

TNF-ALPHA BINDING AGENTS AND METHODS OF USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/483839, filed February 8, 2023, the disclosure of which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing XML associated with this application is provided in XML format and is hereby incorporated by reference into the specification. The name of the XML file containing the sequence listing is 4298-P1WO_Seq_List_20240207.xml. The XML file is 689,475 bytes; was created on February 7, 2024; and is being submitted electronically via Patent Center with the filing of the specification.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

[0003] This invention was made with government support under SBIR Grant Project Numbers 1R43DK117777-01 by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0004] The FDA-approved anti-TNF α therapies include four antibodies, HUMIRA® (adalimumab), REMICADE® (infliximab), CIMZIA® (certolizumab pegol), and SIMPONI ARIA® (golimumab) and one soluble form of TNFR, ENBREL® (etanercept). These drugs are used to treat a wide range of inflammatory conditions, including ulcerative colitis, Crohn's disease, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and psoriasis among others. The value of anti-TNF α therapies in treating chronic inflammatory disease is evident since HUMIRA® is the top selling drug in the world. However, approved anti-TNF α biologics are limited by the requirement for parenteral administration, systemic distribution associated with immunosuppression and heightened risk for infection, and immunogenicity associated with anti-drug antibodies (ADAs) responsible for loss of therapeutic activity. The present invention addresses this and other needs.

SUMMARY

[0005] This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary

is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

[0006] Provided herein are D-peptide-based inhibitors of TNF α . In some embodiments, the peptides inhibit TNF α activity by binding to TNF α . In some embodiments, the peptides inhibit TNF α activity by binding to TNF α and blocking its binding to its receptors, TNFR1 and/or TNFR2. The TNFi peptides comprise a core TNF α binding domain composed of D-amino acids.

[0007] In some embodiments, provided is a D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C (SEQ ID NO:111), wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) or Tyr (Y); X¹¹ is the D form of Trp (W), Gln (Q), Tyr, or His (H); and C denotes the D form of Cysteine.

[0008] In some embodiments, provided is a D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C*-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C* (SEQ ID NO:112), wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) and Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), and Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) and Tyr (Y); X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H); C denotes the D form of Cysteine; and * denotes an optional intramolecular bond.

[0009] In some embodiments, provided is a D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following

amino acid sequence, C*-X²-[W/F/Y]-[Polar]-X⁵-X⁶-F-N-N-[W/Y]-W-C* (SEQ ID NO:113), wherein X² is the D form of any of the canonical L-amino acids other than Cys, X⁵ is the D form of any of the canonical L-amino acids other than cysteine, X⁶ is the D form of any of the canonical L-amino acids other than cysteine, Polar represents a D-amino acid comprising one of R, K, H, E, D, Q, N, T, S, P, A, or G, and the * indicate an optional intramolecular disulfide bond.

[0010] In some embodiments, provided is a D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C-X²-X³-X⁴-X⁵-X⁶-F-F-N-X¹⁰-X¹¹-C (SEQ ID NO:1), wherein each of X² through X⁶, X¹⁰ is a D-amino acid or a D- α -amino acid analog thereof; and

- a. X² is the D form of any of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D- α -amino acid analog thereof;
- b. X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L), or a D- α -amino acid analog thereof;
- c. X⁴ is a Polar amino acid comprising the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D- α -amino acid analog thereof;
- d. X⁵ is the D form of Pro, Trp, His, Gln, Gly, Arg, Ala, Val or Leu, or a D- α -amino acid thereof;
- e. X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof;
- f. X¹⁰ is the D form of Trp (W) or Tyr (Y), or a D- α -amino acid analog thereof;
- g. X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H) or a D- α -amino acid analog thereof; and
- h. C denotes the D form of Cysteine, F denotes the D form of phenylalanine, and N denotes the D form of asparagine, or a D- α -amino acid analog of C, F or N.

[0011] In some embodiments, the TNF β peptides comprise a core TNF α binding domain of D-amino acids and having the following amino acid sequence: C*-X²-X³-X⁴-X⁵-X⁶-F-N-N-X¹⁰-X¹¹-C* (SEQ ID NO:2), wherein each of X¹ through X⁶, X¹⁰, and X¹¹ is a D-amino acid; and

- a. X² is the D form of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D- α -amino acid analog thereof;

- b. X^3 is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L) , or a D- α -amino acid analog thereof;
 - c. X^4 is a Polar amino acid comprising the D forms of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D- α -amino acid analog thereof;
 - d. X^5 is the D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), or a D- α -amino acid analog thereof;
 - e. X^6 is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof;
 - f. X^{10} is the D form of Trp (W) or Tyr (Y), or a D- α -amino acid analog thereof;
- and
- g. X^{11} is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H); C denotes the D form of Cysteine;

wherein F denotes the D form of phenylalanine; N denotes the D for of asparagine; and the * indicate an optional intramolecular disulfide bond.

[0012] In some embodiments, provided is a D-peptide or a salt thereof, wherein the core TNF α binding domain has the following amino acid sequence: C- X^2 -[W/F/Y]- X^4 - X^5 - X^6 -F-N-N-[W/Y]-W-C (SEQ ID NO:3). wherein X^2 is the D form Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W); X^4 is the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G); X^5 is the D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), X^6 is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A); and the * indicate an optional intramolecular disulfide bond between the indicated cysteine residues.

[0013] In some embodiments, provided is a D-peptide or a salt thereof, further comprising an intramolecular disulfide bond between the cysteine residues of the core TNF α binding domain.

[0014] In some embodiments, provided is a D-peptide or a salt thereof having the amino acid sequence set forth in any of SEQ ID Nos:1 to 3, wherein:

- a. X^2 is the D form of Thr, Val, His, Leu, Gln, Ala, Ile, Met or Trp, or a D- α -amino acid analog thereof;
- b. X^2 is the D form of Thr, Val, His, Leu, or Gln, or a D- α -amino acid analog thereof;

- c. X^2 is the D form of Thr, Val, His or Leu, or a D- α -amino acid analog thereof;
- d. X^2 is the D form of Thr, Val or His, or a D- α -amino acid analog thereof;
- e. X^2 is the D form of Thr or Val, or a D- α -amino acid analog thereof;
- f. X^2 is the D form of Thr, or a D- α -amino acid analog thereof; or
- g. X^2 is the D form of Val, or a D- α -amino acid analog thereof.

[0015] In some embodiments, provided is a D-peptide or a salt thereof having the amino acid sequence set forth in any of SEQ ID Nos:1 or 2, wherein:

- a. X^3 is the D form of Trp, Phe, Tyr, or Ser, or a D- α -amino acid analog thereof;
- b. X^3 is the D form of Trp, Phe, or Tyr, or a D- α -amino acid analog thereof;
- c. X^3 is the D form of Trp or Phe, or a D- α -amino acid analog thereof;
- d. X^3 is the D form of Trp or a D- α -amino acid analog thereof; or
- e. X^3 is the D form of Phe, or a D- α -amino acid analog thereof.

[0016] In some embodiments, provided is a D-peptide or a salt thereof having the amino acid sequence set forth in any of SEQ ID Nos:1 to 3, wherein:

- a. X^4 is the D forms of Arg, His, Gln, Asn, Lys, Thr, or Ser, or a D- α -amino acid analog thereof;
 - b. X^4 is the D form of Arg, His, Gln or Asn, or a D- α -amino acid analog thereof;
 - c. X^4 is the D form of Arg, His or Gln, or a D- α -amino acid analog thereof;
 - d. X^4 is the D form of Arg, Gln or Asn, or a D- α -amino acid analog thereof;
- or
- e. X^4 is the D form of Gln, or a D- α -amino acid analog thereof.

[0017] In some embodiments, provided is a D-peptide or a salt thereof having the amino acid sequence set forth in any of SEQ ID Nos:1 to 3, wherein:

- a. X^5 is the D form of Pro, Trp, His, Gln, Gly, Arg, Ala, Val or Leu, or a D- α -amino acid analog thereof;
- b. X^5 is the D form of Pro, Trp, His, Gln, Gly, Arg, or Val, or a D- α -amino acid analog thereof;
- c. X^5 is the D form of Pro, Trp or His, or a D- α -amino acid analog thereof;
- d. X^5 is the D form of Pro or Trp, or a D- α -amino acid analog thereof; or
- e. X^5 is the D form of Pro, or a D- α -amino acid analog thereof.

[0018] In some embodiments, provided is a D-peptide or a salt thereof having the amino acid sequence set forth in any of SEQ ID Nos:1 to 3, wherein:

- a. X^6 is the D-form of Arg, His, Lys, Glu, Gln, Val, Leu, Ser or Ala, or a D- α -amino acid analog thereof;
- b. X^6 is the D-form of Arg, His, Lys, Glu, Gln, Val or Leu, or a D- α -amino acid analog thereof;
- c. X^6 is the D-form of Arg, His, Lys, Glu, or Gln, or a D- α -amino acid analog thereof;
- d. X^6 is the D-form of Arg, His, Lys, or Glu, or a D- α -amino acid analog thereof;
- e. X^6 is the D-form of Arg, His, or Lys, or a D- α -amino acid analog thereof;
- f. X^6 is the D-form of Arg, or a D- α -amino acid analog thereof;
- g. X^6 is the D-form of Lys, or a D- α -amino acid analog thereof, or
- h. X^6 is the D-form of His, or a D- α -amino acid analog thereof.

[0019] In some embodiments, provided is a D-peptide or a salt thereof having the amino acid sequence set forth in any of SEQ ID Nos:1 or 2, wherein:

- a. X^{10} is the D form of Trp, or a D- α -amino acid analog thereof; or
- b. X^{10} is the D form of Tyr, or a D- α -amino acid analog thereof.

[0020] In some embodiments, provided is a D-peptide or a salt thereof having the amino acid sequence set forth in any of SEQ ID Nos:1 to 3, wherein:

- a. X^{11} is the D form of Trp (W), Tyr (Y), or Gln (Q), or a D- α -amino acid analog thereof;
- b. X^{11} is the D form of Tyr (Y), or
- c. X^{11} is the D form of Trp (W), or a D- α -amino acid analog thereof.

[0021] In some embodiments, provided is a D-peptide or a salt thereof, wherein each of X^1 through X^6 , X^{10} and X^{11} is a D-amino acid. And C is the D-form of cysteine. F is the D-form of phenylalanine, and N is the D-form of asparagine.

[0022] In some embodiments, provided is a D-peptide or a salt thereof, wherein the core TNF α binding domain has an amino acid sequence selected from:

- a. CVWQPKFNYYWC (SEQ ID NO:4);
- b. CVWQPRFNYYWC (SEQ ID NO:5);
- c. CTFQPRFNYYWC (SEQ ID NO:6);
- d. CTFQPRFNNWWC (SEQ ID NO:7);

- e. CSFQPRFNYYWC (SEQ ID NO:8);
- f. CSFQPRFNNWWC (SEQ ID NO:9);
- g. CVFQPRFNYYWC (SEQ ID NO:10);
- h. CVFQPRFNNWWC (SEQ ID NO:11);
- i. CTFQWRFNYYWC (SEQ ID NO:12);
- j. CLYQPVFNWWC (SEQ ID NO:13);
- k. CVFQAAFNYYWC (SEQ ID NO:14);
- l. CVFQHFNWWC (SEQ ID NO:15);
- m. CHFNPRFNNWWC (SEQ ID NO:16);
- n. CVWQPHFNYYWC (SEQ ID NO:17);
- o. CVFQGRFNNWWC (SEQ ID NO:18);
- p. CVFQHRFNNWWC (SEQ ID NO:19);
- q. CVFNPRFNNWWC (SEQ ID NO:20);
- r. CVFKPRFNNWWC (SEQ ID NO:21);
- s. CAYQRQFNNWWC (SEQ ID NO:22);
- t. CWFEHRFNNWHC (SEQ ID NO:23);
- u. CHFQHRFNNWWC (SEQ ID NO:24);
- v. CHFQPRFNNWWC (SEQ ID NO:25);
- w. CTYQPRFNNWWC (SEQ ID NO:26);
- x. CQFQPRFNNWQC (SEQ ID NO:27); or
- y. CHFSQRFNNWWC (SEQ ID NO:28).

[0023] In some embodiments, provided is a D-peptide or a salt thereof, wherein the core TNF α binding domain has comprises an amino acid amino acid sequence set forth in SEQ ID NO:77 to 110.

[0024] In some embodiments, provided is a D-peptide or a salt thereof, further comprising a tag sequence attached to the N-terminus of the peptide. In some embodiments, provided is a D-peptide or a salt thereof, wherein the tag comprises the amino acid sequence D-Asp or D-Asp D-Asp (DD). In some embodiments, provided is a D-peptide or a salt thereof, further comprising a tag sequence attached to the C-terminus of the peptide. In some embodiments, provided is a D-peptide or a salt thereof, wherein the tag comprises the amino acid sequence D-GGEEEEK (SEQ ID NO:30) or D-GGRRRK (SEQ ID NO:31), wherein each amino acid residue is a D-amino acid.

[0025] In some embodiments, provided is a D-peptide or a salt thereof, wherein the N-terminus of the peptide comprises a cap. In some embodiments, provided is a D-peptide or a salt thereof, wherein the cap comprises an acetyl group or a protecting group. In some embodiments, provided is a D-peptide or a salt thereof, wherein the C-terminus of the peptide comprises a cap. In some embodiments, provided is a D-peptide or a salt thereof, wherein the cap comprises an amide group or a protecting group.

[0026] In some embodiments, provided is D-peptide or a salt thereof, wherein the peptide comprises a cap and a tag, each as described herein.

[0027] In some embodiments, provided is a D-peptide or a salt thereof, further comprising a polyethylene glycol (PEG) group. In some embodiments, provided is a D-peptide or a salt thereof, further comprising a Linker. In some embodiments, provided is a D-peptide or a salt thereof, wherein the Linker comprises a PEG group. In some embodiments, provided is a D-peptide or a salt thereof, wherein the PEG group is attached to the N-terminus of the D-peptide. In some embodiments, provided is a D-peptide or a salt thereof, wherein the PEG group is attached to the C-terminus of the D-peptide. In some embodiments, provided is a D-peptide or a salt thereof, wherein each PEG group is selected from a PEG group having from 1 to 48 subunits, 1 to 30 subunits, 1 to 24 subunits, or 1 to 12 subunits. In some embodiments, provided is a D-peptide or a salt thereof, wherein each PEG group is selected from a PEG group having from 6 subunits, 8 subunits, 10 subunits, or 12 subunits.

[0028] In some embodiments, provided is a multimer of any of the D-peptides described herein, or a pharmaceutically acceptable salt thereof. In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, wherein the multimer is a dimer. In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, wherein the multimer is a trimer. In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, further comprising a multimer scaffold attached to the D-peptides, optionally via a Linker. In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, wherein the multimer scaffold is trimeric. In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, wherein the multimer scaffold is a tetrameric.

[0029] In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, wherein the trifunctional cross-linker is

tris(succinimidyl) aminotriacetate (TSAT), tris-succinimidyl (6-aminocaproyl)aminotriacetate (LC-TSAT), an Fmoc scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-(((9H-fluoren-9-yl)methoxy)carbonyl) amino)-4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)heptanedioate, an Fmoc scaffold having a PEG27 chain, a cyclohex scaffold (tris(2,5-dioxopyrrolidin-1-yl) cyclohexane-1,3,5-tricarboxylate), a nitro scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)-4-nitroheptanedioate), amine scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-amino-4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)heptanedioate), an amine-PEG27 scaffold, a cholesterol scaffold, a heterotetrameric PEG scaffold based on 3-{2-amino-3-(2-carboxyethoxy)-2-[(2-carboxyethoxy)methyl]propoxy}propionic acid scaffold, a multimeric scaffold based on 4-amino-4-(2-carboxyethyl)heptanedioic acid, or 3-{2-amino-3-(2-carboxyethoxy)-2-[(2-carboxyethoxy)methyl]propoxy}propionic acid.

[0030] In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, wherein the multimer has a structure selected from:

- a. Fmoc-[Peptide-PEG12-K-amide]₃;
- b. Fmoc-[Peptide-PEG4-K-amide]₃;
- c. Fmoc-[Peptide -PEG8-K-amide]₃;
- d. Fmoc-[Ac-K-PEG12-Peptide-amide]₃;
- e. [Peptide-PEG6-K-amide]₃-Fmoc;
- f. [Peptide-PEG12-K-amide]₃-PEG27-Fmoc;
- g. [Peptide-PEG12-K-amide]₃-Fmoc;
- h. [Peptide-PEG12-K-amide]₃-cyclohex;
- i. [Peptide-PEG12-K-amide]₃-nitro;
- j. [Peptide-PEG12-K-amide]₃-PEG27-amine;
- k. [Peptide-PEG12-K-amide]₃-amine;
- l. [Peptide-PEG12-K-amide]₃-PEG27-biotin; and
- m. [Peptide-PEG12-K-amide]₃-PEG27-cholesterol; and wherein Peptide is a

D-peptide.

[0031] In some embodiments, provided is a D-peptide or a salt thereof having an amino acid sequence selected from at least one of:

- a. DDCVWQPKFNYYWC (SEQ ID N:32);
- b. DGACVWQPKFNYYWC (SEQ ID NO:38);
- c. DDCVWQPRFNYYWC (SEQ ID NO:39);

- d. DDCVWQPRFNYYWCGRRRK (SEQ ID NO:40);
- e. DCVWQPKFNYYWC (SEQ ID NO:56);
- f. DDDCVWQPRFNYYWC (SEQ ID NO:60);
- g. DDCTFQPRFNNWWC (SEQ ID NO:61);
- h. DDCVFQPRFNYYWC (SEQ ID NO:62);
- i. DDCSFQPRFNYYWC (SEQ ID NO:63); and
- j. DDCVFQPRFNNWWC (SEQ ID NO:64).

[0032] In some embodiments, provided is a multimer of any of the D-peptides described herein, or a salt thereof, comprising a D-peptide having an amino acid sequence selected from at least one of:

- a. DDCVWQPKFNYYWC (SEQ ID NO:32);
- b. DGACVWQPKFNYYWC (SEQ ID NO:38);
- c. DDCVWQPRFNYYWC (SEQ ID NO:39);
- d. DDCVWQPRFNYYWCGRRRK (SEQ ID NO:40);
- e. DCVWQPKFNYYWC (SEQ ID NO:56);
- f. DDDCVWQPRFNYYWC (SEQ ID NO:60);
- g. DDCTFQPRFNNWWC (SEQ ID NO:61);
- h. DDCVFQPRFNYYWC (SEQ ID NO:62);
- i. DDCSFQPRFNYYWC (SEQ ID NO:63); and
- j. DDCVFQPRFNNWWC (SEQ ID NO:64).

[0033] In some embodiments, provided is a pharmaceutical composition comprising at least one D-peptide described herein or multimer thereof, or pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable excipient or carrier. In some embodiments, provided is such a pharmaceutical composition which is formulated for parenteral administration. In some embodiments, provided is such a pharmaceutical composition which is formulated for intravenous, intramuscular, or subcutaneous administration. In some embodiments, provided is such a pharmaceutical composition, which is formulated for oral administration. In some embodiments, provided is such a pharmaceutical composition which is formulated for topical administration. In some embodiments, provided is such a pharmaceutical composition which is formulated for topical administration to the skin (dermal) or to the eye. In some embodiments, provided is such a pharmaceutical composition which is formulated for rectal administration.

[0034] In some embodiments, provided is a lyophilized composition comprising at least one D-peptide or multimer described herein, or a salt or pharmaceutically acceptable salt thereof and a stabilizing agent. In some embodiments, provided is a lyophilized composition of any of the pharmaceutical compositions described herein and a stabilizing agent. In some embodiments, provided is a re-hydrated solution of any of the lyophilized compositions described herein.

[0035] In some embodiments, provided is a method of treating a TNF α -mediated disease, comprising administering an effective amount of any of the D-peptides described herein, or a salt or pharmaceutically acceptable salt thereof, or any of the multimers described herein, or a salt thereof or any of the pharmaceutical compositions described herein. In some embodiments, the TNF α -mediated disease is adult Crohn's Disease, pediatric Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile idiopathic arthritis (polyarticular), Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or non-radiographic axial spondyloarthritis.

[0036] In some embodiments, the TNF α -mediated disease is an inflammatory bowel disease. In some embodiments, the inflammatory bowel disease is adult Crohn's Disease, pediatric Crohn's Disease, or Ulcerative Colitis. In some embodiments, the administration of any of the D-peptides described herein, or pharmaceutically acceptable salt thereof, or any of the multimers described herein, or pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, is oral. In some embodiments, the administration of any of the D-peptides described herein, or pharmaceutically acceptable salt thereof, or any of the multimers described herein, or pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, is rectal. In some embodiments, the administration of any of the D-peptides described herein, or pharmaceutically acceptable salt thereof, or any of the multimers described herein, or pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, is parenteral.

[0037] In some embodiments, the TNF α -mediated disease is an inflammatory skin disease. In some embodiments, the inflammatory skin disease is selected from Plaque Psoriasis and Cutaneous Lupus. In some embodiments, the administration of any of the D-peptides described herein, or pharmaceutically acceptable salt thereof, or any of the multimers described herein, or pharmaceutically acceptable salt thereof or any of the

pharmaceutical compositions described herein, is topical. In some embodiments, the administration of any of the D-peptides described herein, or pharmaceutically acceptable salt thereof, or any of the multimers described herein, or pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, is parenteral. In some embodiments, the administration of any of the D-peptides described herein, or a pharmaceutically acceptable salt thereof, or any of the multimers described herein, or a pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, is oral.

[0038] In some embodiments, the TNF α -mediated disease is an inflammatory disease, such as, for example, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, idiopathic arthritis (polyarticular), or non-radiographic axial spondyloarthritis. In some embodiments, the administration of any of the D-peptides described herein, or pharmaceutically acceptable salt thereof, or any of the multimers described herein, or pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, is parenteral.

[0039] In any of the embodiments described herein, parenteral administration can be intravenous, subcutaneous, and intramuscular.

[0040] In some embodiments, provided is a method of reducing TNF α -mediated inflammation, comprising administering any of the D-peptides described herein, or a pharmaceutically acceptable salt thereof, or any of the multimers described herein, or a pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, to a subject. In some embodiments, provided is a method of inhibiting TNF α , comprising administering any of the D-peptides described herein, or a pharmaceutically acceptable salt thereof, or any of the multimers described herein, or a pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, to a subject. In some embodiments, provided is a method of reducing an inflammatory response mediated by TNF α , comprising administering any of the D-peptides described herein, or a pharmaceutically acceptable salt thereof, or any of the multimers described herein, or a pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, to a subject. In some embodiments, the administering is by oral administration, by parenteral administration, by topical (dermal), or by rectal administration. In some embodiments, any of the D-peptides described herein, or a pharmaceutically acceptable salt thereof, or any of the multimers described herein, or a

pharmaceutically acceptable salt thereof or any of the pharmaceutical compositions described herein, are administered locally to reduce TNF α activity or inflammation or an inflammatory response. In some embodiments, the subject has a TNF α - mediated disease. In some embodiments, the TNF α - mediated disease is adult Crohn's Disease, pediatric Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile idiopathic arthritis (polyarticular), Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or non-radiographic axial spondyloarthritis.

[0041] In some embodiments, provided is a D-peptide as described herein, a multimer of any of the D-peptides described herein, or a salt thereof, for use as a medicament.

[0042] In some embodiments, provided is a peptide as described herein, a multimer of any of the D-peptides described herein, or pharmaceutically acceptable salt thereof, for use in a method of treating a subject by therapy. In some embodiments, the subject has a TNF α - mediated disease. In some embodiments, the TNF α - mediated disease is adult Crohn's Disease, pediatric Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile idiopathic arthritis (polyarticular), Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or non-radiographic axial spondyloarthritis.

[0043] These and other aspects of the present invention may be more fully understood by reference to the following detailed description, non-limiting examples of specific embodiments and the appended drawings.

DESCRIPTION OF THE DRAWINGS

[0044] The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

[0045] FIGURE 1 shows a comparison of the TNF α binding by selected peptides isolated by mirror image phage screening from all libraries.

[0046] FIGURE 2 shows a comparison of the binding of Peptide 18 and two Peptide 18 variants to TNF α in a solid phase binding assay.

[0047] FIGURE 3 shows that Peptide 18 can block binding of TNF α to its receptor, TNFR1, in a solid phase binding assay.

[0048] FIGURE 4A shows that Peptide 18 can block cellular activity of TNF α in an L-929 cell killing assay.

[0049] FIGURE 4B shows that Peptide 18 can block cellular activity of TNF α in a concentration-dependent manner in the L-929 cell killing assay.

[0050] FIGURE 5 shows the effects of amino acid changes at the N-terminus, C-terminus and an internal position of Peptide 18 on TNF α binding.

[0051] FIGURE 6 shows the effects of amino acid changes at the N-terminus, C-terminus and an internal position of Peptide 18 using the TNF α /TNFR blocking assay.

