising the CKP of any of claims 1-104 and PLGA.

123. The formulation of claim 122, wherein the PLGA is a PLGA rod.

124. A non-naturally occurring cystine knot peptide (CKP) that binds to human low density lipoprotein receptor-related protein 6 (LRP6), wherein the CKP comprises the cystine scaffold structure:

$$Z_1C_1L1C_2L2C_3L3C_4L4C_5L5C_6Z_2;$$

wherein:

Z_1 and Z_2 are any amino acid;

L1 is Loop 1 and has a structure selected from the group consisting of: $X_1X_2X_3X_4X_5X_6$, $X_1X_2X_3X_4X_5X_6X_7$, $X_1X_2X_3X_4X_5X_6X_7X_8$, $X_1X_2X_3X_4X_5X_6X_7X_8X_9$, and $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}$, wherein each of X_1 - X_{10} is any amino acid;

L2 is Loop 2 and has the structure: $X_1X_2X_3X_4X_5$, wherein each of $X_1 - X_5$ is any amino acid;

L3 is Loop 3 and has the structure: $X_1X_2X_3$ wherein each of $X_1 - X_3$ is any amino acid;

L4 is Loop 4 and has the structure: X_1, wherein X_1 is any amino acid; and

L5 is Loop 5 and has the structure: $X_1X_2X_3X_4X_5$, wherein each of $X_1 - X_5$ is any amino acid.

125. The CKP of claim 124, wherein Z_1 and/or Z_2 is G.

126. The CKP of any one of claims 124 or 125, wherein in L1:
X_1 is an amino acid selected from R, V, M, A, G, N, S, and E;
X_2 is an amino acid selected from T, N, S, G, R, and A;
X_3 is an amino acid selected from N, R, H, V, K, S, G, I, and Y;
X_4 is an amino acid selected from R, V, N, I, K, S, and T;
X_5 is an amino acid selected from V, R, K, I, T, S, L, and N; and
X_6 is an amino acid selected from K, G, A, I, R, N, S, and V.

127. The CKP of any one of claims 124-126, wherein in L1:
X_7 is an amino acid selected from G, R, K, E, P, and T.

128. The CKP of any one of claims 124-127, wherein in L1:
X_8 is an amino acid selected from G, R, K, Q, A, and S.

129. The CKP of any one of claims 124-128, wherein in L1:
X_9 is an amino acid selected from R or G.

130. The CKP of any one of claims 124-129, wherein in L1:
X_{10} is an amino acid selected from E, W, and G.

131. The CKP of any one of claims 124-130, wherein in L5:
X_1 is an amino acid selected from G, S, N, Y, A, and R;
X_2 is an amino acid selected from P, G, S, V, E, R, F, and D;
X_3 is an amino acid selected from N, G, S, E, P, K, H, and R;
X_4 is an amino acid selected from G, R, H, S, Q, V, and D; and
X_5 is an amino acid selected from F, D, N, R, G, Y, S, and T.

132. The CKP of any one of claims 124-131, wherein in L2, X_1 is K, X_2 is Q, X_3 is D, X_4 is S, and X_5 is D.

133. The CKP of any one of claims 124-132, wherein in L3, X_1 is L, X_2 is A, and X_3 is G.

134. The CKP of any one of claims 124-133, wherein in L4, X_1 is V.