[0052] FIGURE 7 shows the effects of amino acid changes at the N-terminus, C-terminus and internal positions of Peptide 18 using the TNF α /TNFR blocking assay.

[0053] FIGURE 8 shows the effects of amino acid changes at the N-terminus, C-terminus and internal positions of Peptide 18 using the L-929 cell killing assay.

[0054] FIGURE 9 shows the effects of insertion of an N-terminal PEG4 spacer and insertion or deletion of an amino acid residue on Peptide 18 using the TNF α binding assay.

[0055] FIGURE 10 shows the effects of insertion of an N-terminal PEG spacer and insertion or deletion of an amino acid residue on Peptide 18 using the L-929 cell killing assay.

[0056] FIGURE 11 compares the activity of Peptide 18 KtoR dimers and trimers using the L-929 cell killing assay.

[0057] FIGURE 12 compares the activity of Peptide 18 mutants and other library hits using the TNF α binding assay.

[0058] FIGURE 13 compares the activity of Peptide 18 KtoR trimers having different PEG lengths using the L-929 cell killing assay.

[0059] FIGURE 14 compares the activity of Peptide 18 and Peptide 18 mutants using the L-929 cell killing assay.

[0060] FIGURE 15A shows the structures of three multimer scaffolds used to make peptide trimers.

[0061] FIGURE 15B compares the activity of three Peptide 18 KtoR trimers using the L-929 cell killing assay.

[0062] FIGURE 16A shows the structures of six multimer scaffolds used to make peptide trimers.

[0063] FIGURE 16B compares the activity of six Peptide 18 KtoR trimers using the L-929 cell killing assay.

[0064] FIGURE 17 compares the activity of Peptide 18 KtoR trimers having different PEG lengths using the L-929 cell killing assay.

[0065] FIGURE 18 compares the activity of Peptide 18 mutants using the L-929 cell killing assay.

[0066] FIGURE 19 shows the effects of replacement of an N-terminal aspartate residue with succinic acid using the L-929 cell killing assay.

[0067] FIGURE 20 shows the activity of Peptide 18 mutants that contain non-standard amino acids using the L-929 cell killing assay.

[0068] FIGURE 21 shows that fluorescently-labeled Peptide DD-018 binds to native TNF α .

[0069] FIGURES 22A and B compare the activity of Peptide TF-018-KtoR-WW and oligomers thereof and an anti-TNF α antibody using the TNF α /TNFR blocking assay.

[0070] FIGURES 23A and B compare the activity of Peptide TF-018-KtoR-WW and oligomers and an anti-TNF α antibody using the L-929 cell killing assay.

[0071] FIGURE 24 shows that Peptide TF-18-KtoR-WW C-Trimer can block the activity of membrane-bound TNF α .

[0072] FIGURE 25 shows the inhibition of IL-8 levels by Peptide TF-18-KtoR-WW C-Trimer following stimulation of blood samples with recombinant human TNF, LPS and anti-CD3⁺ anti-CD28 antibodies.

[0073] FIGURE 26 shows the results of Peptide TF-18-KtoR-WW C-Trimer administration in a human TNF α mouse challenge model.

[0074] FIGURES 27 and 28, show Peptide TF-18-KtoR-WW C-Trimer plasma levels following a single subcutaneous dose in CD1 mice.

[0075] FIGURE 29 shows Peptide TF-18-KtoR-WW C-Trimer levels in plasma following administration of 5.0 mg/kg IV or 50 mg/kg SC.

[0076] FIGURE 30 shows Peptide TF-18-KtoR-WW C-Trimer levels in small intestine, liver, plasma, large intestine and kidney following oral administration.

[0077] FIGURE 31 shows inhibition of IL-6 following subcutaneous administration of Peptide TF-18-KtoR-WW C-Trimer to Tg1278 mice.

[0078] FIGURE 32 shows inhibition of mouse KC following subcutaneous administration of Peptide TF-18-KtoR-WW C-Trimer to Tg1278 mice.

DEFINITIONS

[0079] For convenience, certain terms in the specification, examples and claims are defined here. Unless stated otherwise, or implicit from context, the following terms and phrases have the meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0080] As used herein and unless otherwise indicated, the terms “a” and “an” are taken to mean “one”, “at least one” or “one or more”. Unless otherwise required by context, singular terms used herein shall include pluralities and plural terms shall include the singular.

[0081] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

[0082] The terms “decrease,” “reduce,” “reduced”, “reduction”, “decrease,” and “inhibit” are all used herein generally to mean a decrease by a statistically significant amount relative to a reference.

[0083] The terms “increased”, “increase” or “enhance” or “activate” are all used herein to generally mean an increase by a statically significant amount relative to a reference.

[0084] As used herein, the terms “protein” and “polypeptide” are used interchangeably herein to designate a series of amino acid residues each connected to each other by peptide bonds between the alpha-amino and carboxyl groups of adjacent residues. The terms “protein” and “polypeptide” also refer to a polymer of amino acids, including modified amino acids (*e.g.*, phosphorylated, glycosylated, and the like) and amino acid analogs, regardless of its size or function. “Protein” and “polypeptide” are often used in reference to relatively large polypeptides, whereas the term “peptide” is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms “protein” and “polypeptide” are used interchangeably herein when referring to an encoded gene product and fragments thereof. Thus, exemplary polypeptides or proteins include

gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

[0085] TNF α (also referred to as TNF α or TNF- α or TNF) is a protein that is expressed on a variety of cell types, including monocytes, macrophages, NK cells and regulatory T cells. TNF α has both a membrane and a soluble form. TNF α proteins include, but are not limited to, those having the amino acid sequences set forth in accession numbers AQY77150.1, P01375-1, and NP_000585.2; these sequences are incorporated by reference herein.

[0086] The terms “D-amino acid” and D-amino acid residue”, as used herein, refer to an α -amino acid residue having the same absolute configuration as D-glyceraldehyde.

[0087] As used herein, amino acids are named herein using either their 1-letter or 3-letter code according to the recommendations from IUPAC. Unless otherwise indicated by context, an amino acid is of the D-form.

[0088] The phrases “D-forms of the canonical L-amino acids” and “D-forms of any of the canonical L-amino acids” refer to D-forms of alanine, arginine, asparagine, aspartic acid (aspartate), cysteine, glutamine, glutamic acid (glutamate), histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. For convenience, this phrase also refers to glycine, although this amino acid is achiral.

[0089] The term “D-peptide”, as used herein, refers to a peptide composed of D-amino acid residues.

[0090] As used herein, “specifically binds” refers to the ability of a binding agent (*e.g.*, a D-peptide as described herein) to bind to a target with a K_D 10^{-5} M (10000 nM) or less, *e.g.*, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. Specific binding can be influenced by, for example, the affinity and avidity of the binding agent and the concentration of target polypeptide. A person of ordinary skill in the art can determine appropriate conditions under which the binding agent selectively bind to a target using any suitable methods, such as titration of a binding agent in a suitable cell binding assay or in a suitable solid phase binding assay. A binding agent specifically bound to a target is not displaced by a non-similar competitor. In certain embodiments, a binding agent is said to specifically bind to its target when it preferentially recognizes its target in a complex mixture of proteins and/or macromolecules.

[0091] As used herein, the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment.

[0092] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0093] Other than in the examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The term “about” when used in connection with percentages can mean $\pm 1\%$.

[0094] The term “statistically significant” or “significantly” refers to statistical significance and generally means a two-standard deviation (2SD) difference, above or below a reference value.

[0095] The term “pharmaceutically acceptable salt” generally means those salts which retain the biological effectiveness and properties of the free bases and which is not biologically or otherwise undesirable formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and organic acids, such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid. “Pharmaceutically acceptable salt” includes solvates, particularly hydrates, of such salts.

[0096] Other terms are defined herein within the description of the various aspects of the invention.

DETAILED DESCRIPTION

[0097] The present disclosure is based on the discovery of a highly potent and specific class of D-peptide-based inhibitors of TNF α (also referred to herein as TNFi peptides). In some embodiments, the peptides inhibit TNF α activity by binding to TNF α . In some embodiments, the peptides inhibit TNF α activity by binding to TNF α and blocking its binding to its receptors, TNFR1 and/or TNFR2. In some embodiments, the peptides inhibit TNF α activity by blocking its binding to TNFR1. In some embodiments, the peptides inhibit TNF α activity by blocking its binding to TNFR2. The TNFi peptides comprise a core TNF α binding domain composed of D-amino acids. In some

embodiments, the TNFi peptides specifically bind to both soluble and membrane bound TNF α . In some embodiments, the TNFi peptides specifically bind to soluble TNF α . In some embodiments, the TNFi peptides specifically bind to membrane bound TNF α . In some embodiments, the TNFi-peptides are resistant to proteolysis in the environment of the gastrointestinal tract.

[0098] Also provided are methods of using the TNFi peptides for the treatment of TNF α -mediated diseases, including inflammatory and autoimmune diseases and disorders. As used herein, the term “TNF α -mediated diseases” means diseases or disorders in which TNF α signaling pathways and/or the cell biological effects of TNF α are involved in the disease or a symptom thereof. In some embodiments, the TNFi peptides are administered by parenteral administration, by intravenous administration, by intramuscular administration, by subcutaneous administration, by oral administration, by topical administration or by rectal administration. These and other embodiments are further described herein.

[0099] Peptides

[0100] Provided herein are D-peptide-based inhibitors of TNF α . In some embodiments, the peptides inhibit TNF α activity by binding to TNF α . In some embodiments, the peptides inhibit TNF α activity by binding to TNF α and blocking its binding to its receptors, TNFR1 and/or TNFR2. The TNFi peptides comprise a core TNF α binding domain composed of D-amino acids.

[0101] In some embodiments, provided is a D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C (SEQ ID NO:111), wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) or Tyr (Y); X¹¹ is the D form of Trp (W), Gln (Q), Tyr, or His (H); and C denotes the D form of Cysteine.

[0102] In some embodiments, provided is a D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C*-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C* (SEQ ID NO:112), wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) and Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), and Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) and Tyr (Y); X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H); C denotes the D form of Cysteine; and * denotes an optional intramolecular bond.

[0103] In some embodiments, provided is a D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C*-X²-[W/F/Y]-[Polar]-X⁵-X⁶-F-N-N-[W/Y]-W-C* (SEQ ID NO:113), wherein X² is the D form of any of the canonical L-amino acids other than Cys, X⁵ is the D form of any of the canonical L-amino acids other than cysteine, X⁶ is the D form of any of the canonical L-amino acids other than cysteine, Polar represents a D-amino acid comprising one of R, K, H, E, D, Q, N, T, S, P, A, or G, and the * indicate an optional intramolecular disulfide bond.

[0104] In some embodiments, the TNFi peptides comprise a core TNF α binding domain of D-amino acids and having the following amino acid sequence: C-X²-X³-X⁴-X⁵-X⁶-F-N-N-X¹⁰-X¹¹-C (SEQ ID NO:1), wherein each of X¹ through X⁶, X¹⁰, and X¹¹ is a D-amino acid; and X² is the D form of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D- α -amino acid analog thereof; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L), or a D- α -amino acid analog thereof; X⁴ is the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D- α -amino acid analog thereof; X⁵ is the D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), or a D- α -amino acid analog thereof; X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof; X¹⁰ is the D form of Trp (W) or Tyr (Y) or a D- α -amino acid analog thereof; X¹¹ is the D form of Trp

(W), Tyr (Y), Gln (Q) or His (H), or a D- α -amino acid analog thereof; and C denotes the D form of Cysteine.

[0105] In some embodiments, the TNFi peptides comprise a core TNF α binding domain of D-amino acids and having the following amino acid sequence: C*-X²-X³-X⁴-X⁵-X⁶-F-N-N-X¹⁰-X¹¹-C* (SEQ ID NO:2), wherein each of X¹ through X⁶, X¹⁰, and X¹¹ is a D-amino acid; and X² is the D form of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D- α -amino acid analog thereof; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L), or a D- α -amino acid analog thereof; X⁴ is a Polar amino acid comprising the D forms of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D- α -amino acid analog thereof; X⁵ is the D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), or a D- α -amino acid analog thereof; X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof; X¹⁰ is the D form of Trp (W) or Tyr (Y), or a D- α -amino acid analog thereof; X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H); C denotes the D form of Cysteine; F denotes the D form of phenylalanine; N denotes the D form of asparagine; and the * indicate an optional intramolecular disulfide bond.

[0106] In some embodiments of the TNFi peptides set forth in SEQ ID NO:1 or 2, X² is the D-form of Thr, Val, His, Leu, Gln, Ala, Ile, Met or Trp. In some embodiments, X² is the D form of Thr, Val, His, Leu, or Gln. In some embodiments, X² is the D form of Thr, Val, His or Leu. In some embodiments, X² is the D form of Thr, Val or His. In some embodiments, X² is the D form of Thr or Val. In some embodiments, X² is the D form of Thr. In some embodiments, X² is the D form of Val.

[0107] In some embodiments of the TNFi peptides set forth in SEQ ID NO:1 or 2, X³ is the D form of Trp, Phe, Tyr, or Ser. In some embodiments, X³ is the D form of Trp, Phe, or Tyr. In some embodiments, X³ is the D form of Trp or Phe. In some embodiments, X³ is the D form of Trp. In some embodiments, X³ is the D form of Phe.

[0108] In some embodiments of the TNFi peptides set forth in SEQ ID NO:1 or 2, X⁴ is the D form of Arg, His, Gln, Lys, Asn, Thr, or Ser. In some embodiments, X⁴ is the D form of Arg, His, Gln, Lys or Asn. In some embodiments, X⁴ is the D form of Arg, His or Gln. In some embodiments, X⁴ is the D form of Arg, Gln or Asn. In some embodiments, X⁴ is the D form of Gln.

[0109] In some embodiments of the TNFi peptides set forth in SEQ ID NO:1 or 2, X⁵ is the D form of Pro, Trp, His, Gln, Gly, Arg, Ala, Val or Leu. In some embodiments, X⁵ is the D form of Pro, Trp, His, Gln, Gly, Arg, or Val. In some embodiments, X⁵ is the D form of Pro, Trp or His. In some embodiments, X⁵ is the D form of Pro or Trp. In some embodiments, X⁵ is the D form of Pro.

[0110] In some embodiments of the TNFi peptides set forth in SEQ ID NO:1 or 2, X⁶ is the D-form of Arg, His, Lys, Glu, Gln, Val, Leu, Ser or Ala. In some embodiments, X⁶ is the D-form of Arg, His, Lys, Glu, Gln, Val or Leu. In some embodiments, X⁶ is the D-form of Arg, His, Lys, Glu, or Gln. In some embodiments, X⁶ is the D-form of Arg, His, Lys, or Glu. In some embodiments, X⁶ is the D-form of Arg, Lys, or His. In some embodiments, X⁶ is the D-form of Arg. In some embodiments, X⁶ is the D-form of Lys. In some embodiments, X⁶ is the D-form of His.

[0111] In some embodiments of the TNFi peptides set forth in SEQ ID NO:1 or 2, X¹⁰ is the D form of Trp. In some embodiments, X¹⁰ is the D form of Tyr.

[0112] In some embodiments of the TNFi peptides set forth in SEQ ID NO:1 or 2, X¹¹ is the D form of Trp (W), Tyr (Y), or Gln (Q). In some embodiments, X¹¹ is the D form of Trp (W).

[0113] In some embodiments, the TNFi peptides comprise a core TNF α binding domain of D-amino acids and having the following amino acid sequence: C*-X²-[W/F/Y]-X⁴-X⁵-X⁶-F-N-N-[W/Y]-W-C* (SEQ ID NO:3), wherein X² is the D form Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W); X⁴ is the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G); X⁵ is the D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A); and the * indicate an optional intramolecular disulfide bond between the indicated cysteine residues.

[0114] In some embodiments of the TNFi peptides set forth in SEQ ID NO:3, X² is the D form of Thr, Val, His, Leu, Gln, Ala, Ile, Met or Trp. In some embodiments, X² is the D form of Thr, Val, His, Leu, or Gln. In some embodiments, X² is the D form of Thr, Val, His or Leu. In some embodiments, X² is the D form of Thr, Val or His. In some embodiments, X² is the D form of Thr or Val. In some embodiments, X² is the D form of Thr. In some embodiments, X² is the D form of Val.

[0115] In some embodiments of the TNFi peptides set forth in SEQ ID NO:3, X⁴ is selected from the D forms of Arg, His, Lys, Gln, Asn, Thr, or Ser. In some embodiments, X⁴ is the D form of Arg, His, Lys, Gln or Asn. In some embodiments, X⁴ is the D form of Arg, His, Lys or Gln. In some embodiments, X⁴ is the D form of Arg, Gln or Asn. In some embodiments, X⁴ is the D form of Arg, Gln or Lys. In some embodiments, X⁴ is the D form of Gln.

[0116] In some embodiments of the TNFi peptides set forth in SEQ ID NO:3, X⁵ is the D form of Pro, Trp, His, Gln, Gly, Arg, Ala, Val or Leu. In some embodiments, X⁵ is the D form of Pro, Trp, His, Lys, Gln, Gly, Arg, or Val. In some embodiments, X⁵ is the D form of Pro, Trp, or His. In some embodiments, X⁵ is the D form of Pro or Trp. In some embodiments, X⁵ is the D form of Pro.

[0117] In some embodiments of the TNFi peptides set forth in SEQ ID NO:3, X⁶ is the D form of Arg, His, Lys, Glu, Gln, Val, Leu, Ser or Ala. In some embodiments, X⁶ is the D form of Arg, His, Lys, Glu, Gln, Val or Leu. In some embodiments, X⁶ is the D form of Arg, His, Lys, Glu, or Gln. In some embodiments, X⁶ is selected from the D form of Arg, His, Lys, or Glu. In some embodiments, X⁶ is the D form of Arg or His. In some embodiments, X⁶ is the D form of Arg or Lys. In some embodiments, X⁶ is the D form of Arg. In some embodiments, X⁶ is the D form of His. In some embodiments, X⁶ is the D-form of Lys.

[0118] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVWQPKFNYYWC (SEQ ID NO:4), optionally comprising an intramolecular disulfide bond.

[0119] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVWQPRFNYYWC (SEQ ID NO:5), optionally comprising an intramolecular disulfide bond.

[0120] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQPRFNYYWC (SEQ ID NO:6), optionally comprising an intramolecular disulfide bond.

[0121] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQPRFNNWWC (SEQ ID NO:7), optionally comprising an intramolecular disulfide bond.

[0122] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CSFQPRFNYYWC (SEQ ID NO:8), optionally comprising an intramolecular disulfide bond.

[0123] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CSFQPRFNNWWC (SEQ ID NO:9), optionally comprising an intramolecular disulfide bond.

[0124] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQPRFNYYWC (SEQ ID NO:10), optionally comprising an intramolecular disulfide bond.

[0125] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQPRFNNWWC (SEQ ID NO:11), optionally comprising an intramolecular disulfide bond.

[0126] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQWRFNYYWC (SEQ ID NO:12), optionally comprising an intramolecular disulfide bond.

[0127] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CLYQPVFNNWWC (SEQ ID NO:13), optionally comprising an intramolecular disulfide bond.

[0128] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQAAFNNYYWC (SEQ ID NO:14), optionally comprising an intramolecular disulfide bond.

[0129] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQHFFNNWWC (SEQ ID NO:15), optionally comprising an intramolecular disulfide bond.

[0130] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CHFNPRFNNWWC (SEQ ID NO:16), optionally comprising an intramolecular disulfide bond.

[0131] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVWQPHFNYYWC (SEQ ID NO:17), optionally comprising an intramolecular disulfide bond.

[0132] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQGRFNNWWC (SEQ ID NO:18), optionally comprising an intramolecular disulfide bond.

[0133] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQHRFNNWWC (SEQ ID NO:19), optionally comprising an intramolecular disulfide bond.

[0134] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFNPRFNNWWC (SEQ ID NO:20), optionally comprising an intramolecular disulfide bond.

[0135] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFKPRFNNWWC (SEQ ID NO:21), optionally comprising an intramolecular disulfide bond.

[0136] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CAYQRQFNNWWC (SEQ ID NO:22), optionally comprising an intramolecular disulfide bond.

[0137] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CWFQHRFNNWHC (SEQ ID NO:23), optionally comprising an intramolecular disulfide bond.

[0138] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CHFQHRFNNWWC (SEQ ID NO:24), optionally comprising an intramolecular disulfide bond.

[0139] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CHFQPRFNNWWC (SEQ ID NO:25), optionally comprising an intramolecular disulfide bond.

[0140] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTYQPRFNNWWC (SEQ ID NO:26), optionally comprising an intramolecular disulfide bond.

[0141] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CQFQPRFNNWQC (SEQ ID NO:27), optionally comprising an intramolecular disulfide bond.

[0142] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CHFSQRFNNWWC (SEQ ID NO:28), optionally comprising an intramolecular disulfide bond.

[0143] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CIFQQQFNYYWC (SEQ ID NO:77), optionally comprising an intramolecular disulfide bond.

[0144] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CMHQQRFNWWC (SEQ ID NO:78), optionally comprising an intramolecular disulfide bond.

[0145] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFRVRFNNYWC (SEQ ID NO:79), optionally comprising an intramolecular disulfide bond.

[0146] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CIFQWRFNNYWC (SEQ ID NO:80), optionally comprising an intramolecular disulfide bond.

[0147] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQWHFNNYWC (SEQ ID NO:81), optionally comprising an intramolecular disulfide bond.

[0148] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQHLFNNWWC (SEQ ID NO:82), optionally comprising an intramolecular disulfide bond.

[0149] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQWLFNNYWC (SEQ ID NO:83), optionally comprising an intramolecular disulfide bond.

[0150] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CAFQWRFNNYWC (SEQ ID NO:84), optionally comprising an intramolecular disulfide bond.

[0151] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFRWRFNNYWC (SEQ ID NO:85), optionally comprising an intramolecular disulfide bond.

[0152] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTSQWRFNNYWC (SEQ ID NO:86), optionally comprising an intramolecular disulfide bond.

[0153] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQLRFNNYWC (SEQ ID NO:87), optionally comprising an intramolecular disulfide bond.

[0154] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQVRFNNYWC (SEQ ID NO:88), optionally comprising an intramolecular disulfide bond.

[0155] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CIWQPKFNYYWC (SEQ ID NO:89), optionally comprising an intramolecular disulfide bond.

[0156] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQRRFNYYWC (SEQ ID NO:90), optionally comprising an intramolecular disulfide bond.

[0157] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTFQWSFNYYWC (SEQ ID NO:91), optionally comprising an intramolecular disulfide bond.

[0158] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQRHFNNWWC (SEQ ID NO:92), optionally comprising an intramolecular disulfide bond.

[0159] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVSTHHFNWWC (SEQ ID NO:93), optionally comprising an intramolecular disulfide bond.

[0160] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CAFQHGFNNWWC (SEQ ID NO:94), optionally comprising an intramolecular disulfide bond.

[0161] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CAYQRHFNNWWC (SEQ ID NO:95), optionally comprising an intramolecular disulfide bond.

[0162] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CHFNPWFNNWWC (SEQ ID NO:96), optionally comprising an intramolecular disulfide bond.

[0163] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CHFNRWFNNWWC (SEQ ID NO:97), optionally comprising an intramolecular disulfide bond.

[0164] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CHFSQWFNNWWC (SEQ ID NO:98), optionally comprising an intramolecular disulfide bond.

[0165] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CLYQLWFNNWWC (SEQ ID NO:99), optionally comprising an intramolecular disulfide bond.

[0166] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CQFRPRFNNWQC (SEQ ID NO:100), optionally comprising an intramolecular disulfide bond.

[0167] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTLQQQFNNYWC (SEQ ID NO:101), optionally comprising an intramolecular disulfide bond.

[0168] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CTSRWRFNNYWC (SEQ ID NO:102), optionally comprising an intramolecular disulfide bond.

[0169] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQASFNNYWC (SEQ ID NO:103), optionally comprising an intramolecular disulfide bond.

[0170] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFQHSENNWWC (SEQ ID NO:104), optionally comprising an intramolecular disulfide bond.

[0171] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFRHHFNNWWC (SEQ ID NO:105), optionally comprising an intramolecular disulfide bond.

[0172] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVFTHHFNNWWC (SEQ ID NO:106), optionally comprising an intramolecular disulfide bond.

[0173] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVSQHHEFNNWWC (SEQ ID NO:107), optionally comprising an intramolecular disulfide bond.

[0174] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVWQPEFNNYWC (SEQ ID NO:108), optionally comprising an intramolecular disulfide bond.

[0175] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVWQQKFNNYWC (SEQ ID NO:109), optionally comprising an intramolecular disulfide bond.

[0176] In some embodiments, the TNFi peptide comprises a core TNF α binding domain having the following amino acid sequence: CVWRPKFNNYWC (SEQ ID NO:110), optionally comprising an intramolecular disulfide bond.

[0177] In some embodiments, a TNFi peptide comprises a core TNF α binding domain selected from the following amino acid sequences: CVWQPKFNYYWC (SEQ ID NO:4); CVWQPRFNYYWC (SEQ ID NO:5); CTFQPRFNYYWC (SEQ ID NO:6); CTFQPRFNNWWC (SEQ ID NO:7); CSFQPRFNYYWC (SEQ ID NO:8); CSFQPRFNNWWC (SEQ ID NO:9); CVFQPRFNYYWC (SEQ ID NO:10); CVFQPRFNNWWC (SEQ ID NO:11); CTFQWRFNYYWC (SEQ ID NO:12); CHFNPFRNWWC (SEQ ID NO:16); CVFQGRFNNWWC (SEQ ID NO:18); CVFQHRFNNWWC (SEQ ID NO:19); CVFNPRFNNWWC (SEQ ID NO:20); CVFKPRFNNWWC (SEQ ID NO:21); in each case optionally comprising an intramolecular disulfide bond.

[0178] In some embodiments, a TNFi peptide comprises a core TNF α binding domain selected from the amino acid sequences set forth in SEQ ID NO:4-28 and 77-110.

[0179] TNFi Peptides Comprising D-Amino Acid Analogs

[0180] In some embodiments, one or more D-amino acids in a TNFi peptide can be replaced with a D- α -amino acid analog of the D-amino acid(s). A D- α -amino acid analog is a D- α -amino acid analog that has both an amine functional group, either as NH₂, NHR, or NR₂, and a carboxylic acid functional group. In some embodiments, a TNFi peptide having a core TNF α binding domain set forth in any of SEQ ID NOs:1 to 3 and 111 to 113 has at least one D- α -amino acid analog substitution in the core TNF α binding domain. In some embodiments, a TNFi peptide having a core TNF α binding domain set forth in any of SEQ ID NOs:1 to 3 and 111 to 113 has at least two D- α -amino acid analog substitutions in the core TNF α binding domain. In some embodiments, a TNFi peptide having a core TNF α binding domain set forth in any of SEQ ID NOs:1 to 3 and 111 to 113 has at least three D- α -amino acid analog substitutions in the core TNF α binding domain.

[0181] Charged D- α -amino acid analogs include the D forms of 4-methyl glutamate, mono-4-fluoro glutamate, 4,4-difluoro-glutamate, gamma-hydroxy glutamate, L-threo- β -hydroxyaspartate, epsilon-N,N,N-trimethyllysine, epsilon-N-acetyllysine, azaleucine, O-phosphoserine, 3-methylhistidine, 5-hydroxylysine, and methylarginine.

[0182] Polar uncharged D-amino acid analogs include the D forms of L-Glu γ -hydrazide, L-albizziin, L-theanine, β -hydroxy norvaline, aspartate methyl ester and glutamate methyl ester.

[0183] Aromatic D-amino acid analogs include the D forms of β -2-thiazolyl-alanine, triazole alanine, 3-fluoro-L-tyrosine, 3-nitro-L-tyrosine, 3-fluoro phenylalanine, 2-

thienyl alanine, β -methyl phenylalanine), β -thienyl serine, N-acetylserine, N-formylmethionine, p-azido phenylalanine, p-ethynyl phenylalanine, p-nitro-phenylalanine, 7-aza-tryptophan, 5-hydroxy tryptophan, 5-fluoro tryptophan, 5-methoxy tryptophan, 3-(thianapthen-3-yl)-L-alanine, 2-thienyl glycine and L-phenylglycine.

[0184] Non-polar D-amino acid analogs include the D forms of 2-amino hex-5-ynoic acid, norleucine, norvaline, ethionine, β -azidohomoalanine, trifluoro norleucine, trifluoro norvaline, L-C-propargyl glycine, L-allyl glycine, β -cyclopropyl alanine, 3-fluoro-valine, methyl ether L-threonine, methyl ether L-allo-threonine, 4-thia-isoleucine, L-cyclohexyl-glycine, 5',5',5'-trifluoro leucine, β -cyclopentyl alanine, thiazolidine-2-carboxylic acid, thiazolidine-4-carboxylic acid, pseudoproline, 3,4-dehydro proline, and 4-hydroxyproline.

[0185] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having a D- α -amino acid analog has the following amino acid sequence: XTFQPRFNNWWC (SEQ ID NO:114), optionally comprising an intramolecular disulfide bond, wherein X is Penicillamine (Pen).

[0186] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having a D- α -amino acid analog has the following amino acid sequence: CTFQPRFNNWWX (SEQ ID NO:115), optionally comprising an intramolecular disulfide bond, wherein X is Penicillamine (Pen).

[0187] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having a D- α -amino acid analog has the following amino acid sequence: CXFQPRFNNWWC (SEQ ID NO:74), optionally comprising an intramolecular disulfide bond, wherein X is homoleucine (homoLeu).

[0188] In some embodiments, a TNFi peptide comprises a core TNF α binding domain having a D- α -amino acid analog has the following amino acid sequence: CXFQPRFNNWWC (SEQ ID NO:75), optionally comprising an intramolecular disulfide bond, wherein X is norleucine (norLeu).

[0189] Other Modifications to TNFi Peptides

[0190] In some embodiments, the TNFi peptides are capped at the N-terminus and/or the C-terminus. In some embodiments, a TNFi peptide is capped at the N-terminus. In some embodiments, a TNFi peptide is capped at the N-terminus with, for example, an acetyl group. In some embodiments, a TNFi peptide is capped at the N-terminus with, for example, a protecting group. In some embodiments, a TNFi peptide is capped at the C-

terminus. In some embodiments, a TNFi peptide is capped at the C-terminus with, for example, an amide group. In some embodiments, a TNFi peptide is capped at the C-terminus with, for example, a protecting group.

[0191] In some embodiments, a TNFi peptide has a core TNF α binding domain that is flanked by an additional amino acid residue(s) attached to the N-terminus and/or the C-terminus (a flanking sequence or tag). In some embodiments, a TNFi peptide has a core TNF α binding domain that is flanked by an additional D-amino acid residue(s) attached to the N-terminus and/or the C-terminus (a flanking sequence or tag). A tag can, for example, increase the solubility of a TNFi peptide (*e.g.*, in an aqueous solution). When more than one tag is present, the tags can be the same or different. In some embodiments, a tag is attached to the N-terminus of the core-TNF α binding domain. In some embodiments, a tag is attached to the C-terminus of the core TNF α binding domain. In some embodiments, a tag is attached to the N-terminus and to the C-terminus of the core TNF α binding domain.

[0192] In some embodiments, a tag is attached to the N-terminus of the core TNF α binding domain. In some embodiments, a tag attached to the N-terminus of the core TNF α binding domain is 1 to 20 amino acids in length, 1 to 10 amino acids in length, 1 to 6 amino acids in length, or 1 to 3 amino acids in length, 2 to 3 amino acids in length or 1 to 2 amino acids in length or 2 amino acids in length. In some embodiments, a tag having 1, 2 or 3 D-aspartate residues (D, DD, or DDD, respectively) is attached to the N-terminus of the core-TNF α peptide binding domain. In some embodiments, a tag having the amino acid sequence DGA is attached to the N-terminus of the core TNF α binding domain. In some embodiments, a tag of 1 or 2 D-aspartate residues is attached to the N-terminus of the core TNF α binding domain. In some embodiments, a tag of 2 D-aspartate residues is attached to the N-terminus of the core TNF α binding domain. In some embodiments, a tag of 1 D-aspartate residue is attached to the N-terminus of the core TNF α binding domain.

[0193] In some embodiments, a tag is attached to the C-terminus of the core TNF α binding domain. In some embodiments, a tag attached to the C-terminus of the core TNF α binding domain is 1 to 10 amino acids in length, or 1 to 8 amino acids in length, 2 to 8 amino acids in length or 2 to 6 amino acids in length. In some embodiments, a tag comprises a single glycine attached to the C-terminus of the core TNF α binding domain. In some embodiments, a tag comprises a pair of glycine residues attached to the C-terminus of the core TNF α binding domain. In some embodiments, a tag has the D-amino acid sequence GGEEEEK (SEQ ID NO:30) and is attached to the C-terminus of the core TNF α

binding domain. In some embodiments, a tag has the D-amino acid sequence GGRRRK (SEQ ID NO:31) and is attached to the C-terminus of the core TNF α binding domain.

[0194] In some embodiments, the TNFi peptides are capped at the N-terminus and/or the C-terminus of a tag. In some embodiments, the TNFi peptides are capped at the N-terminus of a tag. In some embodiments, the TNFi peptides are capped at the N-terminus with, for example, an acetyl group. In some embodiments, the TNFi peptides are capped at the C-terminus of a tag. In some embodiments, the TNFi peptides are capped at the C-terminus with, for example, an amide group.

[0195] In some embodiments, a TNFi peptide can comprise additional amino acids in addition to the core TNF α binding domain and any attached tags. Such additional amino acids can be D-amino acids or L-amino acids. In some embodiments, a TNFi peptide, including a tag at the N-terminus and/or C-terminus, is from 12 to 50 amino acids in length, or 12 to 40 amino acids in length, or 12 to 30 amino acids in length, or 12 to 20 amino acids in length. In some embodiments, a TNFi peptide, including a tag at the N-terminus and/or C-terminus, is from 12 to 50 amino acids in length. In some embodiments, a TNFi peptide, including a tag at the N-terminus and/or C-terminus, is from 12 to 40 amino acids in length. In some embodiments, a TNFi peptide, including a tag at the N-terminus and/or C-terminus, is from 12 to 30 amino acids in length. In some embodiments, a TNFi peptide, including a tag at the N-terminus and/or C-terminus, is from 12 to 20 amino acids in length.

[0196] Linkers

[0197] In some embodiments, a TNFi peptide includes a Linker attached to its N-terminus and/or its C-terminus. In some embodiments, a Linker comprises L-amino acids. In some embodiments, a Linker comprises D-amino acids. In some embodiments, a Linker comprises chemical groups other than amino acids. In some embodiments, a Linker comprises chemical groups (other than amino acids) and an amino acid(s). In some embodiments, a Linker comprises both chemical groups (other than amino acids) and an L-amino acid(s). In some embodiments, a Linker comprises chemical groups (other than amino acids) and a D-amino acid(s).

[0198] In some embodiments, a Linker comprises a repeating polymer unit. In some embodiments, a polymer unit is attached to the N-terminus of a TNFi peptide. In some embodiments, a polymer unit is attached to the C-terminus of a TNFi peptide. In some embodiments, a polymer unit is attached to the N-terminus and to the C-terminus of

a TNFi peptide. The polymer units attached to the N-terminus and the C-terminus can be the same or different.

[0199] In some embodiments, a polymer unit comprises a polyethylene glycol chain (PEG group). In some embodiments, a polymer unit comprises a polysaccharide chain. In some embodiments, a polymer unit comprises an alkyl polyol chain. In some embodiments, a polymer unit comprises an elastin-like polypeptide. In some embodiments, a polymer unit comprises a poly-sarcosine chain. In some embodiments, a polymer unit comprises an alkyl chain. In some embodiments, a polymer unit comprises a polypeptide chain, such as albumin or other polypeptide.

[0200] In some embodiments, a Linker comprises a polymer unit having a polyethylene glycol chain (PEG group). In some embodiments, a Linker comprises a polyethylene glycol chain (PEG group) that is linear or branched. In some embodiments, a PEG group is attached to the N-terminus of the TNFi peptide. In some embodiments, a PEG group is attached to the C-terminus of the TNFi peptide. In some embodiments, a PEG group is attached to the N-terminus and to the C-terminus of a TNFi peptide. PEG groups attached to the N-terminus and the C-terminus can be the same or different.

[0201] In some embodiments, a PEG group has from 1 to 48 (ethylene glycol) subunits and is either linear or branched. In some embodiments, a PEG group has from 1 to 30 subunits and is either linear or branched. In some embodiments, a PEG group has from 1 to 24 subunits and is either linear or branched. In some embodiments, a PEG group has from 1 to 12 subunits. In some embodiments, a PEG group has from 4 to 30 subunits and is either linear or branched. In some embodiments, a PEG group has from 4 to 24 subunits and is either linear or branched. In some embodiments, a PEG group has from 4 to 12 subunits and is either linear or branched. In some embodiments, a PEG group has from 4 to 10 subunits and is either linear or branched. In some embodiments, a PEG group has 4 subunits and is either linear or branched. In some embodiments, a PEG group has 6 subunits and is either linear or branched. In some embodiments, a PEG group has 8 subunits and is either linear or branched. In some embodiments, a PEG group has 10 subunits and is either linear or branched. In some embodiments, a PEG group has 12 subunits and is either linear or branched.

[0202] In some embodiments, a Linker includes a functional group at an end opposite from its attachment site to a TNFi peptide. A functional group can serve as a cap and/or provide an attachment site for another molecule, such as a multimer scaffold group

or an auxiliary molecule. In some embodiments, the functional group is, for example, an acetate group, a carboxylic acid group, an amine group, an amide group, a carboxamide group, a thiol group, a hydrazide group, an NHS-ester or a o-pentafluorophenyl ester. In some embodiments, the functional group is an amino acid residue or residues. In some embodiments, the amino acid residue or residues of the functional group are in the L-configuration. In some embodiments, the amino acid residues of the functional group are in the D-configuration. In some embodiments, the functional group is an amino acid residue, such as a lysine residue. In some embodiments, the functional group is an attachment site for a multimer scaffold. In some embodiments, the functional group comprises one or more lysine residues, one or more hydrazide groups or one or more thiols or other groups.

[0203] Multimers

[0204] In some embodiments, the TNFi peptides can be in the form of a multimer, such as a dimer or a trimer or a higher order multimer. For example, when a multimer is a dimer, the dimer can be comprised of two identical TNFi peptides (*i.e.*, a homodimeric), or can be comprised of two different TNFi peptides (*i.e.*, heterodimeric). A multimer can also be a trimer. When the multimer is a trimer, the trimer can be comprised of two identical TNFi peptides and one different TNFi peptide (*i.e.*, heterotrimeric), or three identical TNFi peptides (*i.e.*, homotrimeric), or three different TNFi peptides, each of which is distinct from each other (*i.e.*, heterotrimeric).

[0205] In some embodiments, two or more TNFi peptides can be linked via a Linker (*e.g.*, a linker of amino acid residues or other chemical moieties, as described herein or as known to the skilled artisan) to form a multimer, such a dimer, trimer, or higher order multimer.

[0206] In some embodiments, the TNFi peptides form a dimer. In some embodiments, the TNFi peptides form a trimer. In some embodiments, the TNFi peptides form a multimer comprising at least four TNFi peptides. In some embodiments, a higher order multimer can include 5, 6, 7, 8, 9, 10, 11, 12, or more TNFi peptides. A multimer can be heteromeric or homomeric.

[0207] In some embodiments, TNFi peptides are joined in a dimeric format and form a linear chain. In some embodiments, the TNFi peptides are linked via the C-terminus of one peptide joined to the N-terminus of a second peptide, optionally comprising a Linker connecting the two TNFi peptides. In some embodiments, the TNFi peptides are linked

via the C-terminus of one peptide joined to the C-terminus of the second peptide, optionally comprising a Linker connecting the two TNFi peptides. In some embodiments, the TNFi peptides are linked via the N-terminus of one peptide joined to the N-terminus of the second peptide, optionally comprising a Linker connecting the two TNFi peptides. In any of these embodiments, the TNFi peptides may be the same or different.

[0208] In some embodiments, TNFi peptides are joined in a trimeric format and form a linear chain. In some embodiments, the TNFi peptides are linked via the C-terminus of one peptide joined to the N-terminus of the next peptide, the C-terminus of which is connected to the N-terminus of the third peptide, optionally comprising a Linker connecting the pairs of TNFi peptides. In some embodiments, the TNFi peptides are linked in any suitable orientation of N-termini and C-termini. In any of these embodiments, the TNFi peptides may be the same or different.

[0209] In some embodiments, TNFi peptides are joined in a linear multimeric format comprising at least four TNFi peptides and form a linear chain. In some embodiments, a higher order multimer can include 5, 6, 7, 8, 9, 10, 11, 12 or more peptides per chain. In some embodiments, the multimers are linked via the C-terminus of one peptide joined to the N-terminus of the next peptide, optionally comprising a Linker connecting the pairs of TNFi peptides. In some embodiments, the multimers are linked in any suitable orientation of N-termini and C-termini, optionally comprising a Linker connecting the pairs of TNFi peptides. In any of these embodiments, the peptides may be the same or different.

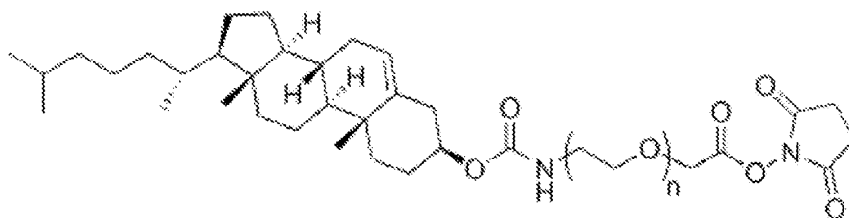
[0210] In some embodiments, the TNFi peptides are crosslinked to form a multimer, such as a branched multimer. TNFi peptides are crosslinked via their N- and/or C-termini. In some embodiments, a crosslinker is a polyethylene glycol (PEG) group derivatized with a reactive group, such as an N-hydroxysuccinimide (NHS)-ester (which reacts with a Lys residue) and/or a maleimide group (which reacts with a thiol group). In some embodiments, a crosslinker can contain two distinct linkage chemistries (*e.g.*, NHS-ester on one end and maleimide on the other end). In any of these embodiments, the peptides may be the same or different.

[0211] In some embodiments, TNFi peptides are crosslinked through the use of a multimer scaffold(s). A multimer scaffold can include two or more functional groups for attachment of TNFi peptides, each TNFi peptide optionally attached to a multimer scaffold via a Linker. In some embodiments, each TNFi peptide is attached to a reactive group on

a multimer scaffold via a Linker, such as a PEG group. In some embodiments, each TNFi peptide is attached to a reactive group on a multimer scaffold via a Linker, such as a PEG group.

[0212] In some embodiments, a multimer scaffold can be a dimeric scaffold comprising two functional groups, such as NHS ester groups. In some embodiments, a multimer scaffold may comprise two of the same functional groups, such as NHS ester groups. In some embodiments, a multimer scaffold may comprise at least two different functional groups, such as an NHS ester group and a maleimide group. In some embodiments, a multimer scaffold can be a trimeric scaffold comprising three functional groups, such as NHS ester groups or o-pentafluorophenyl ester groups. In some embodiments, a multimer scaffold may comprise three of the same functional groups, such as NHS ester groups or o-pentafluorophenyl ester groups. In some embodiments, a multimer scaffold may comprise at least two different functional groups, such as NHS ester groups or o-pentafluorophenyl ester groups and maleimide groups. In some embodiments, a multimer scaffold may be a tetrameric scaffold comprising four functional groups, such as three NHS ester groups, or o-pentafluorophenyl ester groups and a fourth orthogonal group. In some embodiments, a multimer scaffold may comprise different functional groups, for example, NHS ester groups, o-pentafluorophenyl ester groups, maleimide groups and a fourth orthogonal group. In some embodiments, a functional group can be Fmoc-amine, nitro, amine, or carboxylate.

[0213] In some embodiments, an orthogonal group can be a functional group for attachment of an auxiliary molecule. In some embodiments, an orthogonal group can be, for example, a PEG chain, a cholesterol moiety, a biotin group, a lipid (fatty acid group), a nitro group, or a protected amine. In some embodiments, a PEG chain of an orthogonal group has from 2 to 48 ethylene glycol repeats, or 2 to 24 ethylene glycol repeats. In some embodiments, an orthogonal group is a cholesteryl-PEG4-NHS ester, as shown in the following figure where $n = 4$:



[0214] In some embodiments, a TNFi peptide is attached to a multimer scaffold via a Linker. In some embodiments, the Linker comprises a PEG group having functional groups at its termini. In some embodiments, the PEG group has an NHS ester at two termini. In some embodiments, the PEG group has an o-pentafluorophenyl ester at two termini. In some embodiments, the PEG group has an amine group at one terminus and an NHS ester at the other terminus. In some embodiments, the PEG group has an amine group at one terminus and an o-pentafluorophenyl ester at the other terminus. In some embodiments, the PEG group has a carboxyl group at one terminus and an o-pentafluorophenyl ester at the other terminus. In some embodiments, the PEG group has an amine or carboxyl group at one terminus and an NHS ester or o-pentafluorophenyl ester at the other terminus. In some embodiments, the PEG group has an amine or carboxyl group at one terminus and a carboxyl or amine group at the other terminus.

[0215] In some embodiments, a multimer scaffold is a trifunctional cross-linker, such as tris(succinimidyl) aminotriacetate (TSAT), which contains three N-hydroxysuccinimide (NHS) ester groups. In some embodiments, a multimer scaffold is a trifunctional cross-linker, such as Tris-succinimidyl (6-aminocaproyl)aminotriacetate (LC-TSAT). In some embodiments, a multimer scaffold is a heterotetrameric PEG scaffold, having three functional groups of one type and a further reactive group orthogonal to the other functional groups. In some embodiments, a multimer scaffold is a heterotetrameric PEG scaffold, having three NHS ester functional groups and a further maleimide reactive group. In some embodiments, the Linkers connecting the TNFi peptides to the scaffold have different PEG lengths than a PEG group attached to the orthogonal reactive group.

[0216] In some embodiments, a multimer scaffold is an Fmoc scaffold (bis(2,5-dioxypyrrolidin-1-yl) 4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(3-((2,5-dioxypyrrolidin-1-yl)oxy)-3-oxopropyl)heptanedioate. In some embodiments, the multimer scaffold is an Fmoc scaffold having a PEG27 chain (see Figure 15A). In some embodiments, the multimer scaffold is a cyclohex scaffold (tris(2,5-dioxypyrrolidin-1-yl) cyclohexane-1,3,5-tricarboxylate). In some embodiments, the multimer scaffold is a nitro scaffold (bis(2,5-dioxypyrrolidin-1-yl) 4-(3-((2,5-dioxypyrrolidin-1-yl)oxy)-3-oxopropyl)-4-nitroheptane dioate). In some embodiments, the multimer scaffold is an Amine scaffold (bis(2,5-dioxypyrrolidin-1-yl) 4-amino-4-(3-((2,5-dioxypyrrolidin-1-yl)oxy)-3-oxopropyl) heptane dioate). In some embodiments, the multimer scaffold is an

Amine-PEG27 scaffold, as shown in Figure 16A. In some embodiments, the multimer scaffold is a cholesterol scaffold, as shown in Figure 16A. In some embodiments, a heterotetrameric PEG scaffold is based on a 3-{2-Amino-3-(2-carboxyethoxy)-2-[(2-carboxyethoxy)-methyl]propoxy}propionic acid scaffold. In some embodiments, the multimer scaffold is based on 4-amino-4-(2-carboxyethyl)heptanedioic acid. In some embodiments, a TNFi peptide comprises a C-terminal D-lysine residue that is attached to a carboxyl group of 4-amino-4-(2-carboxyethyl)heptanedioic acid. In some embodiments, the TNFi peptide multimer comprises three TNFi peptides, each TNFi peptide having a C-terminal D-lysine residue that is attached to a carboxyl group of 4-amino-4-(2-carboxyethyl)heptanedioic acid. In some embodiments, such a TNFi peptide multimer further comprises an auxiliary molecule attached to the free amino group of the carboxyl group of 4-amino-4-(2-carboxyethyl)heptanedioic acid scaffold. In some further embodiments, the auxiliary molecule is a PEG group.

[0217] In some embodiments, a PEG group of a Linker attached to a multimer scaffold has from 1 to 48 subunits. In some embodiments, the PEG group of a Linker has from 1 to 30 subunits. In some embodiments, the PEG group of a Linker has from 1 to 24 subunits. In some embodiments, the PEG group of a Linker has from 1 to 12 subunits. In some embodiments, the PEG group of a Linker has from 4 to 30 subunits. In some embodiments, the PEG group of a Linker has from 4 to 24 subunits. In some embodiments, the PEG group of a Linker has from 4 to 12 subunits. In some embodiments, the PEG group of a Linker has from 4 to 10 subunits. In some embodiments, the PEG group of a Linker has 4 subunits. In some embodiments, the PEG group of a Linker has 6 subunits. In some embodiments, the PEG group of a Linker has 8 subunits. In some embodiments, the PEG group of a Linker has 10 subunits. In some embodiments, the PEG group of a Linker has 12 subunits. In some embodiments, the PEG group of a Linker as part of the multimer can have the same or different length. In some embodiments, the PEG group may be composed of a single PEG chain, or a first PEG chain and a second PEG chain linked in series. In some embodiments, the PEG group may include an internal NHS-ester or other bond, such as is formed by linking multiple PEG groups together.

[0218] In some embodiments, a Linker or multimer scaffold can comprise a tris, di-lysine, benzene ring, phosphate, or peptide core as a functional or reactive group. In some embodiments, a cross-linking group includes a bis(sulfosuccinimidyl)suberate (B3S), a Disuccinimidyl glutarate (DSG) or a bis(sulfosuccinimidyl)suberate (DST) group. In

some embodiments, a crosslinking group comprises a thiol-reactive group, *e.g.*, haloacetyls (*e.g.*, iodoacetate), pyridyl disulfides (*e.g.*, HPDP), and other thiols.

[0219] Auxiliary Molecules Attached to TNFi Peptides or Multimers Thereof

[0220] In some embodiments, a TNFi peptide or multimer thereof can be modified or linked to an auxiliary molecule, such as a potency-enhancing molecule, a stabilizing molecule or other molecule that provides an increase in activity, potency, binding, pharmacokinetic, membrane-localizing, or other property(ies).

[0221] In some embodiments, a multimer scaffold can include auxiliary molecule, such as a PEG molecule (*e.g.*, linear or branched), sterol (*e.g.*, cholesterol) or analog thereof (*e.g.*, thiocholesterol), a sugar, a maltose binding protein, a biotin group, a lipid (a fatty acid), serum albumin, ubiquitin, streptavidin, immunoglobulin domains, keyhole limpet hemacyanin, sperm whale myoalbumin, green fluorescent protein, gold particle, magnetic particle, agarose bead, lactose bead, an alkane chain (*e.g.*, C₈, C₁-C₆, or a C₁-C₈ alkyl chain or the like), or a fatty acid (*e.g.*, C₈ fatty acid, C₁₆ fatty acid, C₁₈ fatty acid, palmitate, or the like). In other embodiments, the auxiliary molecule can be the linking of multiple multimers, such as the linking of multiple trimers (*e.g.*, to increase molecular weight and reduce renal filtration).

[0222] In some embodiments, a TNFi peptide or a multimer thereof comprises an auxiliary molecule such as a label or other detectable marker. In some embodiments, such a label or other detectable marker is attached at the N-terminus and/or the C-terminus of the TNFi peptide. In some embodiments, the TNFi peptide is labeled at the N-terminus. In some embodiments, the TNFi peptide is labeled at the N-terminus with, for example, a biotin group. In some embodiments, the TNFi peptide is labeled at the C-terminus. In some embodiments, the TNFi peptide is labeled at the C-terminus with, for example, a biotin group.

[0223] In some embodiments, a TNFi peptide multimer comprises a label or other detectable marker. In some embodiments, such a label or other detectable marker is attached at the N-terminus and/or the C-terminus of the TNFi peptide or to an orthogonal arm of a multimer scaffold. In some embodiments, the label or other detectable marker is a biotin group.

[0224] In some embodiments, an auxiliary molecule is attached to a TNFi peptide or a multimer thereof via a PEG group. Various chemistries that are known in the art may be used to attach an auxiliary molecule to a PEG group. For example, the auxiliary

molecule may be attached to a PEG group via carbamate, formed via a halide formate molecule reacting with an amine. In another example, the auxiliary molecule may be attached to a PEG group via an amide bond, formed by condensation between a carboxylic acid molecule and an amine. In another example, the auxiliary molecule may be attached to a PEG group via an amide bond, formed by an-NHS molecule or any other active ester. In another example, the auxiliary molecule may be attached to a PEG molecule chain via an amide bond, formed by reaction of a ketone with an amine (isourea). In another example, the auxiliary molecule may be attached to a PEG chain via a thioether bond, formed by reaction of a thiol with a maleimide ester. In another example, the auxiliary molecule may be attached to a PEG group via an ether bond, for example via a dehydration reaction with a terminal hydroxyl on an auxiliary molecule and a PEG group. In yet another example, the auxiliary molecule may be attached to a PEG group via click chemistry, for example Huisgen 1,3-dipolar cycloaddition between an azide and an alkyne.

[0225] In some embodiments, a PEG group attached to an auxiliary molecule may be composed of a single PEG chain or a first PEG chain and a second PEG chain linked in series. In some embodiments, the PEG group auxiliary molecule may include an internal NHS-ester or other bond, such as is formed by linking multiple PEG groups together.

[0226] Avidity of Multimers

[0227] Multimers of the TNFi peptides can have an increased affinity for a TNF α molecule, when compared with the affinity of a single TNFi peptide, or control peptide, for the TNF α molecule. In some embodiments, multimers of the TNFi peptides can have an increased affinity for trimeric TNF α , when compared with the affinity of a single TNFi peptide, or control peptide, for trimeric TNF α . In some embodiments, the TNFi peptide multimer is a dimer. In some embodiments, the TNFi peptide multimer is a trimer. The single peptide, or control peptide, can be identical to one of the components of the multimer, or the single peptide can be a different peptide which is not contained in the multimer.

[0228] A TNFi peptide multimer can exhibit about a 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 1000-fold, 2000-fold, 3000-fold, 4000-fold, 5000-fold, or 10,000-fold increase in affinity for trimeric TNF α when compared with the affinity of a TNFi peptide alone.

[0229] Pharmaceutical Compositions

[0230] The TNFi peptides and multimers thereof can be administered *in vivo* in a pharmaceutical composition. A pharmaceutical composition typically includes a pharmaceutically acceptable excipient, carrier and/or other components. By “pharmaceutically acceptable” is meant that the components of the composition are not biologically or otherwise undesirable, *i.e.*, the components may be administered to a subject (*e.g.*, a human) along with TNFi peptide or multimer thereof, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. A pharmaceutically acceptable excipient is selected to minimize any degradation of the active ingredient (*e.g.*, TNFi peptides or multimers thereof) and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

[0231] Pharmaceutical compositions may be formulated for any suitable form of administration. Pharmaceutical compositions may be formulated for oral administration, for parenteral administration (*e.g.*, for intravenous administration, for intramuscular administration, or for subcutaneous administration), for topical administration (*e.g.*, dermal), for rectal administration, and the like. The dosage of a pharmaceutical composition required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disease, its mode of administration and the like. An appropriate dosage can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

[0232] Parenteral administration of pharmaceutical composition is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Parenteral administration may also include use of a slow release or sustained release system (*i.e.*, depot) such that a constant dosage is maintained.

[0233] Suitable excipients and carriers and formulations thereof are described in Remington: The Science and Practice of Pharmacy (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically acceptable salt is used in a formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer’s solution, and dextrose solution. The pH of the solution may be from about 5 to about 8, and alternatively from about 7 to about 7.5. Further carriers include sustained

release preparations such as semipermeable matrices of solid hydrophobic polymers containing TNFi peptides or multimers thereof, which matrices are in the form of shaped articles, *e.g.*, films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of the composition being administered.

[0234] Carriers for inclusion in a pharmaceutical composition are known to those skilled in the art. These most typically are standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously.

[0235] Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents, and the like, in addition to the TNFi peptides or multimers thereof. A pharmaceutical composition may also include one or more other active ingredients such as an antimicrobial agent, an anti-inflammatory agent, an anesthetic, and the like.

[0236] Preparations for parenteral administration include sterile aqueous or nonaqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions, or suspensions including, for example, saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, an antimicrobial, an anti-oxidant, a chelating agent, an inert gas, and the like.

[0237] Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable. Additionally, it is contemplated herein that compositions designed for oral administration can further comprise a gut permeabilizing agent.

[0238] In some embodiments, a TNFi peptide or multimers thereof may be administered as a pharmaceutically acceptable acid- or base-addition salt, formed by reaction with an inorganic acid such as hydrochloric acid, hydrobromic acid, perchloric

acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and an organic base such as, for example, a mono-, di-, trialkyl and aryl amine and a substituted ethanolamine.

[0239] In some embodiments, a pharmaceutical composition of a TNFi peptide or a multimer thereof is lyophilized. Formulations of the TNFi peptide or multimers thereof can be lyophilized for reconstitution or in liquid form. The terms “lyophilization,” “lyophilized,” and “freeze-dried” refer to a process by which the material to be dried is first frozen and then the ice or frozen solvent is removed by sublimation in a vacuum environment. An excipient may be included in a pre-lyophilized formulation to enhance stability of the lyophilized product upon storage. A “reconstituted” formulation is one that has been prepared by dissolving a lyophilized protein formulation in a diluent such that the protein is dispersed in the reconstituted formulation. The reconstituted formulation is suitable for administration, (*e.g.*, parenteral administration), and may optionally be suitable for subcutaneous administration or other route of administration.

[0240] Uses

[0241] A TNFi peptide and multimers thereof can be used to reduce TNF α -mediated inflammation in a subject. In some embodiments, the TNFi peptide or multimer thereof is administered to reduce TNF α -mediated inflammation in a subject. In some embodiments, the TNFi peptide or multimer thereof is administered to reduce TNF α activity in a subject. In some embodiments, the TNFi peptide or a multimer thereof is administered to reduce an inflammatory response mediated by TNF α . A TNFi peptide or a multimer thereof can be administered by oral administration, by parenteral administration, by intravenous administration, by intramuscular administration, by subcutaneous administration, by topical administration, by rectal administration, or the like. In some embodiments, the TNFi peptide or a multimer thereof is administered locally, so as to achieve local inhibition of TNF α activity or TNF α -mediated inflammation or a TNF α -mediated inflammatory response.

[0242] A TNFi peptide and multimers thereof can be used to treat subjects having a TNF α -mediated disease. In some embodiments, the TNFi peptide or multimers thereof are administered to a subject in need thereof for the treatment of a TNF α -mediated disease.

In some embodiments, the TNF α - mediated disease is an inflammatory disease or condition. In some embodiments, the TNF α - mediated disease is an autoimmune disorder.

[0243] In some embodiments, the TNFi peptide or multimers thereof are administered orally. In some embodiments, the TNFi peptide or multimers thereof are administered parenterally. In some embodiments, the TNFi peptide or multimers thereof are administered intravenously. In some embodiments, the TNFi peptide or multimers thereof are administered intramuscularly. In some embodiments, the TNFi peptide or multimers thereof are administered subcutaneously. In some embodiments, the TNFi peptides or multimers thereof are administered topically. In some embodiments, the TNFi peptide or multimers thereof are administered by rectal administration.

[0244] As used herein, the term “subject” refers to a human or an animal. Usually, the animal is a vertebrate such as a primate, rodent, domestic animal, or game animal. Primates include chimpanzees, cynomolgus monkeys, spider monkeys, and macaques, *e.g.*, Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits, and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, *e.g.*, domestic cat, canine species, *e.g.*, dog, fox, wolf, avian species, *e.g.*, chicken, emu, ostrich, and fish, *e.g.*, trout, catfish, and salmon. In some embodiments, the subject is a mammal, *e.g.*, a primate, *e.g.*, a human. In some embodiments, the subject is a human, monkey, and dog. In some embodiments, the subject is human.

[0245] In some embodiments, a TNFi peptide or multimers thereof are administered to a subject in need thereof for the treatment of a TNF α -mediated disease, such as adult Crohn’s Disease, pediatric Crohn’s Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile idiopathic arthritis (polyarticular), Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or non-radiographic axial spondyloarthritis.

[0246] In some embodiments, a TNFi peptide or a multimer thereof is administered to a subject in need thereof having an inflammatory bowel disease, such as adult or pediatric Crohn’s Disease or Ulcerative Colitis. In some embodiments, the TNFi peptide is administered orally to the subject.

[0247] In some embodiments, a TNFi peptide or a multimer thereof is administered to a subject in need thereof having an inflammatory bowel disease, such as adult or pediatric

Crohn's Disease or Ulcerative Colitis. In some embodiments, the TNFi peptide is administered rectally (*e.g.*, by enema) to the subject.

[0248] In some embodiments, a TNFi peptide or a multimer thereof is administered to a subject in need thereof having an inflammatory skin disease, such as Plaque Psoriasis, Hidradenitis suppurativa, or Cutaneous Lupus. In some embodiments, the TNFi peptide is administered topically to the subject.

[0249] In some embodiments, a TNFi peptide or a multimer thereof is administered to a subject in need thereof having an inflammatory skin disease, such as Plaque Psoriasis, Hidradenitis suppurativa, or Cutaneous Lupus. In some embodiments, the TNFi peptide or a multimer thereof is administered parenterally (such as subcutaneously, intramuscularly, or intravenously) to the subject. In some embodiments, the TNFi peptide or a multimer thereof is administered subcutaneously to the subject. In some embodiments, the TNFi peptide or a multimer thereof is administered intramuscularly to the subject. In some embodiments, the TNFi peptide or a multimer thereof is administered intravenously to the subject.

[0250] In some embodiments, a TNFi peptide or a multimer thereof is administered to a subject in need thereof having an inflammatory disease, such as Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, or Ankylosing Spondylitis. In some embodiments, the TNFi peptide or a multimer thereof is administered parenterally (such as subcutaneously, intramuscularly, or intravenously) to the subject. In some embodiments, the TNFi peptide or a multimer thereof is administered subcutaneously to the subject. In some embodiments, the TNFi peptide or a multimer thereof is administered intramuscularly to the subject. In some embodiments, the TNFi peptide or a multimer thereof is administered intravenously to the subject.

[0251] Effective dosages and schedules for administering a TNFi peptide or a multimer thereof may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the TNFi peptide or multimer and compositions thereof are those large enough to produce the desired effect in which the symptoms/disorder is affected. The dosage is typically not so large as to cause serious adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex, and extent of the disease in the subject, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by

the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one, or several days.

[0252] In some embodiments, a typical daily dosage of the TNFi peptide or multimer thereof used might range from about 1 µg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. In some embodiments, a typical daily dosage of the TNFi peptide or multimer thereof used might range from about 0.01 µg/kg to up to 1 mg/kg of body weight or more per day. In some embodiments, the TNFi peptide or multimer thereof can be administered several times daily, daily, weekly, monthly, or yearly, depending on the condition of the subject, other modes of therapy, and the like. One of skill in the art could readily ascertain an appropriate dosing schedule.

[0253] The TNFi peptide or multimer thereof may be administered prophylactically to a patient or a subject who is at risk for a TNFα-mediated disease or at risk for recurrence of a TNFα-mediated disease.

[0254] Methods of Making the TNFi peptides and Multimers Thereof

[0255] A TNFi peptide or a multimer thereof can be made using any method known to those of skill in the art for preparation of a D-peptide or a multimer thereof. The TNFi peptide can be linked, for example, by disulfide crosslinks. For example, the D-peptides disclosed herein have two Cys residues connected by a disulfide bond, which circularizes the peptide and creates a more compact and structured peptide. This disulfide is known to have enhanced TNFα binding activity.

[0256] Two or more TNFi peptides can also be linked together by protein chemistry techniques. For example, a peptide or a polypeptide can be chemically synthesized using currently available laboratory equipment using either Fmoc (fluorenylmethyloxycarbonyl) or Boc (tert butyloxycarbonyl) chemistry (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide corresponding to any of the disclosed TNFi peptides, for example, can be synthesized by standard chemical reactions. For example, a TNFi peptide can be synthesized and not cleaved from its synthesis resin whereas another fragment of a peptide can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form a TNFi peptide. ((Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M. and Trost B.,

Ed. (1993) Principles of Peptide Synthesis. SpringerVerlag Inc., NY (both of which are herein incorporated by reference for material related to peptide synthesis)). Once isolated, these independent peptides may be linked to form a peptide via similar peptide condensation reactions.

[0257] For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger a peptide (Abrahmsen L., *et al.*, *Biochemistry*, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct larger peptides from shorter peptide fragments. This method consists of a two-step chemical reaction (Dawson *et al.*, Synthesis of Proteins by Native Chemical Ligation. *Science*, 266:776779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide thioester with another unprotected peptide segment containing an amino terminal Cys residue to give a thioester linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes a spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolini M. *et al.*, (1992) *FEBS Lett.* 307:97-101; Clark-Lewis I. *et al.*, *J. Biol. Chem.*, 269:16075 (1994); Clark-Lewis I. *et al.*, *Biochemistry*, 30:3128 (1991); Rajarathnam K. *et al.*, *Biochemistry* 33 :6623-6630 (1994)).

[0258] Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (nonpeptide) bond (Schnolzer, M. *et al.*, *Science*, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC *et al.*, Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257267 (1992)).

[0259] The present invention is further illustrated by the following embodiments which should not be construed as limiting.

1. A D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C-X²-X³-X⁴-X⁵-X⁶-F-N-N-X¹⁰-X¹¹-C (SEQ ID NO:1), wherein each of X¹ through X⁶, X¹⁰ and X¹¹ is a D-amino acid or a D α -amino acid analog thereof, and C, F, and N are the D forms of cysteine, phenylalanine, and asparagine, or a D- α -amino acid analogs thereof; and

- a. X^2 is the D form of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D- α -amino acid analog thereof;
 - b. X^3 is selected from the D forms of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L), or a D- α -amino acid analog thereof;
 - c. X^4 is a Polar amino acid selected from the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D- α -amino acid analog thereof;
 - d. X^5 is D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), or a D- α -amino acid analog thereof;
 - e. X^6 is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof;
 - f. X^{10} is the D form of Trp (W) or Tyr (Y), or a D- α -amino acid analog thereof;
 - g. X^{11} is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H), or a D- α -amino acid analog thereof; and
 - h. C denotes the D form of Cysteine, or a D- α -amino acid analog thereof, F denotes the D form of phenylalanine, or a D- α -amino acid analog thereof; and N denotes the D form of asparagine, or a D- α -amino acid analog thereof.
2. The D-peptide or a salt thereof of embodiment 1, wherein the core TNF α binding domain has the following amino acid sequence: C- X^2 -[W/F/Y]- X^4 - X^5 - X^6 -F-N-N-[W/Y]-W-C (SEQ ID NO:3).
 3. The D-peptide or a salt thereof of any of embodiments 1 to 2, further comprising an intramolecular disulfide bond between the cysteine residues of the core TNF α binding domain.
 4. The D-peptide or a salt thereof of any of embodiments 1 to 3, wherein:
 - a. X^2 is the D form of Thr, Val, His, Leu, Gln, Ala, Ile, Met or Trp, or a D- α -amino acid analog thereof;
 - b. X^2 is the D form of Thr, Val, His, Leu, or Gln, or a D- α -amino acid analog thereof;
 - c. X^2 is selected from the D form of Thr, Val, His and Leu, or a D- α -amino acid analog thereof;
 - d. X^2 is selected from the D form of Thr, Val and His, or a D- α -amino acid analog thereof;

- e. X^2 is selected from the D form of Thr and Val, or a D- α -amino acid analog thereof;
 - f. X^2 is the D form of Thr, or a D- α -amino acid analog thereof; or
 - g. X^2 is the D form of Val, or a D- α -amino acid analog thereof.
5. The D-peptide or a salt thereof of any of embodiments 1 or 3 to 4, wherein:
- a. X^3 is selected from the D forms of Trp, Phe, Tyr, and Ser, or a D- α -amino acid analog thereof;
 - b. X^3 is selected from the D forms of Trp, Phe, and Tyr, or a D- α -amino acid analog thereof;
 - c. X^2 is selected from the D forms of Trp and Phe, or a D- α -amino acid analog thereof;
 - d. X^3 is the D form of Trp or a D- α -amino acid analog thereof; or
 - e. X^3 is the D form of Phe, or a D- α -amino acid analog thereof.
6. The D-peptide or a salt thereof of any of embodiments 1 to 5, wherein:
- a. X^4 is the D form of Arg, His, Gln, Asn, Lys, Thr, or Ser, or a D- α -amino acid analog thereof;
 - b. X^4 is selected from the D forms of Arg, His, Gln and Asn, or a D- α -amino acid analog thereof;
 - c. X^4 is the D form of Arg, His or Gln, or a D- α -amino acid analog thereof;
 - d. X^4 is the D form of Arg, Gln or Asn, or a D- α -amino acid analog thereof; or
 - e. X^4 is the D form of Gln, or a D- α -amino acid analog thereof.
7. The D-peptide or a salt thereof of any of embodiments 1 to 6, wherein:
- a. X^5 is the D form of Pro, Trp, His, Gln, Gly, Arg, Ala, Val or Leu, or a D- α -amino acid analog thereof;
 - b. X^5 is the D form of Pro, Trp, His, Gln, Gly, Arg, or Val, or a D- α -amino acid analog thereof;
 - c. X^5 is the D forms of Pro, Trp or His, or a D- α -amino acid analog thereof;
 - d. X^5 is the D forms of Pro or Trp, or a D- α -amino acid analog thereof;
- or
- e. X^5 is the D form of Pro, or a D- α -amino acid analog thereof.

8. The D-peptide or a salt thereof of any of embodiments 1 to 7, wherein:
 - a. X^6 is the D-form of Arg, His, Lys, Glu, Gln, Val, Leu, Ser or Ala, or a D- α -amino acid analog thereof;
 - b. X^6 is the D-form of Arg, His, Lys, Glu, Gln, Val or Leu, or a D- α -amino acid analog thereof;
 - c. X^6 is the D-form of Arg, His, Lys, Glu, or Gln, or a D- α -amino acid analog thereof;
 - d. X^6 is the D-form of Arg, His, Lys, or Glu, or a D- α -amino acid analog thereof;
 - e. X^6 is the D-form of Arg or His, or a D- α -amino acid analog thereof;
 - f. X^6 is the D-form of Arg, or a D- α -amino acid analog thereof; or
 - g. X^5 is the D-form of His, or a D- α -amino acid analog thereof.
9. The D-peptide or a salt thereof of any of embodiments 1 and 3 to 8, wherein:
 - a. X^{10} is the D form of Trp, or a D- α -amino acid analog thereof; or
 - b. X^{10} is the D form of Tyr, or a D- α -amino acid analog thereof.
10. The D-peptide or a salt thereof of any of embodiments 1 and 3 to 9, wherein:
 - a. X^{11} is the D form of Trp (W), Tyr (Y), or Gln (Q), or a D- α -amino acid analog thereof; or
 - b. X^{11} is the D form of Trp (W), or a D- α -amino acid analog thereof.
11. The D-peptide or a salt thereof of any of embodiments 1 to 10, wherein each of X^1 through X^6 , X^{10} , and X^{11} is a D- α -amino acid.
12. The D-peptide or a salt thereof of any of the embodiments 1 to 11, wherein the core TNF α binding domain has an amino acid sequence of:
 - a. CVWQPKFNYYWC (SEQ ID NO:4);
 - b. CVWQPRFNYYWC (SEQ ID NO:5);
 - c. CTFQPRFNYYWC (SEQ ID NO:6);
 - d. CTFQPRFNNWWC (SEQ ID NO:7);
 - e. CSFQPRFNYYWC (SEQ ID NO:8);
 - f. CSFQPRFNNWWC (SEQ ID NO:9);
 - g. CVFQPRFNYYWC (SEQ ID NO:10);
 - h. CVFQPRFNNWWC (SEQ ID NO:11);
 - i. CTFQWRFNYYWC (SEQ ID NO:12);
 - j. CLYQPVFNWWC (SEQ ID NO:13);

- k. CVFQAAFNNYWC (SEQ ID NO:14);
- l. CVFQHHFNNWWC (SEQ ID NO:15);
- m. CHFNPFRFNNWWC (SEQ ID NO:16);
- n. CVWQPHFNNYWC (SEQ ID NO:17);
- o. CVFQGRFNNWWC (SEQ ID NO:18);
- p. CVFQHRFNNWWC (SEQ ID NO:19);
- q. CVFNPRFNNWWC (SEQ ID NO:20);
- r. CVFKPRFNNWWC (SEQ ID NO:21);
- s. CAYQRQFNNWWC (SEQ ID NO:22);
- t. CWFHRFNNWHC (SEQ ID NO:23);
- u. CHFQHRFNNWWC (SEQ ID NO:24);
- v. CHFQPRFNNWWC (SEQ ID NO:25);
- w. CTYQPRFNNWWC (SEQ ID NO:26);
- x. CQFQPRFNNWQC (SEQ ID NO:27); or
- y. CHFSQRFNNWWC (SEQ ID NO:28).

13. The D-peptide or a salt thereof of any of the preceding embodiments, wherein the core TNF α binding domain has an amino acid sequence of SEQ ID NO:77 to 110.

14. The D-peptide or a salt thereof of any of the preceding embodiments, further comprising a tag sequence attached to the N-terminus of the peptide.

15. The D-peptide or a salt thereof of embodiment 14, wherein the tag comprises the amino acid sequence D-Asp or D-AspAsp (DD).

16. The D-peptide or a salt thereof of any of the preceding embodiments, further comprising a tag sequence attached to the C-terminus of the peptide.

17. The D-peptide or a salt thereof of embodiment 16, wherein the tag comprises the amino acid sequence D-GGEEEK (SEQ ID NO:30) or D-GGRRRK (SEQ ID NO:31).

18. The D-peptide or a salt thereof of any of the preceding embodiments, wherein the N-terminus of the peptide comprises a cap.

19. The D-peptide or a salt thereof of embodiment 18, wherein the cap comprises an acetyl group or a protecting group.

20. The D-peptide or a salt thereof of any of the preceding embodiments, wherein the C-terminus of the peptide comprises a cap.

21. The D-peptide or a salt thereof of embodiment 20, wherein the cap comprises an amide group or a protecting group.
22. The D-peptide or a salt thereof of any of the preceding embodiments, further comprising a polyethylene glycol (PEG) group.
23. The D-peptide or a salt thereof of any of the preceding embodiments, further comprising a Linker.
24. The D-peptide or a salt thereof of embodiment 23, wherein the Linker comprises a PEG group.
25. The D-peptide or a salt thereof of any of embodiments 22 or 24, wherein the PEG group is attached to the N-terminus of the D-peptide.
26. The D-peptide or a salt thereof of any of embodiments 22 or 24, wherein the PEG group is attached to the C-terminus of the D-peptide.
27. The D-peptide or a salt thereof of any of embodiments 22 and 24 to 26, wherein each PEG group is selected from a PEG group having from 1 to 48 subunits, 1 to 30 subunits, 1 to 24 subunits or 1 to 12 subunits.
28. The D-peptide or a salt thereof of embodiment 27, wherein each PEG group is selected from a PEG group having from 6 subunits, 8 subunits, 10 subunits or 12 subunits.
29. A multimer of the D-peptide of any of the preceding embodiments, or a salt thereof.
30. The multimer or a salt thereof of embodiment 29, wherein the multimer is a dimer.
31. The multimer or a salt thereof of embodiment 29, wherein the multimer is a trimer.
32. The multimer or a salt thereof of any of embodiments 29 to 31, further comprising a multimer scaffold attached to the D-peptides, optionally via a Linker.
33. The multimer or a salt thereof of embodiment 32, wherein the multimer scaffold is trimeric.
34. The multimer or a salt thereof of embodiment 32, wherein the multimer scaffold is tetrameric.
35. The multimer or a salt thereof of any of embodiments 33 or 34, wherein the trifunctional cross-linker is tris(succinimidyl) aminotriacetate (TSAT), tris-succinimidyl (6-aminocaproyl)aminotriacetate (LC-TSAT), an Fmoc scaffold (bis(2,5-dioxopyrrolidin-

1-yl) 4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)heptanedioate, an Fmoc scaffold having a PEG27 chain, a cyclohex scaffold (tris(2,5-dioxopyrrolidin-1-yl) cyclohexane-1,3,5-tricarboxylate), a nitro scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)-4-nitroheptanedioate), an amine scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-amino-4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)heptanedioate), an amine-PEG27 scaffold, a cholesterol scaffold, a heterotetrameric PEG scaffold based on 3-{2-amino-3-(2-carboxyethoxy)-2-[(2-carboxyethoxy)methyl]propoxy}propionic acid scaffold, a multimeric scaffold based on 4-amino-4-(2-carboxyethyl)heptanedioic acid, or 3-{2-Amino-3-(2-carboxyethoxy)-2-[(2-carboxyethoxy)methyl]propoxy}propionic acid.

36. The multimer or a pharmaceutically acceptable salt thereof of embodiment 35, wherein the multimer has a structure selected from:

- a. Fmoc-[Peptide-PEG12-K-amide]₃;
- b. Fmoc-[Peptide-PEG4-K-amide]₃;
- c. Fmoc-[Peptide-PEG8-K-amide]₃;
- d. Fmoc-[Ac-K-PEG12-Peptide-amide]₃;
- e. [Peptide-PEG6-K-amide]₃-Fmoc;
- f. [Peptide-PEG12-K-amide]₃-PEG27-Fmoc;
- g. [Peptide-PEG12-K-amide]₃-Fmoc;
- h. [Peptide-PEG12-K-amide]₃-cyclohex;
- i. [Peptide-PEG12-K-amide]₃-nitro;
- j. [Peptide-PEG12-K-amide]₃-PEG27-amine;
- k. [Peptide-PEG12-K-amide]₃-amine;
- l. [Peptide-PEG12-K-amide]₃-PEG27-biotin; and
- m. [Peptide-PEG12-K-amide]₃-PEG27-cholesterol; and wherein

Peptide is a D-peptide.

37. The D-peptide or a salt thereof of any of embodiments 1 to 28 having an amino acid sequence selected from at least one of the following:

- a. DDCVWQPKFNYYWC (SEQ ID N:32);
- b. DGACVWQPKFNYYWC (SEQ ID NO:38);
- c. DDCVWQPRFNYYWC (SEQ ID NO:39);
- d. DDCVWQPRFNYYWCGGRRRK (SEQ ID NO:40);
- e. DCVWQPKFNYYWC (SEQ ID NO:56);

- f. DDDCVWQPRFNYYWC (SEQ ID NO:60);
- g. DDCTFQPRFNNWWC (SEQ ID NO:61);
- h. DDCVFQPRFNYYWC (SEQ ID NO:62);
- i. DDSCFQPRFNYYWC (SEQ ID NO:63); and
- j. DDCVFQPRFNNWWC (SEQ ID NO:64).

38. The multimer or a salt thereof of any of embodiments 29 to 36, comprising the D-peptide having an amino acid sequence selected from at least one of the following:

- a. DDCVWQPKFNYYWC (SEQ ID NO:32);
- b. DGACVWQPKFNYYWC (SEQ ID NO:38);
- c. DDCVWQPRFNYYWC (SEQ ID NO:39);
- d. DDCVWQPRFNYYWCGGRRRK (SEQ ID NO:40);
- e. DCVWQPKFNYYWC (SEQ ID NO:56);
- f. DDDCVWQPRFNYYWC (SEQ ID NO:60);
- g. DDCTFQPRFNNWWC (SEQ ID NO:61);
- h. DDCVFQPRFNYYWC (SEQ ID NO:62);
- i. DDSCFQPRFNYYWC (SEQ ID NO:63); and
- j. DDCVFQPRFNNWWC (SEQ ID NO:64).

39. A pharmaceutical composition comprising at least one D-peptide or multimer, or a pharmaceutically acceptable salt thereof, as set forth in any of the preceding embodiments, and at least one pharmaceutically acceptable excipient or carrier.

40. The pharmaceutical composition of embodiment 39, which is formulated for parenteral administration.

41. The pharmaceutical composition of embodiment 40, which is formulated for intravenous, intramuscular, or subcutaneous administration.

42. The pharmaceutical composition of embodiment 39, which is formulated for oral administration.

43. The pharmaceutical composition of embodiment 39, which is formulated for topical administration.

44. The pharmaceutical composition of embodiment 43, which is formulated for topical administration to the skin (dermal) or to the eye.

45. The pharmaceutical composition of embodiment 39, which is formulated for rectal administration.

46. A lyophilized composition comprising at least one D-peptide or multimer, or a salt or pharmaceutically acceptable salt thereof, of any of embodiments 1 to 38, and a stabilizing agent.

47. A lyophilized composition of the pharmaceutical composition of any of embodiments 39 to 45 and a stabilizing agent.

48. A re-hydrated solution of the lyophilized composition of embodiment 46 or 47.

49. A method of treating a TNF α -mediated disease, comprising administering an effective amount of the D-peptide of any of embodiments 1 to 28 or 37, the multimer of any of embodiments 29 to 36 and 38 or the pharmaceutical composition of any of embodiments 39 to 45, or a pharmaceutically acceptable salt thereof.

50. The method of embodiment 49, wherein the TNF α -mediated disease is adult Crohn's Disease, pediatric Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile idiopathic arthritis (polyarticular), Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or non-radiographic axial spondyloarthritis.

51. The method of embodiment 49, wherein the TNF α -mediated disease is an inflammatory bowel disease.

52. The method of embodiment 51, wherein the inflammatory bowel disease is adult Crohn's Disease, pediatric Crohn's Disease, or Ulcerative Colitis.

53. The method of embodiment 51 or 52, wherein the administration is oral.

54. The method of embodiment 51 or 52, wherein the administration is rectal.

55. The method of embodiment 51 or 52, wherein the administration is parenteral.

56. The method of embodiment 49, wherein the TNF α -mediated disease is an inflammatory skin disease.

57. The method of embodiment 56, wherein the inflammatory skin disease is Plaque Psoriasis, Hidradenitis suppurativa, or Cutaneous Lupus.

58. The method of embodiment 56 or 57, wherein the administration is topical.

59. The method of embodiment 56 or 57, wherein the administration is parenteral.

60. The method of embodiment 49, wherein the TNF α -mediated disease is an inflammatory disease and wherein the inflammatory disease is Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, or Juvenile idiopathic arthritis (polyarticular).

61. The method of embodiment 60, wherein the administration is parenteral.

62. The method of embodiment 65, 59 or 61, wherein the parenteral administration is selected from intravenous, subcutaneous, and intramuscular.

63. A method of reducing TNF α -mediated inflammation, comprising administering a D-peptide or multimer thereof, or a pharmaceutically acceptable salt thereof, of any of embodiments 1 to 38, or a pharmaceutical composition of any of embodiments 39 to 45, to a subject.

64. A method of inhibiting TNF α , comprising administering a D-peptide or multimer thereof, or a pharmaceutically acceptable salt thereof, of any of embodiments 1 to 38, or a pharmaceutical composition of any of embodiments 39 to 45, to a subject.

65. A method of reducing an inflammatory response mediated by TNF α , comprising administering a D-peptide or multimer thereof, or a pharmaceutically acceptable salt thereof, of any of embodiments 1 to 38, or a pharmaceutical composition of any of embodiments 39 to 45, to a subject.

66. The method of any of embodiments 63 to 65, wherein the administering is by oral administration, by parenteral administration, by topical (dermal), or by rectal administration.

67. The method of any of embodiments 63 to 65, wherein the D-peptide or the multimer thereof, or a pharmaceutically acceptable salt thereof is administered locally to reduce TNF α activity or inflammation or an inflammatory response.

68. The method of any of embodiments 63 to 67, wherein the subject has a TNF α - mediated disease.

69. The method of embodiment 68, wherein the TNF α - mediated disease is adult Crohn's Disease, pediatric Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile idiopathic arthritis (polyarticular), Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or non-radiographic axial spondyloarthritis.

70. The D-peptide or the multimer thereof, or the pharmaceutically acceptable salt thereof, according to any of embodiments 1 to 38 for use as a medicament.

71. The D-peptide or the multimer thereof, or the pharmaceutically acceptable salt thereof, according to any of embodiments 1 to 38 for use in a method of treating a subject by therapy.

72. The D-peptide of embodiment 71, wherein the subject has a TNF α -mediated disease.

73. The D-peptide of embodiment 72, wherein the TNF α -mediated disease is adult Crohn's Disease, pediatric Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile idiopathic arthritis (polyarticular), Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or non-radiographic axial spondyloarthritis.

[0260] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. These and other changes can be made to the disclosure in light of the detailed description.

[0261] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[0262] All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the

present disclosure. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to Applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0263] EXAMPLES

[0264] EXAMPLE 1: Screening of Phage Display Libraries for TNF α Antagonist Peptides

[0265] To identify D-peptides that bind to and inhibit human TNF α activity, peptide libraries were screened using an enantiomeric screening technique, mirror-image phage display. A synthetic, biotinylated D-amino acid form of human TNF α (*i.e.*, the mirror image of the natural L-amino acid form) was used to identify L-amino acid peptides (L-peptides) that specifically bound to that D-form of TNF α . The sequences of the L-peptides that bound to the D-amino acid form of human TNF α (the “hits”) were determined by deep sequencing and Sanger sequencing. Based on the sequencing results, D-amino acid peptides of selected L-peptide hits were synthesized and screened for their ability to bind to native human TNF α (L-amino acid form) and to inhibit its activity.

[0266] Synthesis of the L-form of TNF α

[0267] The L-version of biotinylated-human TNF α was synthesized and shown to possess native TNF α protein-like stability and activity. The human TNF α protein had the following amino acid sequence: VRSSSRTPSDKPVAVHVVANPQAEGQLQWLNR RANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVS YQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDY LDFAESGQVYFGIHAL (SEQ ID NO:29); corresponding to Ref Seq NP_000585.2.

[0268] Chemical approaches, based on an established solid-phase peptide synthesis (*e.g.*, Merrifield *et al.*, 1963, *J. Am. Chem. Soc.* 85:2149-2154; Merrifield, 1986, *Science* 232:341-347) and native chemical ligation methods (*e.g.*, Dawson *et al.*, 2000, *Annu. Rev. Biochem.* 69:923-960; Dawson *et al.*, 1994, *Science* 266:776-779), were used to synthesize the L-form of human TNF α . Each synthetic intermediate was purified to homogeneity by reverse-phase HPLC and characterized by HPLC and LCMS analyses. The final linear protein target was then refolded by first forming the single intramolecular disulfide and

then dialyzing step-by-step from 6 M, 2 M, 0.5 M, to 0 M guanidine hydrochloride buffers. The folded, biotinylated L-TNF α trimer was isolated by SEC (size-exclusion chromatography) to remove any aggregated or misfolded species (*i.e.*, separation of trimer from monomer or higher-order aggregates). Synthetic and recombinant L-TNF α proteins were further characterized by biophysical (LC and ESI-MS), biochemical (multiple assays on binding to TNFR), and activity assays (L-929 cell killing assay) to ensure they were equivalent (data not shown).

[0269] Synthesis of D-Form of TNF α

[0270] A biotinylated D-TNF α protein for screening in mirror-image phage display was synthesized using D-amino acids. The folded conformation of the synthetic D-form of the TNF α protein was confirmed by electrospray-mass spectrometry. Folding was confirmed by a difference of -2 Da due to formation of one disulfide bond and a different ionization pattern. The D-protein was purified by size exclusion chromatography and the trimeric form used in the following studies.

[0271] Mirror Image Phage Display Selection Process

[0272] Mirror-image phage display was performed against the folded biotinylated D-TNF α trimeric protein by screening phage libraries of L-peptides having nine different geometries (one linear and eight disulfide-constrained). The L-peptide libraries were as follows: a linear random 12-amino acid L-peptide library (X₁₂), where each X is any L-amino acid other than cysteine; an L-peptide library having the sequence X₂CX₈CX₂, where C is cysteine, each X is any amino acid other than cysteine, and the subscripted numbers refer to the number of L-amino acids in each portion of the peptide; an L-peptide library having the sequence CX₁₀C, where C is cysteine, each X is any L-amino acid other than cysteine, and the subscripted number refers to the number of L-amino acids between the cysteine residues; and six libraries of lariat L-peptides, having the general formula CX_nC, where C is cysteine, each X is any L-amino acid, and n refers to the number of L-amino acids between the cysteine residues (n = 2, 4, 6, 8, 10, 12). The total unique sequence diversity was $\sim 4 \times 10^{10}$.

[0273] Both next generation deep-sequencing and Sanger sequencing of phage that bound to the biotinylated D-TNF α trimeric protein were used to identify “hit” sequences (highest affinity binders). Bioinformatic analyses of the sequences identified >10 different consensus sequences (related families of peptides that have certain residues in common) (data not shown). Multiple hits from each family were tested in a phage ELISA assay to

identify the best binders to biotinylated D-TNF α trimeric protein. Twenty-eight (28) that were identified during the selection process were further validated using an assay that quantified binding of different phage clones to the biotinylated D-TNF α protein. Multiple hits from each family, prioritized based on abundance and the strength of consensus sequence, were synthesized as D-peptides for testing of binding to L-TNF α and blocking of L-TNF α binding to its receptor, as described below. 28 phage that were identified during the selection process were further validated using an assay that quantified binding of different phage clones to the biotinylated D-TNF α protein. Multiple hits from each family, prioritized based on abundance and the strength of consensus sequence, were synthesized as D-peptides for testing of binding to L-TNF α protein and blocking of L-TNF α binding to its receptor, as described below. The peptides identified by sequencing include those having the core TNF α binding domains set forth below in the following Examples as well as in SEQ ID NOs:78 to 111.

[0274] Identification of Initial D-Peptides Binders to TNF α

[0275] The top 28 hits from phage display library screen were synthesized as biotinylated D-peptides (Biotin-(D-AspAsp-(D-peptide)-amide) and tested for binding to recombinant human L-TNF α using a sequential solid phase ELISA binding assay in which the D-peptides were captured onto a Neutravidin-coated plate. Soluble His6-tagged TNF α was detected using an anti-His-HRP secondary antibody. Referring to Figure 1, TNF α -018 emerged as the leading hit based on its binding to recombinant TNF α (L-form) and its high aqueous solubility. Another hit, TNF α -001, was insoluble in aqueous buffers although it showed strong binding to TNF α based on phage display.

[0276] Validation of TNF α -binding by the D-peptide hits was confirmed in three different ELISA formats: “solid-phase” (as described above), “solution-phase” (all components mixed in solution then captured onto Neutravidin surface), and “sequential solid-phase” (mixed biotinylated D-peptide with TNF α in solution, captured on Neutravidin surface, and then a secondary Ab was added). Binding to L-TNF α by the D-peptide hits was also confirmed using a direct fluorescent readout assay (immobilized recombinant biotinylated TNF α was treated with fluorescently-labeled D-peptides). These same fluorescent peptides were also used to document TNF α -binding by co-elution with recombinant TNF α (L-form) on an analytical size exclusion column (SEC) under native conditions. As described below, select D-peptide point mutants abrogated binding in these assays. A thermal shift dye-binding assay (Lavinder *et al.*, 2009, *J. Am. Chem. Soc.*

131:3794-3795), using the SYPRO™ Orange dye, was also employed to confirm binding, which showed that interaction of the D-peptides (TNF-018α, specifically) to TNFα increased its thermal stability (T_m). In addition, SPR (surface plasmon resonance) demonstrated that immobilized TNFα bound to the initial TNFα-018 D-peptide.

[0277] EXAMPLE 2: Binding of Peptide 18 to TNFα *In Vitro*

[0278] Peptide 18 (also designed TNFα-018 and Peptide 018) as well as the variants described below were tested for binding to human TNFα protein (L-form) using a sequential solid phase binding assay. A BSA control was also included in the assay.

[0279] Peptide 18 contained a core TNFα binding domain, CVWQPKFNYYWC (SEQ ID NO:4), with an N-terminal biotin molecule attached to a D-AspAsp (DD) dipeptide tag attached to the N-terminus of the peptide core TNFα binding domain; the dipeptide tag increased the solubility of the peptide. Peptide 18 also contained a C-terminal amide group. Peptide 18-V1 and Peptide 18-V2 contained the same core TNFα binding domain, were acetylated at their N-termini and contained a C-terminal GGEEEEK (SEQ ID NO:30) or GGRRRK (SEQ ID NO:31) peptide, respectively, and a biotin-amide group. These C-terminal sequences were included in Peptide 18-V1 and Peptide 18-V2 to determine whether they increased solubility and/or binding. A Peptide 18 mutant was prepared that had similar structure to Peptide 18, except for a W to G substitution at the penultimate C-terminal residue of the core TNFα binding domain. The sequences of the peptides were as follows, where amino acid sequence differences from Peptide 18 are underlined:

Peptide Biotin DD-18: Biotin-DDCVWQPKFNYYWC-amide (SEQ ID NO:32)

Peptide 18-V1: Ac-CVWQPKFNYYWCGGEEEEK(Biotin)-amide (SEQ ID NO:33)

Peptide 18-V2: Ac-CVWQPKFNYYWGGRRRK(Biotin)-amide (SEQ ID NO:34)

Peptide Biotin DD-18 (mutant): Biotin-DDCVWQPKFNYYGC-amide (SEQ ID NO:35)

[0280] In the assay, the peptides were immobilized on a neutravidin plate and washed with wash buffer (PBS pH 7.4 containing 0.1% BSA and 0.01% Tween-20). His6-TEV-TNFα was then added followed by washing. The TNFα used in the assay was recombinantly expressed human TNFα with an N-terminal His6 tag. The amount of TNFα bound to the peptides was then measured using an anti-His6 tag antibody conjugated to

Horse Radish Peroxidase (HRP) followed by detection using a QuantaBlu™ fluorogenic peroxidase substrate (Thermo Scientific).

[0281] Referring to Figure 2, Peptides 18, 18-V1 and 18-V2 showed significant binding to TNFα by this capture assay. For the Peptide 18 mutant, the Trp (W) to Gly (G) substitution adjacent the C-terminal Cys residue of the core TNFα binding domain abolished binding of that peptide to TNFα. The BSA control also showed negligible TNFα binding. In Figure 2, the number above each bar indicates the fold increase in peptide binding to TNFα, as compared to the BSA control. In this assay, differences in the amount of peptide binding to TNFα were dependent, in part, on the relative position and composition of the peptide tags and the location of the biotin molecule.

[0282] EXAMPLE 3: Peptide 18 Blocked Binding of TNFα to TNFR1

[0283] Peptide Biotin DD-18 (Biotin-DDCVWQPKFNYYWC-amide (SEQ ID NO:32)) was tested in a binding assay that measures disruption of binding between TNFα and one of its receptors, TNFR1 (Tumor Necrosis Factor Receptor 1). In this assay, a soluble, extracellular domain of TNFR1 with C-terminal biotin tag was immobilized onto neutravidin plates. In parallel, recombinant TNFα with an N-terminal His6 tag (as described in Example 2) was pre-incubated with Peptide 18 at concentrations of 10, 25 and 50 micromolar. Following preincubation, the TNFα-Peptide 18 mixtures were then added to the immobilized TNFR1. BSA protein was included as a negative control. A llama anti-TNFα nanobody VHH2 was recombinantly expressed (Beirnaert *et al.*, 2017, *Front. Immunol.* 8:867) was used as a positive control.

[0284] Referring to Figure 3, Peptide 18 showed concentration-dependent blocking of TNFα-TNFR1 binding. BSA protein did not disrupt TNFα-TNFR1 binding, while the positive control, anti-TNFα nanobody VHH2, blocked TNFα-TNFR1 binding. In Figure 3, the percentages above each bar indicate the percentage reduction in TNFα binding to TNFR1, as compared to the negative control. This study showed that Peptide 18 could bind to TNFα and block its binding to TNFR1 binding.

[0285] EXAMPLE 4: Peptide 18 Blocked Cellular Activity of TNFα by L-929 Cells

[0286] The ability of Peptide 18 to block TNFα-mediated cell killing was demonstrated using an L-929 cell killing assay (Trost and Lemasters, 1994, *Anal. Biochem.* 220(1):149-153). TNFα in the presence of actinomycin D causes TNFα-TNFR-mediated

cell death in this cell line. The sequence of Peptide 18 was: Ac-DDCVWQPKFNYYWC-amide (SEQ ID NO:32; Peptide DD-18).

[0287] Cell death measured by absorbance at 450 nm. Media alone (no TNF α) was used as a negative control. TNF α plus BSA (no Peptide 18) was used as a positive control.

[0288] Referring to Figure 4A, TNF α induced L-929 cell killing (compare first and second columns on left). The addition of Peptide 18 at 50 micromolar blocked TNF α -induced cell death (compare columns 2 and 3 from left). Reduction of TNF α induced cell killing was comparable to that shown by soluble TNFR1 (2.5 microgram/mL) while the anti-TNF α VHH2 nanobody (30 microgram/mL) and anti-TNF α monoclonal antibody (research grade adalimumab; R&D Systems) exhibited greater inhibition of TNF α -induced L-929 cell killing.

[0289] The concentration of Peptide 18 was also varied in this assay from 50 micromolar to 0.78125 micromolar and cell killing was measured as described above (by absorbance at 450 nm). Referring to Figure 4B, the left bar in each triplet shows the result of Peptide 18 (designated B-D-P 18; Peptide Biotin DD-18; SEQ ID NO:32) alone at the indicated concentration, the middle bar shows the results of addition of recombinant human TNF α at a concentration of 0.5 nM with the indicated amount of Peptide 18 (designated B-D-P 18; Peptide Biotin DD-18; SEQ ID NO:32), and the right bar shows the results of addition of TNF α at a concentration of 0.1 nM with the indicated amount of Peptide 18. (Note: the left most bar is absent in the triplets 8-9 (rTNF α only, and VHH2) because Peptide 18 was not present.)

[0290] Generally, as the amount of Peptide 18 decreased, the amount of TNF α -induced cell killing increased. The control TNF α only condition (rTNF α only) caused cell killing, as expected. TNF α plus anti-TNF α VHH2 nanobody (VHH2) blocked TNF α -induced cell killing. When Peptide DD-18 was replaced with the Peptide DD-18 mutant (Biotin-DDCVWQPKFNYYGC-amide (SEQ ID NO: 35)), TNF α -mediated cell death was not inhibited (18(M) 50 micromolar). The media only control (Media) yielded the anticipated results.

[0291] In this study, the EC₅₀ value for Peptide 18 at the 0.5 nM TNF α and the 0.1 nM TNF α conditions were calculated to be 9.6 micromolar and 6.1 micromolar, respectively.

[0292] EXAMPLE 5: Comparison of the Blocking Activity of Peptide 18 and Other Leads in the L-929 Cell Killing Assay

[0293] Additional peptide variants of Peptide 18 were screened for inhibition of TNF α -induced cell death in the L-929 cell killing assay described in Example 4. The screened peptides were Peptide 18 (Biotin-DDCVWQPKFN NYWC -amide; Peptide Biotin DD-18; SEQ ID NO:32), a Peptide 18 mutant (Biotin-DDCVWQPKFN NYGC -amide; SEQ ID NO: 35; Peptide Biotin DD-18 mutant), and a second mutant, Peptide 18 di-mutant (Biotin-DDCDWQDKFN NYWC -amide; SEQ ID NO:36; Peptide Biotin DD-18 di-mutant). The differences between Peptide 18 and Peptide 18 mutant and Peptide 18 di-mutant are underlined in the Peptide 18 mutant and Peptide 18 di-mutant sequences.

[0294] Figure 5, shows the results. In each pair of bars, the left bar shows the result with peptide alone. The right bar shows the result with peptide plus 0.5 mM recombinant human TNF α . In comparison to Peptide 18, neither Peptide 18 mutant nor the di-mutant showed any significant inhibition of TNF α -induced cell killing.

[0295] A trimerized version of Peptide 18, Peptide 18-IZ (CVWQPKFN NYWC -PEG12-IKKEIEAIKKEQEAIKKIEAIEKEA-hydrazide; comprising peptide 18 (SEQ ID NO:4) plus PEG12 plus IZ coiled coil (SEQ ID NO:46)), was tested in this assay. This peptide induces a naturally forming trimeric IZ-coiled-coil sequence that forms a trimer of Peptide 18 in solution. Referring again to Figure 5, trimeric Peptide 18-IZ exhibited less inhibition than Peptide 18 as a monomer in this assay.

[0296] EXAMPLE 6: Modification of Peptide 18 with Peptide Tags

[0297] In Peptide 18 (Biotin-DDCVWQPKFN NYWC -amide; SEQ ID NO:32; Peptide Biotin DD-18), the N-terminal DD dipeptide tag was added to the core TNF α binding domain (CVWQPKFN NYWC ; SEQ ID NO: 4) to increase solubility of the peptide. In Example 2, two other variants of Peptide 18 having C-terminal tags (GGEEEEK (SEQ ID NO:30) and GGRRRK (SEQ ID NO:31)), Peptide 18-V1 (SEQ ID NO:33) and Peptide 18-V2 (SEQ ID NO:34), respectively, were tested and shown to retain TNF α binding activity. To determine whether the tags affected the binding activity of the core TNF α binding domain to TNF α , Peptide 18-V1 (designated Peptide 18-E3 (Ac-CVWQPKFN NYWCGGEEEEK (Biotin)-amide; SEQ ID NO: 33)) and Peptide 18-V2 (designated Peptide 18-R3 (Ac-CVWQPKFN NYWCGGRRRK (Biotin)-amide; SEQ ID NO:34) were tested in the L-929 cell killing assay described in Example 4. (The differences between Peptide 18 and the variants are underlined in the variant sequences.) In addition, an extended peptide based on the sequence of the peptide isolated from the phage library

(Example 1), Peptide Biotin DD-18-ex (Biotin-DDCVWQPKFN~~NY~~WCGGGSAETVE-amide; SEQ ID NO:37) was tested.

[0298] Referring again to Figure 5, Peptide 18, having an N-terminal DD dipeptide, exhibited greater inhibition of TNF α -induced cell death than Peptide 18-E3 and Peptide 18-R3. Peptide 18-ex exhibited inhibition of TNF α -induced cell killing that was about the same as or slightly greater than that of Peptide 18.

[0299] EXAMPLE 7: Comparing the Binding of Additional Peptide 18 Sequence Variants in TNF α Binding Assays

[0300] The TNF α binding assay described above (Example 2) was used to compare the binding of Peptide 18 with additional sequence variants. In addition, another peptide from the same CX₁₀C library, Hit 16, was tested. The following peptides were tested, where the sequence differences from Peptide 18 are underlined.

Peptide 18: Biotin-DD-18 - Biotin-DDCVWQPKFN~~NY~~WC-amide (SEQ ID NO:32)

Biotin-DGA-18: Biotin-DGACVWQPKFN~~NY~~WC-amide (SEQ ID NO:38)

Biotin-DD-18-KtoR: Biotin-DDCVWQPRFN~~NY~~WC-amide (SEQ ID NO:39)

Biotin-DD-18-KtoR-R3: Biotin-DDCVWQPRFN~~NY~~WCGGRRRK-amide (SEQ ID NO:40)

Biotin2-DD-18-KtoR-R3: Biotin-DDCVWQPRFN~~NY~~WCGGRRRK(biotin)-amide (SEQ ID NO:40)

Biotin-DD-hit16: Biotin-DDCHFNPRFNNWWC-amide (SEQ ID NO:41)

[0301] The results of these binding assays are shown in Figure 6. Initially, the binding of two different preparations of Peptide 18 (Biotin-DD-18; SEQ ID NO:32) were compared (first and second columns on the left). Peptides from both preparations showed comparable binding activity.

[0302] Comparing Peptide 18 (Biotin-DD-18; SEQ ID NO:32) with the DGA N-terminal variant (Biotin-DGA-18; SEQ ID NO:38), Peptide 18 exhibited better binding (first three columns on the left), suggesting that a DD peptide tag is preferred to a DGA peptide tag.

[0303] Comparing Peptide 18 (Biotin-DD-18; SEQ ID NO:32) with the Peptide 18 variant having a Lys to Arg substitution (KtoR) in the core TNF α binding domain (Biotin-DD-18-KtoR; SEQ ID NO:39), the Lys to Arg substitution enhanced binding (compare first two columns on the left with the fourth column).

[0304] Comparing the binding of Peptide Biotin-DD-18-KtoR (having a DD N-terminal tag; SEQ ID NO:39) with Peptide Biotin-DD-18-KtoR-R3 (SEQ ID NO:40), the addition of a C-terminal GRRRRK (SEQ ID NO:31) appeared to further increase binding. Addition of a second biotin molecule to Peptide Biotin-DD-18-KtoR-R3 (SEQ ID NO:40) to make Peptide Biotin2-DD-18-KtoR-R3 (SEQ ID NO:41) did not appear to significantly affect binding. Binding of Hit 16 (SEQ ID NO:42) was similar to that of Peptide 18.

[0305] EXAMPLE 8: Comparing Additional Peptide 18 Sequence Variants in TNF α -TNFR Blocking Assays

[0306] The TNF α /TNFR blocking assay described above (Example 3) was used to compare the blocking ability of Peptide 18 with additional sequence variants of Peptide 18. Hit 16 from the same library, was also tested. The peptides tested are shown below. The sequence differences from Peptide 18 are underlined. In addition, some peptides that were not biotinylated were tested in this study.

DD-18: DDCVWQPKFN~~NY~~WC-amide (SEQ ID NO:32)

Biotin-DD-18: Biotin-DDCVWQPKFN~~NY~~WC-amide (SEQ ID NO:32)

Biotin-DD-18-ex: Biotin-DDCVWQPKFN~~NY~~WCGGGSAETVE-amide (SEQ ID NO:37)

Biotin-DGA-18: Biotin-DGACVWQPKFN~~NY~~WC-amide (SEQ ID NO:38)

DGA-18: DGACVWQPKFN~~NY~~WC-amide (SEQ ID NO:38)

DD-18-KtoR: DDCVWQPR~~F~~N~~NY~~WC-amide (SEQ ID NO:39)

Biotin-DD-18-KtoR: Biotin-DDCVWQPR~~F~~N~~NY~~WC-amide (SEQ ID NO:39)

Biotin-DD-18-KtoR-R3: Biotin-DDCVWQPR~~F~~N~~NY~~WCGGRRRRK-amide (SEQ ID NO:40)

Biotin2-DD-18-KtoR-R3: Biotin-DDCVWQPR~~F~~N~~NY~~WCGGRRRRK(biotin)-amide (SEQ ID NO:40)

Biotin-DD-Hit16: Biotin-DDCHFNPRFNNWWC-amide (SEQ ID NO:41)

DD-Hit16: DDCHFNPRFNNWWC-amide (SEQ ID NO:41)

[0307] Referring to Figure 7, the percentages above each bar indicate the percentage of peptide-dependent blocking of TNF α -TNFR1 binding. Initially, the two different preparations of Peptide 18 (Biotin-DD-18) were tested (Example 7) and both exhibited similar blocking activity (compare second and third bars from the left).

[0308] In this blocking assay, both unbiotinylated Peptide 18 (DD-18) and biotinylated Peptide 18 (Biotin-DD-18) exhibited better blocking than Peptide Biotin-DD-18-ex (compare four columns on the left). Changing the N-terminal tag of Peptide 18 from DD to DGA did not significantly affect blocking of TNF α -TNFR1 binding (compare second to sixth columns from the left). The presence or absence of biotin on the N-terminal DGA variants did not appear to significantly affect blocking by that peptide.

[0309] When the sequence of the core TNF α binding domain was modified by a Lys to Arg substitution (DD-18-KtoR and Biotin-DD-18-KtoR; SEQ ID NO:39), the blocking ability of these peptides increased as compared to Peptide Biotin-DD-18 (compare columns 2 and 3 with columns 11 and 12 from left).

[0310] When a C-terminal tag (GGRRRK (SEQ ID NO:31)) was added to the core modified peptides, without or without an additional biotin molecule (Biotin-DD-18-KtoR-R3; SEQ ID NO:40 and Biotin2-DD-18-KtoR-R3; SEQ ID NO:40), the blocking activity of the peptides decreased (compare columns 11-14 from left).

[0311] Hit 16 with a DD N-terminal addition (DDCHFNPRFNNWWC-amide (SEQ ID NO:41), with or without an additional biotin molecule (DD-Hit16 and Biotin-DD-Hit16), exhibited comparable blocking compared to Peptide DD-18; SEQ ID NO:32) (compare columns 4 and 15-16 from the left).

[0312] In summary, these results suggest that biotin does not contribute to the blocking ability of the peptides and that the Lys to Arg substitution in the core TNF α binding domain increases binding and blocking by the peptides.

[0313] EXAMPLE 9: Comparing Additional Peptide 18 Sequence Variants in L-929 Cell Killing Assay

[0314] The L-929 cell killing assay described above (Example 4) was used to compare the ability of Peptide 18 (designated DD-18; SEQ ID NO:32) and additional sequence variants to block TNF α -induced cell killing. Biotinylated and non-biotinylated forms of Hit 16 were also included. The peptides tested are shown below. The amino acid sequence differences from Peptide 18 are underlined. Some peptides were not biotinylated in this study.

DD-18: DDCVWQPKFN~~NY~~WC-amide (SEQ ID NO:32)

Biotin-DD-18: Biotin-DDCVWQPKFN~~NY~~WC-amide (SEQ ID NO:32)

Biotin-DD-18-ex: Biotin-DDCVWQPKFN~~NY~~WCGGGSAETVE-amide (SEQ ID NO:37)

Biotin-DGA-18: Biotin-DGACVWQPKFN^YWC-amide (SEQ ID NO:38)

DGA-18: DGACVWQPKFN^YWC-amide (SEQ ID NO:38)

DD-18-KtoR: DDCVWQPRFN^YWC-amide (SEQ ID NO:39)

Biotin-DD-18-KtoR: Biotin-DDCVWQPRFN^YWC-amide (SEQ ID NO:39)

Biotin-DD-18-KtoR-R3: Biotin-DDCVWQPRFN^YWCGGRRRK-amide (SEQ ID NO:40)

Biotin2-DD-18-KtoR-R3: Biotin-DDCVWQPRFN^YWCGGRRRK(biotin)-amide (SEQ ID NO:40)

DD-hit16: DDCHFNPRFN^NWC-amide (SEQ ID NO:41)

Biotin-DD-hit16: Biotin-DDCHFNPRFN^NWC-amide (SEQ ID NO:41)

[0315] Referring to Figure 8, initially, the two different preparations of Peptide 18 (Biotin-DD-18; SEQ ID NO:32) were tested, and both preparations of peptide exhibited comparable although somewhat different blocking activity (compare second and third bars from the left). Peptide 18 without a biotin tag (DD-18) blocked cell killing more than Peptide 18 with biotin (Biotin-DD-18) (compare second, third and fourth columns from the left). Similarly, Peptide 18 without biotin (DD-18) blocked more cell killing than Peptide Biotin-DD-18-ex (SEQ ID NO:37) (compare first and fourth columns from the left).

[0316] Changing the N-terminal tag of Peptide 18 from DD to DGA, without or without biotin, (Biotin-DGA-18 and DGA-18; SEQ ID NO:38), reduced the ability of the peptides to block cell killing in this assay (compare column 4 with columns 5 and 6). This result is consistent with the results of these peptides in TNF α binding and TNF α /TNFR assays in Examples 7 and 8 (above).

[0317] The Lys to Arg substitution in the core TNF α binding domain of Peptide 18, with or without biotin (Biotin-DD-18-KtoR and DD-18-KtoR; SEQ ID NO:39), increased the ability of the peptides to block cell death (compare column 4 with columns 7 and 8). This result is consistent with the results of these peptides in the TNF α binding and TNF α /TNFR assays in Examples 7 and 8 (above).

[0318] When a C-terminal tag (GGRRRK (SEQ ID NO:31)) was added to the core modified peptides, without or without an additional biotin molecule (Biotin-DD-18-KtoR-R3; SEQ ID NO:40 and Biotin2-DD-18-KtoR-R3; SEQ ID NO:41), the ability of the peptides to block cell killing decreased (compare columns 7 to 10). This result is consistent with the results of these peptides in the TNF α binding and TNF α /TNFR assays in Examples 7 and 8 (above).

[0319] Hit 16 with a DD N-terminal addition (DDCHFNPFRNNWWC-amide (SEQ ID NO:41), with or without an additional biotin molecule (DD-Hit16 and Biotin-DD-Hit 16), exhibited comparable blocking compared to Peptide DD-18 and Peptide DD-18 (SEQ ID NO:32) (compare columns 2, 3, and 4 with columns 11 and 12 from left).

[0320] In summary, the results of the studies in Examples 7 to 9 indicate modification of the core TNF α binding domain by a Lys to Arg substitution increased the activity of the peptides. The addition of N and C terminal peptide tags modulated the activity of the peptides. Biotinylation had little effect on the activity of the peptides.

[0321] EXAMPLE 10: Determination of an EC₅₀ Value for Peptide 18 and Variants

[0322] The three most active peptides from the above assays were tested in the L-929 cell killing assay (described above in Example 4) at peptide concentrations ranging from 50 micromolar to 0.78125 micromolar (in serial two-fold dilutions) and the EC₅₀ value for each peptide was determined (data not shown). The following peptides were tested:

DD-18: DDCVWQPKFN $\underline{N}$ NYWC-amide (SEQ ID NO:32)

DD-18-KtoR: DDCVWQPRFN $\underline{N}$ NYWC-amide (SEQ ID NO:39)

Biotin-DD-18-KtoR: Biotin-DDCVWQPRFN $\underline{N}$ NYWC-amide (SEQ ID NO:39)

[0323] The EC₅₀ value for each peptide was as follows:

DD-18: 16.1 micromolar

DD-18 KtoR: 6.16 micromolar

Biotin-DD-18 KtoR: 8.7 micromolar

[0324] EXAMPLE 11 Results from Sequence Analysis From Peptide Phage Library Lariat CX₁₀C

[0325] Peptide 18 was isolated from a phage library lariat CX₁₀C (CX₂X₃X₄X₅X₆X₇X₈X₉X₁₀X₁₁C). Deep sequence analysis of the peptide hits from that library revealed that the most frequent amino acids at each position of the peptides (from left to right between the cysteine residues), were as follows:

X₂ T, V, H, L, Q, A, I, M, W,

X₃ F, W, Y, S, H, L

X₄ Q, R, N, S, T

X₅ P, W, H, Q, R, A, V, L

X₆ R, H, K, E, Q, V, L, S, A
 X₇ F
 X₈ N
 X₉ N
 X₁₀ W, Y
 X₁₁ W, Y, Q, H

[0326] The peptides from this library consistently had an FNN(W/Y) amino acid sequence at peptide positions X₇ to X₁₀.

[0327] EXAMPLE 12: Comparison of Peptide 18 and Variant Binding in the TNF α Binding Assay

[0328] Peptide 18 and the variants described below with altered spacing between the biotin molecule and the AspAsp (DD) N-terminal tag were tested for TNF α binding using the (TNF α binding assay described in Example 2. N-terminal spacing variants with PEG4 (4 PEG subunits) were prepared by insertion of a PEG4 at the N-terminus between biotin and the N-terminus of the DD-core TNF α binding domain of Peptide 18. The following peptides were tested:

Biotin-DD-18-KtoR: Biotin-DDCVWQPRFNNYWC-amide (SEQ ID NO:39)

Biotin-DD-18: Biotin-DDCVWQPKFNNYWC-amide (SEQ ID NO:32)

Biotin-PEG4-DD-18: Biotin-PEG4-DDCVWQPKFNNYWC-amide (SEQ ID NO:54)

[0329] Referring to Figure 9, addition of a PEG4 spacer at the N-terminus of Peptide 18 did not significantly affect the binding of the peptides to TNF α (compare columns 1 to 3 from the left). In addition, TNF α binding of two different preparations of Peptide Biotin-DD-18-KtoR was tested. Little difference in TNF α binding was detected (compare first column on left with second column on right).

[0330] Peptide 18 cyclizes by formation of a disulfide bond between the terminal cysteine residues of the core TNF α binding domain. To determine whether a single amino acid deletion or insertion between the cysteine residues might alter the activity of the peptide, a valine deletion (Biotin-DD-18-noVal) and an alanine insertion (Biotin-DD-18-plusAla) were prepared. The sequences are as follows:

Biotin-DD-18-noVal: Biotin-DDCWQPKFNNYWC-amide (SEQ ID NO:42)

Biotin-DD-18-plusAla: Biotin-DDCAVWQPKFNNYWC-amide (SEQ ID NO:43)

[0331] Referring again to Figure 9, deletion or insertion of an amino acid residue adjacent the conserved N-terminal cysteine residue significantly reduced binding of the peptides to TNF α (compare column 2 with columns 4 and 5). Peptides Biotin-DD-18-noVal SEQ ID NO:43) and Biotin-DD-18-plusAla (SEQ ID NO:44) were also tested in the L-929 cell killing assay and exhibited little ability to inhibit TNF α -induced cell killing (data not shown). This result suggested that the spacing of certain residues in the core TNF α binding domain as well as the conformation of the peptide may be important for binding to TNF α .

[0332] EXAMPLE 13: Comparison of PEG-modified Peptide 18 and Trimeric Versions of Peptide 18 in the L-929 Cell Killing Assay.

[0333] TNF α can associate as a trimer *in vivo*. To determine whether multimerization affected the activity of the peptides, Peptide 18 and its variants were tested in the L-929 cell killing assay described in Example 4. Initially, monomeric Peptide 18 having the Lys to Arg substitution in the core TNF α binding domain (Peptide DD-18KtoR; SEQ ID NO:39) was tested, with and without an N-terminal Lys-PEG12 (12 PEG subunits) or C-terminal peg12-Lys addition. The monomeric peptides had the following structures:

DD-18KtoR: DDCVWQPRFNYYWC-amide (SEQ ID NO:39)

DD-18-KtoR-PEG12-K: Ac-DDCVWQPRFNYYWC-PEG12-K-amide (SEQ ID NO:44)

K-PEG12-DD-18-KtoR: Ac-K-PEG12-DDCVWQPRFNYYWC-amide (SEQ ID NO:45)

[0334] Referring to Figure 10, addition of a Lys-PEG12 at the N-terminus or PEG12-Lys at the C-terminus did not significantly alter blocking of TNF α -induced cell killing (compare column 1 and columns 8 and 9).

[0335] The activity of Peptide 18 (Biotin-DD-18) and an N-terminal PEG4 version thereof were also tested. The peptides had the following structures:

Biotin-DD-18: Biotin-DDCVWQPKFNYYWC-amide (SEQ ID NO:32)

Biotin-PEG4-DD-18: Biotin-PEG4-DDCVWQPKFNYYWC-amide (SEQ ID NO:54)

[0336] Referring again to Figure 10, addition of a PEG4 to the N-terminus of monomeric Peptide 18 did not significantly alter blocking of TNF α -induced cell death (compare columns 2 and 3 from the left).

[0337] Peptide 18 (DD-18) was trimerized by the method in which it was assembled on the solid phase resin to form Peptide DD-18-tricasso. Semi-orthogonal protecting groups (Dde and Fmoc) were used to assemble the trimeric peptide DD-18 trimer (tricasso) directly (see WO2017/040350 for a general description of the tricasso trimerization method). In addition, Peptide 18 with the Lys to Arg substitution (DD-18-KtoR; SEQ ID NO:39) was trimerized using a naturally forming trimeric IZ coiled-coil sequence (IKKEIEAIKKEQEAIKKKIEAIEKEA (SEQ ID NO:46) and a PEG12 spacer to form Peptide DD-18-KtoR-IZ. The IZ forms a non-covalent trimer. The structures of the resulting peptides were as follows:

DD-18-tricasso: [DDCVWQPKFNYYWC-PEG12]3-KK-amide

DD-18-KtoR-IZ: DDCVWQPRFNYYWC-PEG12-IKKEIEAIKKEQEAIKKKIEAIEKEA-hydrazide (SEQ ID NO:39 linked through PEG12 to SEQ ID NO:47)

[0338] Referring again to Figure 10, trimerization of these peptides did not significantly reduce the ability of the peptides to block TNF α -induced cell death (compare columns 2 and 6 and columns 1 and 7).

[0339] The valine deletion of Peptide 18 (Biotin-DD-18-noVal) and an alanine insertion (Biotin-DD-18-plusAla) were also tested in this assay. The sequences are as follows:

Biotin-DD-18-noVal: Biotin-DDCWQPKFNYYWC-amide (SEQ ID NO:42)

Biotin-DD-18-plusAla: Biotin-DDCAVWQPKFNYYWC-amide (SEQ ID NO:43)

[0340] Referring again to Figure 10, deletion or insertion of an amino acid residue adjacent the conserved N-terminal cysteine residue significantly reduced binding of the peptides to TNF α (compare column 2 with columns 4 and 5 from left).

[0341] The EC₅₀ value of each trimeric Peptides DD-18-tricasso and DD-18-KtoR-IZ in the L-929 cell killing assay were determined by varying the concentrations of peptides from 50 micromolar to 0.78125 micromolar (in serial two-fold dilutions). The EC₅₀ value of trimeric Peptide DD-18-KtoR-IZ in PBS and water was determined to be 2.4 micromolar and 2.1 micromolar, respectively. The EC₅₀ value of Peptide DD-18-tricasso was determined to be 3.4 micromolar.

[0342] EXAMPLE 14: Preparation and Testing of Covalently-Linked Dimeric and Trimeric Peptides

[0343] Covalently linked dimers and trimers of Peptide DD-18-KtoR were prepared and tested for activity in the L-929 cell killing assay. Covalently linked dimers and trimers of DD-18-KtoR were prepared using amine-NHS chemistry. The DD-18-KtoR monomers were prepared with terminal amine groups by installing a lysine residue and a PEG group at the N-terminus or C-terminus of the peptide. Then, dimers and trimers were prepared by NHS-amine chemistry using linkers with multiple NHS groups. The dimers and trimers were purified using standard HPLC purification methods.

[0344] In this study, two different monomers of DD-18-KtoR were prepared: either a N-terminal or C-terminal Lysine was added, separated from the DD-18-KtoR peptide by a PEG12 spacer (SEQ ID NOs: 45 and 46). To prepare dimers, the DD-18-KtoR peptides, with PEG12-Lys, were reacted with an NHS-PEG6-NHS linker. To prepare trimers, the DD-18-KtoR peptides, with PEG12-Lys, were reacted with an Fmoc-amino-tri-NHS linker; in this case, the Fmoc was retained on the amine of the trimeric linker.

[0345] The peptides tested in the L-929 cell killing assay were the following:

DD-18-KtoR: DDCVWQPRFNYYWC-amide SEQ ID NO:39)

DD-18-KtoR-N-dimer: (Ac-K-PEG12-DDCVWQPRFNYYWC-amide)2-PEG6

DD-18-KtoR-C-dimer: (Ac-DDCVWQPRFNYYWC-PEG12-K-amide)2-PEG6

DD-18-KtoR-N-trimer: Fmoc-[Ac-K-PEG12-DDCVWQPRFNYYWC-amide]3

DD-18-KtoR-C-trimer: Fmoc-[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]3

[0346] Referring to Figure 11, both the covalently linked dimers and trimers of Peptide DD-18-KtoR exhibited significantly more inhibition of TNF α -mediated cell killing than monomeric Peptide DD-18-KtoR. The dimers and trimers were also more active than the anti-TNF α VHH2 nanobody control.

[0347] Example 15: Determination of EC₅₀ Values for Dimers and Trimers of Peptide DD-18-KtoR

[0348] To determine the EC₅₀ values of the DD-18-KtoR dimers and trimers prepared in Example 14, the concentrations of the following dimers and trimers of Peptide DD-18-KtoR (SEQ ID NOs: 45 and 46) are varied from 10 micromolar to 0.0015 micromolar (in eight two-fold dilutions) in the L-929 cell killing assay. The peptides tested were the following:

DD-18-KtoR-N-dimer: (Ac-K-PEG12-DDCVWQPRFNYYWC-amide)2-PEG6

DD-18-KtoR-C-dimer: (Ac-DDCVWQPRFNYYWC-PEG12-K-amide)2-PEG6

DD-18-KtoR-N-trimer: Fmoc-[Ac-K-PEG12-DDCVWQPRFNYYWC-amide]3

DD-18-KtoR-C-trimer: Fmoc-[Ac-DDCVWQPRFN^YWC-PEG12-K-amide]₃

[0349] The calculated EC₅₀ values were as follows:

DD-18-KtoR-N-dimer: 10 nM

DD-18-KtoR-C-dimer: 2.6 nM

DD-18-KtoR-N-trimer: 2 nM

DD-18-KtoR-C-trimer: << 1.5nM

[0350] For both the N-linked and C-linked peptides, the trimers were more potent than the dimers. The C-linked peptides, both dimers and trimers, were more potent than the N-linked peptides.

[0351] Example 16: Determination of the Species Selectivity of the DD-18-KtoR Trimer

[0352] The C-terminal linked DD-18-KtoR trimer from Example 14 was tested for binding activity against TNF α proteins from multiple species in the L-929 cell killing assay: Recombinantly expressed soluble TNF α from human, monkey, dog and rat were tested at concentrations ranging from 10 micromolar to 41 nM (in three-fold dilutions). TNF α from these species can bind to human TNFR, so the L-929 cell killing assay could be used to evaluate the blocking activity of Peptide DD-18-KtoR by the different TNF α proteins. The trimer used was DD-18-KtoR-C-trimer: Fmoc-[Ac-DDCVWQPRFN^YWC-PEG12-K-amide]₃.

[0353] The C-terminal linked trimer was active against human, monkey, and dog TNF α in the cell killing assay at all concentrations tested (data not shown). The trimer was not active against rat TNF α in this assay at any of the concentrations tested (data not shown).

[0354] Example 17: Identification of Residues in Monomeric Peptide DD-18 Involved in Interaction with TNF α

[0355] To identify residues of Peptide DD-18 that are involved in its interaction with TNF α , additional mutants of Peptide DD-18 (Peptide 18) were prepared. The peptides were tested in the TNF α binding assay described in Example 2. The peptides tested were the following, where the differences from peptide Bio-DD-18 are underlined:

Biotin-DD-018: biotin-DDCVWQPKFN^YWC-amide (SEQ ID NO:32)

Biotin-DD-018(K2R): biotin-DDCVWQPRFN^YWC-amide (SEQ ID NO:39)

Bio-018-WtoG: biotin-DDCVGQPKFN^YWC-amide (SEQ ID NO:47)

Bio-018-NtoG: biotin-DDCVWQPKFNGYWC-amide (SEQ ID NO:48)

[0356] In addition, the N-terminal DD tag was changed to DDD in the following peptide:

Bio-DDD-018: biotin-DDDCVWQPKFN~~NY~~WC-amide (SEQ ID NO:49)

[0357] Referring to Figure 12, peptides have substitutions of Trp and Asn to Gly (at positions 3 and 9 of the core TNF α binding domain, respectively) significantly reduced binding activity (compare column 11 with columns 1 and 2). Peptide Biotin-DD-18K2R (having the Lys to Arg substitution at position 6 of the core TNF α binding domain; SEQ ID NO:39) exhibited more binding activity than Peptide Bio-DD-18 (SEQ ID NO:32), as shown previously (compare columns 10 and 11). Addition of one more D-Asp residue at the N-terminal end of the Peptide Biotin-DD-018 (Peptide Bio-DDD-018; SEQ ID NO:50) reduced its binding activity to some extent (compare columns 3 and 11 from the left).

[0358] In addition, other hits from the CX₁₀C peptide libraries were tested in the TNF α binding assay. The peptides tested were the following, where differences from Peptide Bio-DD-18 are underlined:

Bio-DD-Hit19: biotin-DDCQFQPRFNNWQC-amide (SEQ ID NO:50)

Bio-DD-Hit33: biotin-DDCHFSQRFNNWWC-amide (SEQ ID NO:51)

Bio-DD-Hit92: biotin-DDCAYQRQFNNWWC-amide (SEQ ID NO:52)

Bio-DD-Hit193: biotin-DDCWFEHRFNNWHC-amide (SEQ ID NO:53)

[0359] Referring again to Figure 12, Peptides Bio-DD-Hit19, Bio-DD-Hit92 and Bio-DD-Hit193 exhibited some, but reduced TNF α binding activity compared to Peptide DD-018, while Peptide Bio-DD-Hit33 exhibited significant binding activity (compare bars 6, 7, and 9 with bar 11 from left).

[0360] To assess the contribution of the proximity of the N-terminal DD tag to the core TNF α binding domain, a PEG4 molecule was inserted between the DD tag and the N-terminus of the core TNF α binding domain. The activity of this peptide was compared to a peptide having the PEG4 molecule added to the N-terminus of the DD tag. The peptides tested were as follows:

Bio-DD-PEG4-018: biotin-DD-PEG4-CVWQPKFN~~NY~~WC-amide (SEQ ID NO:55)

Bio-PEG4-DD-018: biotin-PEG4-DDCVWQPKFN~~NY~~WC-amide (SEQ ID NO:54)

[0361] Referring again to Figure 12, separation of the DD tag from the N-terminus of the core TNF α peptide binding domain reduced TNF α binding activity to some extent (compare bars 4, 5 and 11 from the left).

[0362] Example 18: Modification of the N-terminal DD Tag of Monomeric Peptide Bio-DD-18

[0363] To further assess the effects of changes in sequence and proximity of the N-terminal DD tag to the core TNF α binding domain, the following peptides were tested in the L-929 cell killing assay and the EC₅₀ values were determined:

Biotin-PEG4-DD-018: biotin-PEG4-DDCVWQPKFNYYWC-amide (SEQ ID NO:54)

Biotin-DD-018(K2R): biotin-DDCVWQPRFNYYWC-amide (SEQ ID NO:39)

Bio-DDD-018: biotin-DDDCVWQPKFNYYWC-amide (SEQ ID NO:49)

Bio-DD-018: biotin-DDCVWQPKFNYYWC-amide (SEQ ID NO:32)

Bio-D-018: biotin-DCVWQPKFNYYWC-amide (SEQ ID NO:56)

Bio-DD-PEG4-018: biotin-DD-PEG4-CVWQPKFNYYWC-amide (SEQ ID NO:55)

[0364] The L-929 cell killing assay was performed as described in Example 4.

[0365] The EC₅₀ values were as follows:

Bio-DD-018: 16.6 micromolar

Bio-DDD-018: 4.1 micromolar

Bio-D-018: 38 micromolar

Biotin-DD-018(K2R): 2.1 micromolar

Biotin-PEG4-DD-018: 15 micromolar

Bio-DD-PEG4-018: > 50 micromolar

[0366] Referring to these results, compared to Peptide Bio-DD-018 (SEQ ID NO:32), addition of an additional Asp residue in the N-terminal tag (SEQ ID NO:50) results in a more effective (lower) EC₅₀ value while removing an Asp residue (SEQ ID NO:57) results in a less effective (higher) EC₅₀ value. Increasing the spacing between the DD tag and the core TNF α binding domain results in a less effective (higher) EC₅₀ value. Finally, Peptide Biotin-DD-018(K2R; SEQ ID NO:39) had a lower EC₅₀ value than Peptide Bio-DD-018, as shown previously.

[0367] Example 19: Thermal Stability of Complexes of TNF α and Monomeric Peptide Biotin-DD-18 and Selected Variants

[0368] The thermal stabilities of complexes of TNF α and Peptide Biotin-DD-18 and the following mutants were determined using the high-throughput thermal scanning method described by Lavinder *et al.*, *J. Am. Chem. Soc.* 2009, 131(11):3794-3795. Briefly, a fluorescent dye, SYPROTM, complexes with protein (or protein-peptide) complexes in solution. The dye fluoresces after denaturation of the complexes. The results are determined as TNF α - Δ Tm in degrees centigrade.

[0369] The following peptides were complexed with TNF α for this study, where differences from Biotin-DD-18 are underlined:

Bio-18: Biotin-DDCVWQPKFNNYWC-amide (SEQ ID NO:32)

Bio-18-WtoG-1: Biotin-DDCVWQPKFNNYGC-amide (SEQ ID NO:35)

Bio-18-WtoG-2: Biotin-DDCVGQPKFNNYWC-amide (SEQ ID NO:47)

Bio-18-NtoG: Biotin-DDCVWQPKFNGYWC-amide (SEQ ID NO:48)

[0370] The results were as follows:

Bio-18: 80.5 (+5.7 over TNF α alone)

Bio-18-WtoG-1: 75.1 (+0.3 over TNF α alone)

Bio-18-WtoG-2: 75.0 (+0.2 over TNF α alone)

Bio-18-NtoG: 74.7 (-0.1 under TNF α alone)

TNF α alone 74.8

[0371] Each of the single amino acid substitutions in Biotin-DD-18 reduced the thermostability of the TNF α /peptide complex, which is consistent with the reduced binding activity of these peptides, as shown previously.

[0372] Example 20: Thermostability of Monomeric Peptide Biotin-DD-18 and Spacer Changes

[0373] The thermostability of the DD spacer changes on Peptide Biotin-DD-18 was determined using the thermostability assay described in Example 19. The peptides tested were the following:

Biotin-PEG4-DD-018: biotin-PEG4-DDCVWQPKFNNYWC-amide (SEQ ID NO:54)

Bio-DD-PEG4-018: biotin-DD-PEG4-CVWQPKFNNYWC-amide (SEQ ID NO:55)

[0374] The results were as follows:

Biotin-PEG4-DD-018: 80.3 (+5.5 over TNF α alone)

Bio-DD-PEG4-018: 78.4 (+3.6 over TNF α alone)

TNF α alone: 74.8

[0375] These results are consistent with the analysis of these peptides described above and indicate that introduction of a PEG4 spacer between the DD tag and the N-terminus of the core TNF α binding domain weakens TNF α binding and complex stability.

[0376] Example 21: Thermostability of Monomeric Peptides Having Modifications of the N-terminal DD Tag of Peptide DD-18

[0377] The thermostability assay described in Example 19 was performed on the following peptides to further assess changes in sequence of the N-terminal DD tag of Peptide Biotin-DD-18. The following peptides were tested:

DD-018: biotin-DDCVWQPKFN $\overline{N}$ YWC-amide (SEQ ID NO:32)

D-018: biotin-DCVWQPKFN $\overline{N}$ YWC-amide (SEQ ID NO:56)

DDD-018: biotin-DDDCVWQPKFN $\overline{N}$ YWC-amide (SEQ ID NO:49)

[0378] The results were as follows:

DD-018: 80.5 (+ 5.7 over TNF α alone)

D-018: 78.9 (+ 4.1 over TNF α alone)

DDD-018: 81.6 (+ 6.8 over TNF α alone)

TNF: 74.8

[0379] These results are consistent with the analyses of these peptides described above and indicate that increasing the number of Asp residues in the N-terminal tag increases TNF α binding and complex stability.

[0380] Example 22: Thermostability of Peptide Bio-DD-018 and Variants

[0381] The thermostability assay described in Example 19 was performed on the following peptides from the library screen described in Example 1 to further assess the effects of changes in the sequence of Peptide Biotin-DD-18 on the binding to TNF α . The following peptides were tested, where differences from Peptide Biotin-DD-18 are underlined:

DD-018: Biotin-DDCVWQPKFN $\overline{N}$ YWC -amide (SEQ ID NO:32)

DD-018-KtoR: Biotin-DDCVWQPRF $\overline{N}$ YWC -amide (SEQ ID NO:39)

DD-Hit19: Biotin-DDCQFQPRF $\overline{N}$ NWQC -amide (SEQ ID NO:50)

DD-Hit33: Biotin-DDCHFSQRFN $\overline{N}$ WWC -amide (SEQ ID NO:51)

DD-Hit92: Biotin-DDCAYQRQFN $\overline{N}$ WWC -amide (SEQ ID NO:52)

DD-Hit193: Biotin-DDCWFEHRFN $\overline{N}$ WHC -amide (SEQ ID NO:53)

DD-Hit16: Biotin-DDCHFNPRFN $\overline{N}$ WWC -amide (SEQ ID NO:41)

[0382] The results were as follows (in °C):

DD-018:	80.5 (+5.7 over TNF α alone)
DD-018-KtoR:	81.9 (+8.1 over TNF α alone)
DD-Hit19:	76.6 (+1.8 over TNF α alone)
DD-Hit33:	77.5 (+2.7 over TNF α alone)
DD-Hit92:	77.2 (+2.7 over TNF α alone)
DD-Hit193:	75.9 (+1.1 over TNF α alone)
DD-Hit16:	79.9 (+5.1 over TNF α alone)
TNF α alone:	74.8

[0383] Peptides representing other hits from the library exhibited less thermostability than Peptides Bio-DD-18 and Bio-DD-018-KtoR, although the thermostability of Peptide DD-Hit16 was close to that of Peptide DD-018.

[0384] Example 23: Analysis of the Effects of Changing PEG Spacer Length in Peptide DD-18-KtoR Trimers

[0385] Trimers with different PEG spacers were prepared to determine the effect of PEG spacer length on activity of trimer of Peptide DD-18-KtoR (SEQ ID NO:39) activity in the L-929 cell killing assay. The Peptide DD-018-KtoR-C-trimer (PEG12) from Example 14 was used. Two different preparations of 018-KtoR-C-trimer (PEG12) were tested in this study. They are designated -1 and -2. In addition, trimers with PEG spacers of 4 and 8 PEG subunits were prepared. The trimeric peptides tested were the following:

018-KtoR-C-trimer (PEG12) – 1: Fmoc-[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃

018-KtoR-C-trimer (PEG12) – 2: Fmoc-[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃

018-KtoR-C-trimer (PEG4): Fmoc-[Ac-DDCVWQPRFNYYWC-PEG4-K-amide]₃

018-KtoR-C-trimer (PEG8): Fmoc-[Ac-DDCVWQPRFNYYWC-PEG8-K-amide]₃

[0386] Referring to Figure 13, the PEG8 spacer and the PEG12 spacer were about the same in activity while the PEG4 spacer consistently yielded less binding activity (compare bars 2, 3, and 4 with bar 1 within each series).

[0387] Example 24: Activity of Peptide DD-18-Dimers and Trimers in Cell Killing assay Using Mouse TNF

[0388] In Example 16, the Peptide 018-KtoR-C-trimer and Peptide 018-KtoR-C-dimer were shown to bind to human, monkey, and dog TNF α , but not rat TNF α . In this study, the dimers and trimers were tested against mouse TNF α in the L-929 cell killing assay.

[0389] The peptides tested were the following:

DD-18-KtoR-N-dimer: (Ac-K-PEG12-DDCVWQPRFN $\underline{N}$ YWC-amide)₂-PEG6

DD-18-KtoR-C-dimer: (Ac-DDCVWQPRFN $\underline{N}$ YWC-PEG12-K-amide)₂-PEG6

DD-18-KtoR-N-trimer: Fmoc-[Ac-K-PEG12-DDCVWQPRFN $\underline{N}$ YWC-amide]₃

DD-18-KtoR-C-trimer: Fmoc-[Ac-DDCVWQPRFN $\underline{N}$ YWC-PEG12-K-amide]₃

[0390] In this study, none of the dimers or trimers showed significant activity in binding to mouse TNF α (data not shown).

[0391] Example 25: Modification of the Peptide DD-18 Sequence

[0392] Peptide 001 from the CX₁₀C library was a strong binder in the phage display screen but was insoluble under the aqueous assay conditions used in these studies. Based on the similarity of Peptide 001 and Peptide 018-KtoR, a modified Peptide 018 was designed. The sequences of the core TNF α binding domains of Peptide 001 and Peptide 018-KtoR and the modified Peptide 18 are shown below, where differences from Peptide 018-KtoR are underlined:

Peptide 001: CTFQWRFN $\underline{N}$ YWC (SEQ ID NO:12)

Peptide 018-KtoR: CVWQPRFN $\underline{N}$ YWC (SEQ ID NO:5)

Modified Peptide 18-KtoR: CTFQPRFN $\underline{N}$ YWC (SEQ ID NO:6)

[0393] Another variant of Peptide DD-018 was also tested; this variant had an N to Q substitution near the C-terminal end of the peptide (at the penultimate C-terminal position of the core TNF α binding domain) was prepared. These peptides were tested in the TNF α binding assay (Example 2). Another peptide, Hit 14 from the CX₁₀C library, was tested due to its sequence similarity with Peptide DD-018. The peptides used in this study were as follows, where differences from Peptide Biotin-DD-018 are underlined:

Biotin-DD-018: Biotin-DDCVWQPKFN $\underline{N}$ YWC-amide (SEQ ID NO:32)

Biotin-DD-018-KtoR: Biotin-DDCVWQPRF $\underline{N}$ YWC-amide (SEQ ID NO:39)

Biotin-DD-018-NtoQ: Biotin-DDCVWQPKFNQYWC-amide (SEQ ID NO:57)

Biotin-DD-TF-018-KtoR: Biotin-DDCTFQPRF $\underline{N}$ YWC-amide (SEQ ID NO:58)

Biotin-DD-Hit14: Biotin-DDCVFQHHFNNWWC-amide (SEQ ID NO:59)

[0394] Each peptide was tested using two different preparations of human TNF α in this study.

[0395] Referring to Figure 14, assays with each preparation of TNF α are shown for each peptide tested (left and right bars within each pair). Peptide Biotin-DD-018-KtoR exhibited slightly better TNF α binding than Peptide Biotin-DD-018, which is consistent with previous results. Peptide Biotin-DD-018-NtoQ exhibited little TNF α binding activity as compared to Peptide Biotin-018-KtoR. This result suggests that residues in the C-terminal end of peptide are involved in contact with TNF α . The modified peptide, Biotin-DD-TF-018-KtoR, had slightly better TNF α binding than Peptide Biotin-DD-018-KtoR. Hit14 exhibited binding somewhat less than Peptide Biotin-DD-018.

[0396] Example 26: Determination of EC₅₀ values of Modified DD-018-KtoR Peptides Using the L-929 Cell Killing Assay

[0397] To further analyze the effects of modifications to Peptide Biotin-DD-018, the following peptides were tested in the L-929 cell killing assay to determine the EC₅₀ values, where differences from Peptide Biotin-DD-018 are underlined:

Bio-DD-018: Biotin-DDCVWQPKFNNYWC-amide (SEQ ID NO:32)

Bio-DDD-018-KtoR: Biotin-DDDCVWQPRFNNYWC-amide (SEQ ID NO:60)

Bio-DD-018-KtoR: Biotin-DDCVWQPRFNNYWC-amide (SEQ ID NO:39)

Bio-DD-TF-018-KtoR: Biotin-DDCTFQPRFNNYWC-amide (SEQ ID NO:58)

Bio-DD-Hit14: Biotin-DDCVFQHFFNNWWC-amide (SEQ ID NO:59)

[0398] The EC₅₀ values were the following:

Bio-DD-018: 14.5 micromolar

Bio-DDD-018-KtoR: 2.7 micromolar

Bio-DD-018-KtoR: 5.4 micromolar

Bio-DD-TF-018-KtoR: 1.7 micromolar

Bio-DD-hit14: 14.7 micromolar

[0399] These results indicated that the TF substitution proximal to the N-terminal cysteine residue in Peptide Bio-DD-TF-018-KtoR improved the activity of Peptide Bio-DD-018 KtoR. In addition, the addition of an additional aspartate residue to the N-terminal tag improved (lowered) the EC₅₀ value of the peptide. Hit14 exhibited a similar EC₅₀ value to that of Peptide Biotin-DD-018.

[0400] Example 27: Analysis of Further Modifications of Peptide Bio-DD-018-KtoR

[0401] Based on the results of the previous example, additional peptides were designed that included the TF substitution or variants thereof. These peptides were tested in the L-929 cell killing assay to determine their EC₅₀ values. The peptides tested were the following, where differences from Peptide Bio-DD-018-KtoR are underlined:

Bio-DD-018-KtoR: Biotin-DDCVWQPRFN^YWC-amide (SEQ ID NO:39)

Bio-DD-TF-018-KtoR: Biotin-DDCTTFQPRFN^YWC-amide (SEQ ID NO:58)

Bio-DD-TF-018-KtoR-W: Biotin-DDCTTFQPRFNNWWC-amide (SEQ ID NO:61)

Bio-DD-VF-018-KtoR: Biotin-DDCVFQPRFN^YWC-amide (SEQ ID NO:62)

Bio-DD-SF-018-KtoR: Biotin-DDCSFQPRFN^YWC-amide (SEQ ID NO:63)

[0402] The EC₅₀ values determined were as follows:

Bio-DD-018-KtoR: 2.25 micromolar

Bio-DD-TF-018-KtoR: 1.16 micromolar

Bio-DD-TF-018-KtoR-W: 0.54 micromolar

Bio-DD-VF-018-KtoR: 0.64 micromolar

Bio-DD-SF-018-KtoR: 2.78 micromolar

[0403] These results also showed that the TF substitution and F substitution in the N-terminus of the core TNF α binding domain increased its activity. Both Tyr and Trp at the second C-terminal position of the core TNF α binding domain allow comparable binding activity.

[0404] Example 28: Activity of Peptide DD-TF-018-KtoR Trimer as compared to anti-TNF α antibody and VHH2 in The L-929 Cell Killing Assay

[0405] The activity of a trimeric form of the DD-TF-018-KtoR (SEQ ID NO:59) that has linked to it a PEG8-K spacer in each arm was compared against the activity of other TNF α -blocking agents in the L-929 cell killing assay and the EC₅₀ values were determined. Anti-TNF α antibody was from R&D Systems (research grade). Anti-TNF α VHH2 nanobody was as described in Beirnaert *et al.*, 2017, *Front. Immunol.* 8:867. The peptide tested was the following:

DD-TF-018-KtoR C-trimer (PEG8): Fmoc-[Ac-DDCTFQPRFN^YWC-PEG8-K-amide]₃

[0406] The EC₅₀ values were as follows:

DD-TF-018-KtoR C-trimer (PEG8): 1 pM to 1 fM

Anti-TNF α antibody: 90.5 pM

Anti-TNF α VHH2 nanobody: 16.9 pM

[0407] In this assay, Peptide DD-TF-018-KtoR trimer was more potent than the anti-TNF α antibody and anti-TNF α VHH2.

[0408] Example 29: Analysis of Variations of the Trimeric Scaffold Structure and Spacer Length in the L-929 CELL Killing Assay

[0409] To determine whether and/or how different trimeric scaffolds affect the activity of Peptide DD-018-KtoR (SEQ ID NO:32), three different scaffolds were tested. In addition, Peptide DD-TF-018-KtoR (SEQ ID NO:58) was included in one trimer construct. Referring to Figure 15A, the scaffolds tested were an Fmoc-scaffold (Quanta BioDesign), an Fmoc-PEG27 scaffold (Quanta BioDesign) and a cyclohex scaffold (the cyclohex tri-NHS ester was prepared from cyclohexanetricarboxylic acid, also called 1,3,5-cyclohexanetricarboxylic acid, by conversion of tri-carboxylic acid compounds with NHS (N-hydroxysuccinimide) in presence with a carbodiimide (such as DIC or DCC)). The Fmoc-scaffold was used previously in Example 14. In addition, the length of the PEG spacer was changed from PEG8 to PEG6. The trimeric peptides were tested in the L-929 cell killing assay using peptide concentrations of 0.2 micromolar to 0.51 pM (in five-fold dilutions). The trimeric peptides tested were as follows:

018-KtoR-C-trimer (PEG6)-Fmoc:

[Ac-DDCVWQPRFNYYWC-PEG6-K-amide]₃-Fmoc

018-KtoR-C-trimer (PEG12)-PEG27-Fmoc:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-PEG27-Fmoc

018-KtoR-C-trimer (PEG12)-Fmoc:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-Fmoc

DD-TF-018-KtoR-trimer (PEG12)-Fmoc:

[Ac-DDCTFQPRFNYYWC-PEG12-K-amide]₃-Fmoc

018-KtoR-trimer (PEG12)-cyclohex:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-cyclohex

[0410] Referring to Figure 15B, the choice of scaffold did not appear to significantly affect the activity of the trimers, although the trimer with a PEG6 spacer had decreased activity as compared to the PEG8 spacer tested previously (see Example 23).

[0411] Example 30: Further Analysis of Variations of the Trimeric Scaffold Structure in the L-929 Cell Killing Assay

[0412] To determine whether other trimeric scaffolds increased the activity of Peptide DD-018-KtoR (SEQ ID NO:39) in trimeric form, five different scaffolds were tested in addition to the Fmoc-PEG27 scaffold used in Example 29. Referring to Figure 16A, the scaffolds tested were a Nitro-scaffold (prepared from 4-(2-Carboxyethyl)-4-nitroheptanedioic acid by conversion of tri-carboxylic acid compounds with NHS (N-hydroxysuccinimide) in presence with a carbodiimide (such as DIC or DCC)), an Amine-PEG27-scaffold (Quanta BioDesign), the Fmoc-scaffold (see Example 29), an Amine-scaffold (Quanta BioDesign), a cholesterol-scaffold (Quanta BioDesign) and a Biotin-PEG27-scaffold (Quanta BioDesign). The trimeric peptides were tested in the L-929 cell killing assay using peptide concentrations of 0.2 micromolar to 2.6 pM (in five-fold-dilutions). The trimeric peptides tested were as follows:

018-KtoR-C-trimer (PEG12)-nitro:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-nitro

018-KtoR-C-trimer (PEG12)-PEG27-amine:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-PEG27-amine

018-KtoR-C-trimer (PEG12)-Fmoc:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-Fmoc

018-KtoR-C-trimer (PEG12)-amine:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-amine

018-KtoR-C-trimer (PEG12)-PEG27-biotin:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-PEG27-biotin

018-KtoR-C-trimer (PEG12)-PEG27-cholesterol:

[Ac-DDCVWQPRFNYYWC-PEG12-K-amide]₃-PEG27-cholesterol

[0413] Referring to Figure 16B, generally the choice of trimeric scaffold did not significantly affect the activity of the trimeric peptides, although the cholesterol-scaffold yielded slightly lower activity at lower concentrations.

[0414] Example 31: Activity of Certain Trimeric Peptides at Lower Concentrations

[0415] Trimers of Peptide DD-018-KtoR (SEQ ID NO:39) or Peptide TF-DD-018-KtoR (SEQ ID NO:59) using the Fmoc-scaffold, amine-scaffold or cyclohex-scaffold from Examples 29 and 30 were tested in L-929 cell killing assay at lower concentrations than in prior examples to determine the EC₅₀ values. The trimeric peptide concentrations ranged

from 0.32 nM to 0.16 fM (in 5-fold dilutions). The trimeric peptides tested were the following:

018-KtoR-C-trimer (PEG12)-Fmoc:



018-KtoR-C-trimer (PEG12)-amine:



018-KtoR-C-trimer (PEG12)-cyclohex:



TF-018-KtoR-C-trimer (PEG12)-Fmoc:



[0416] The EC₅₀ values were as follows:

018-KtoR-C-trimer (PEG12)-Fmoc: 5.1 pM

018-KtoR-C-trimer (PEG12)-amine: 0.16 fM

018-KtoR-C-trimer (PEG12)-cyclohex: 4 pM

TF-018-KtoR-C-trimer (PEG12)-Fmoc: 0.16 fM

[0417] The 018-KtoR-C-trimer (PEG12)-amine and TF-018-KtoR-C-trimer (PEG12)-Fmoc yielded comparable activities. In the Fmoc-trimeric format, the TF-018-KtoR trimer yielded better activity than the 018-KtoR trimer. The amine trimer yielded the best activity for the 018-KtoR trimers.

[0418] Example 32: Analysis of Further Modifications to Monomeric DD-VF-018-KtoR-WW Peptide in the L-929 Cell Killing Assay

[0419] Based on the above examples, Peptide TF-018-KtoR-WW (DDCTFQPRFNNWWC; SEQ ID NO:61) yielded the best activity in the L-929 cell killing assay. Using a similar reference sequence, Peptide VF-018-KtoR-WW (DDCVFQPRFNNWWC; SEQ ID NO:64), additional substitutions in the peptides were made and tested in the L-929 cell killing assay. The peptides tested were as follows, where differences from Peptide VF-018-KtoR-WW (SEQ ID NO:65) are underlined. Peptide Bio-018-KtoR (SEQ ID NO:39) was also included as a control.

Bio-VF-018-KtoR-WW: Biotin-DDCVFQPRFNNWWC-amide (SEQ ID NO:64)

Bio-DtoE-VF-018-KtoR-WW: Biotin-EDCVFQPRFNNWWC-amide (SEQ ID NO:65)

Bio-VF-018-QtoK-KtoR-WW: Biotin-DDCVFKPRFNNWWC-amide (SEQ ID NO:66)

VF-018-QtoKbio-KtoR-WW: Ac-DDCVFX⁶PRFNNWWC-amide; wherein X⁶ is K(bio); SEQ ID NO:67)

Bio-018-KtoR: Biotin-DDCVWQPRFNNYWC-amide (SEQ ID NO:39)

[0420] Referring to Figure 17, changing the N-terminal Aspartate to Glutamate in the tag did not significantly modify activity of Peptide Bio-VF-018-KtoR-WW (SEQ ID NO:65). Peptides VF-018-QtoKbio-KtoR-WW and Bio VF-018-QtoKbio-KtoR-WW (SEQ ID NO:67) (with substitution of the Gln (at position 4 of the core TNF α binding domain (from the N-terminus)) to Lys or Lys-biotin had somewhat reduced activity compared to Bio-VF-018-KtoR-WW (SEQ ID NO:65).

[0421] Example 33: Analysis of Further Modifications to Monomeric DD-VF-018-KtoR-WW Peptide in the L-929 Cell Killing Assay

[0422] To determine whether further modifications to Peptide VF-018-KtoR-WW (SEQ ID NO:65) could improve its activity, additional substitutions were made and tested in the L-929 cell killing assay. The peptides tested were as follows, where differences from Peptide VF-018-KtoR-WW (SEQ ID NO:65) are underlined. Peptide Bio-018-KtoR (SEQ ID NO:39) was also included as a control.

Bio-VF-018-KtoR-WW: Biotin-DDCVFQPRFNNWWC-amide (SEQ ID NO:64)

Bio-VF-018-PtoG-KtoR-WW: Biotin-DDCVFQGRFNNWWC-amide (SEQ ID NO:68)

Bio-VF-018-PtoH-KtoR-WW: Biotin-DDCVFQHRFNNWWC-amide (SEQ ID NO:69)

Bio-VF-018-QtoN-KtoR-WW: Biotin-DDCVFNPRFNNWWC-amide (SEQ ID NO:70)

Bio-018-KtoR: Biotin-DDCVWQPRFNNYWC-amide (SEQ ID NO:39)

[0423] Referring to Figure 18, Peptides Bio-VF-018-PtoG-KtoR-WW, Bio-018-KtoR-WW and Bio-VF-018-PtoH-KtoR-WW had comparable activity at the highest concentration. As the concentrations were decreased, Peptide Bio-VF-018-KtoR-WW and Bio-VF-018-PtoH-KtoR-WW retained better activity than the other peptides, although all peptides retained binding activity which was comparable to Peptide Bio-018-KtoR.

[0424] Example 34: Analysis of Further Modifications to Peptide DD-018-KtoR in Monomeric and Dimeric Forms in the L-929 Cell Killing Assay

[0425] The DD tag at the N-terminus of the peptides provides a negative charge at neutral pH, which helps improve the solubility of the peptides. Two Aspartate (D) residues in the N-terminal tag maintained high solubility and good activity. In this study, the N-terminal Asp (D) was replaced by a mimic suc (succinate) group. Succinic acid is a dicarboxylic acid. One acid group is conjugated to the Asp through an amide bond, while the other acid is free to introduce a negatively-charged carboxylate. Both monomeric and dimeric forms of Peptide DD-018-KtoR-PEG8-K were tested in the L-929 cell killing assay. The dimeric forms were made using amine-NHS chemistry and an NHS-PEG8-NHS crosslinker (Quanta BioDesign). Peptide Bio-DD-TF-018-KtoR-WW (SEQ ID NO:62) was also included. The peptides tested were the following:

DD-018-KtoR-PEG8-K:

Ac-DDCVWQPRFNYYWC-PEG4-PEG4-K-amide (SEQ ID NO:39)

sucD-018-KtoR-PEG8-K:

suc-DCVWQPRFNYYWC-PEG4-PEG4-K-amide (SEQ ID NO:71)

DD-018-KtoR-PEG8-K Dimer:

(Ac-DDCVWQPRFNYYWC-PEG4-PEG4-K-amide)₂-PEG6

sucD-018-KtoR-PEG8-K Dimer:

(suc-DCVWQPRFNYYWC-PEG4-PEG4-K-amide)₂-PEG6

Bio-DD-TF-018-KtoR-WW: Biotin-DDCTFQPRFNNWWC-amide (SEQ ID NO:61)

[0426] Referring to Figure 19, replacement of one Asp residue of the tag with a succinate group did not significantly affect monomer and dimer activity, as compared to the unmodified peptide.

[0427] Example 35. Analysis of Further Modifications to Peptide TF-018-KtoR-WW in the L-929 Cell Killing Assay

[0428] In this study, D- α -amino acid analogs were site-selectively introduced to determine whether potency of monomeric Peptide TF-018-KtoR-WW (SEQ ID NO:62) was increased in the L-929 cell killing assay. The D- α -amino acid analogs used were penicillamine (Pen), a methyl cysteine analog; Homoleucine (homoLeu), an analog of Leucine (Leu); and Norleucine (norLeu), an analog of Leucine (Leu). The peptides tested were the following, where differences from Peptide Bio-DD-TF-018-KtoR-WW (SEQ ID NO:62) are underlined:

Bio-Pen-TF-018-KtoR-WW: Biotin-DDXTFQPRFNNWWC-amide (SEQ ID NO:72), where X in this peptide is D-penicillamine

Bio-TF-018-KtoR-WW-Pen: Biotin-DDCTFQPRFNNWWX-amide (SEQ ID NO:73), where X in this peptide is D-penicillamine

Bio-homoLeu-F-018-KtoR-WW:

Biotin-DDCXFQPRFNNWWC-amide (SEQ ID NO:74), where X in this peptide is D-homoleucine

Bio-norLeu-F-018-KtoR-WW:

Biotin-DDCXFQPRFNNWWC-amide (SEQ ID NO:75), where X in this peptide is D-norleucine

Bio-DD-TF-018-KtoR-WW: Biotin-DDCTFQPRFNNWWC-amide (SEQ ID NO:61)

[0429] Referring to Figure 20, substitution with D- α -amino acid analogs did not significantly affect the potency of the monomer at the highest concentration, although Peptide Bio-homoLeu-F-018-KtoR-WW exhibited somewhat lower activity as compared to the other peptides.

[0430] Example 36. Thermostability of Complexes of TNF α with Peptide DD-018-KtoR (SEQ ID NO:39) and Oligomeric Variants

[0431] The thermostability assay described in Example 19 was performed on the following complexes of TNF α and peptides to further assess the effects of oligomerization on TNF α binding stability. The following peptides were tested:

DD-18-KtoR: DD-18-KtoR: DDCVWQPRFN NYWC -amide (SEQ ID NO:39)

DD-18-KtoR-C-dimer: (Ac-DDCVWQPRFN NYWC -PEG12-K-amide)₂-PEG6

DD-18-KtoR-C-trimer: Fmoc-[Ac-DDCVWQPRFN NYWC -PEG12-K-amide]₃

[0432] In addition, TNF α alone was included as a reference.

[0433] [000353] The results were as follows (in °C):

DD-18-KtoR:	81.9 (+8.1)
DD-18-KtoR-C-dimer	84.6 (+9.8)
DD-18-KtoR-C-trimer	86.0 (+10.2)
TNF α alone	74.8

[0434] These results show that increased oligomerization of Peptide DD-018-KtoR increased the thermostability of the peptide complexes with TNF α .

[0435] Example 37. Fluorescently-labeled Peptide DD-018 Was Used to Confirm Binding of the Monomeric Peptide to Native TNF α (trimeric)

[0436] Native TNF α (trimer form) was incubated with either Peptide DD-018 (SEQ ID NO:32) or Peptide DD-018-WtoG2 (SEQ ID NO:77) (a nonbinding control). Both peptides were labeled with an N-terminal fluorescein group (by amide coupling of carboxyfluorescein during solid phase peptide synthesis SPPS). Peptide plus TNF α mixtures were then assayed by analytical SEC in 0.1 M ammonium acetate, pH 8, using an AKTA analytical SEC column. The peptides tested were as follows:

DD-018: Fluor-DDCVWQPKFNNYWC-amide (SEQ ID NO:32)

DD-018-WtoG2: Fluor-DDCVGQPKFNNYGC-amide (SEQ ID NO:76)

[0437] Referring to Figure 21, for each panel, the left Y-axis is absorbance at 280 nm, while the right y-axis is absorbance at 480 nm. P1 identifies the elution peak corresponding to TNF α . P2 identifies the elution peak for free peptide. The top panel shows the elution profile for TNF α alone. The middle panel shows the elution profile for TNF α plus fluorescently labeled Peptide DD-018. Some peptide co-elutes with TNF α (P1), indicating that it bound to TNF α . P2 is the excess peptide. The bottom panel shows the elution profile for TNF α plus fluorescently labeled Peptide DD-018-WtoG2, a non-binding control. Peptide DD-018-WtoG2 does not coelute with TNF α .

[0438] Example 38. Peptide TF-018-KtoR-WW and Oligomers Thereof Were Tested in the TNF α /TNFR blocking assay and L-929 Cell Killing Assay.

[0439] The activity of Peptide TF-018-KtoR-WW (SEQ ID NO:62) as a monomer, dimer, and trimer was determined in the TNF α /TNFR blocking assay (Example 3) and in the L-929 Cell Killing Assay (Example 4). The peptides tested were as follows:

TF-018-KtoR-WW Monomer: Ac-DDCTFQPRFNNWWC-amide (SEQ ID NO:61)

TF-18-KtoR-WW C-Dimer: (Ac-DDCTFQPRFNNWWC-PEG12-K-amide)₂-PEG6

TF-18-KtoR-WW C-Trimer: amine-[Ac-DDCTFQPRFNNWWC-PEG8-K-amide]₃

[0440] In addition, the activities of the peptide monomers and trimers were compared to an anti-TNF α antibody (R&D Systems (research grade)).

[0441] Figure 22A shows a comparison of the binding affinities of monomeric, dimeric and trimeric Peptide TF-018-KtoR-WW in the TNF α /TNFR blocking assay.

Multimerization of the peptide improved the IC₅₀. Figure 22B shows a comparison of the monomeric trimeric Peptide TF-018-KtoR-WW with an anti-TNF α antibody (R&D Systems (research grade)). The IC₅₀ of trimeric Peptide TF-018-KtoR-WW was better than that of the anti-TNF α antibody in the TNF α /TNFR blocking assay.

[0442] Figure 23A shows a comparison of the activities of monomeric, dimeric, and trimeric Peptide TF-018-KtoR-WW in the L-929 cell killing assay. Multimerization of the peptide improved the EC₅₀. Figure 23B shows a comparison of the monomeric and trimeric Peptide TF-018-KtoR-WW with an anti-TNF α antibody (R&D Systems (research grade)). The EC₅₀ value of trimeric Peptide TF-018-KtoR-WW was better than that of the anti-TNF α antibody in this assay.

[0443] Example 39. Binding Affinity Determination of Peptide TF-018-KtoR-WW Monomer and Trimer

[0444] The binding affinities of Peptide TF-018-KtoR-WW (SEQ ID NO:62) monomer and trimer were determined by surface plasmon resonance (SPR). Recombinant biotin-TNF α was immobilized to an SPR chip surface through interaction with neutravidin-modified chips. Then different concentrations of monomer or trimer were introduced across the surface, and relative binding parameters were determined. Peptide TF-018-KtoR-WW monomer had an affinity of ~ 50 nM, as determined using concentrations of 1200 nM, 625 nM, and 312.5 nM of monomer. The affinity of the Peptide TF-18-KtoR-WW C-trimer could not be determined in this assay (using concentrations of 3.75 nM, and 1.88 nM) due to its strong interaction with immobilized TNF α .

[0445] Example 40. Peptide TF-18-KtoR-WW C-Trimer Can Block the Activity of Membrane-Bound TNF α

[0446] Peptide TF-18-KtoR-WW C-Trimer was tested for its ability to specifically block the activity of membrane-bound TNF α using the L-929 cell killing assay. HEK293T cells expressing either wild-type (WT) or membrane-restricted (Δ 1-12 aa) TNF α (see Ruuls *et al. Immunity* 15:533-543, 2001; Decoster *et al. J. Biol. Chem.* 270:18473-18478, 1995). The membrane restricted form of TNF α has a deletion (Δ 1-12 aa) of the substrate cleavage site for TACE (TNF α converting enzyme). To evaluate the blocking activity of TF-18-KtoR-WW C-Trimer against membrane-associated TNF α , L-929 (TNFR-expressing) cells were overlaid against HEK293T cell lines (expressing either WT or membrane-restricted (Δ 1-12 aa) TNF α) in the absence or presence of trimer.

[0447] Referring to Figure 24, in the first (left most) panel, addition of WT TNF α expressing cells resulted in L-929 cell death. In the second panel (from left), addition of cell expressing membrane restricted TNF α (Δ 1-12aa TNF α) expressing cells resulted in L-929 cell death. In the third panel (from left), addition of WT TNF α expressing cells plus TF-18-KtoR-WW C-Trimer rescued L-929 cell death, depending on the amount of TNF α -containing cells added. In the fourth panel (right most panel), addition of TNF α (Δ 1-12aa TNF α) expressing cells plus TF-18-KtoR-WW C-Trimer rescued L-929 cell death.

[0448] In conclusion, the TF-18-KtoR-WW C-Trimer could rescue TNF α -mediated cell killing for both WT and membrane-restricted TNF α .

[0449] Example 41. Stability of Peptide TF-18-KtoR-WW C-Trimer in Simulated Gastric and Intestinal Fluids

[0450] The stability of Peptide TF-18-KtoR-WW C-Trimer was assessed in simulated gastric and intestinal fluids (SGF and SIF, respectively). The TF-18-KtoR-WW C-Trimer has three PEG8 arms and a free amino group. Simulated gastric fluid (SGF – RICCA Chemical Company, Product Number 7108) contained 0.2 % (w/v) sodium chloride in 0.7 % (v/v) hydrochloric acid with 3.2 mg of freshly added purified pepsin (derived from porcine stomach mucosa, with an activity of 800 to 2500 units per mg of protein) added per mL of solution. The pH of the simulated gastric fluid was 1 to 1.6. Simulated intestinal fluid (SIF – RICCA Chemical Company, Product Number 7109) contained 0.68 % (w/v) potassium phosphate, 0.06 % (w/v) sodium hydroxide and 1 % (w/v) pancreatin. The pH of this solution was 6.7 to 6.9.

[0451] 26 μ M of Peptide TF-18-KtoR-WW C-Trimer was incubated in SGF and SIF for 24 hours at 37 °C, followed by HPLC to determine the amount of Peptide TF-18-KtoR-WW C-Trimer remaining after 24 hours by comparing peak areas. An L-amino acid form of Peptide TF-18-KtoR-WW C-Trimer was used as a control. Peptide TF-18-KtoR-WW C-Trimer was stable in these conditions with only minimal degradation apparent after 24 hours, while the L-amino acid form of Peptide TF-18-KtoR-WW C-Trimer was mostly degraded by 8 hours or 1 hour in SGF and SIF, respectively.

[0452] Example 42. Whole Blood Cytokine Release Assay.

[0453] The effects of Peptide TF-18-KtoR-WW C-Trimer on IL-8 release in human whole blood stimulated *ex vivo* was evaluated. Three stimuli were included in the study: recombinant human TNF (CellSystems #CS-C1140), LPS (Invivogen #REP-HEBS-10) and anti-CD3 plus anti-CD28 antibodies (ThermoFisher #16-0037-81 and #16-0289-81).

[0454] Whole blood assays were carried out under sterile conditions in a laminar flow hood. All conditions were tested in triplicate. Fresh human whole blood from three healthy donors (< 4 hours after sampling, French National Blood Service) was anticoagulated with heparin.

[0455] The following controls were included in the assay: non-stimulated blood; non-stimulated blood in the presence of 100 nM Peptide TF-18-KtoR-WW C-Trimer; or TNF – or LPS – or anti-CD3-antibody plus anti-CD28-antibody stimulated blood in the absence of compound. The stimulation of whole blood was done in U bottom 96-well plates as follows. For the stimulation with TNF, Peptide TF-18-KtoR-WW C-Trimer and TNF (10 ng/mL) were preincubated for 30 min at room temperature before being added to diluted blood and further incubated at 37 °C, 5 % CO₂ for 24 hours. For the stimulation with LPS (0.1 EU/mL) or anti-CD3 plus anti-CD28 antibodies (0.1 µg/mL anti-CD3 and 1 µg/mL anti-CD28), Peptide TF-18-KtoR-WW C-Trimer was first mixed with diluted blood before the stimulus was added according to the plate layout. The controls were prepared in a similar manner. The blood samples were incubated at 37 °C, 5 % CO₂ in RPMI containing stabilized L-glutamine. After a 24 hours incubation at 37 °C, 5 % CO₂, supernatants were harvested and stored at -20 °C. The final reaction volume was 300 µL per well and the final blood concentration was 50 %. IL-8 released in the culture supernatants was measured using a specific ELISA kit according to the supplier's instructions (Human IL-8 ELISA kit, R&D Systems, #DY208).

[0456] After 24 hours of incubation, no sign of hemolysis was observed in the harvested supernatants. In the absence of stimulation, IL-8 levels were between 450 and 1070 pg/mL on average. The incubation of blood with Peptide TF-18-KtoR-WW C-Trimer at 100 nM by itself did not induce IL-8. A decrease in spontaneous IL-8 release was observed in the presence of 100 nM Peptide TF-18-KtoR-WW C-Trimer. In the presence of Peptide TF-18-KtoR-WW C-Trimer, a clear dose dependent decrease of IL-8 released by stimulated blood was observed for the three donors. The extent of IL-8 inhibition by Peptide TF-18-KtoR-WW C-Trimer was different between the three stimuli. TNF-induced IL-8 was completely inhibited by Peptide TF-18-KtoR-WW C-Trimer at 100 nM (and 10 nM for donor 2). LPS-induced IL-8 was partly inhibited in blood from donors 1 and donor 3 but was not modulated in blood from donor 2. Anti-CD3 plus anti-CD28-antibodies induced IL-8 was also fully (donors 1 and 3) or partly (donor 2) inhibited in the presence

of Peptide TF-18-KtoR-WW C-Trimer. Referring to Figure 25, the average IL-8 inhibition for the three samples for each condition are shown.

[0457] Example 43. Peptide TF-18-KtoR-WW C-Trimer Promotes Survival *In Vivo* in a Human TNF α Mouse Challenge Model.

[0458] Healthy normal mice were challenged with an LD₅₀ of human TNF α (HuTNF α) at time zero (T = 0) and monitored for 24 hours. 30 minutes prior to challenge, the mice were pre-treated with an anti-TNF α monoclonal antibody (research grade adalimumab; R&D Systems) or Peptide TF-18-KtoR-WW C-Trimer at 1 X or 5 X the amount of huTNF α challenge dose. The mice were treated again with Peptide TF-18-KtoR-WW C-Trimer at 0.5 and 1.5 hours. Referring to Figure 26, the anti-TNF α monoclonal antibody (anti-TNF) inhibits both mouse and human TNF-alpha while Peptide TF-18-KtoR-WW C-Trimer inhibits only human TNF-alpha.

[0459] Example 44. Single Dose Subcutaneous Dosing with Peptide TF-18-KtoR-WW C-Trimer

[0460] Mice were dosed with Peptide TF-18-KtoR-WW C-Trimer diluted in PBS and administered as a single subcutaneous dose in 2 male and 2 female CD1mice. Referring to Figures 27 and 28 show the average amount of Peptide TF-18-KtoR-WW C-Trimer in the plasma for each group of four animals, two males and two females. Panels A and B show two different graphical representations of the data.

[0461] Example 45. Peptide TF-18-KtoR-WW C-Trimer Plasma Levels Following Oral Dosing

[0462] Peptide TF-18-KtoR-WW C-Trimer was diluted in PBS to 50 mg/kg and administered to mice, 2 males and 2 females, by a single oral gavage prior to serum collection. Referring to the following Table 1, the concentration (ng/mL) of Peptide TF-18-KtoR-WW C-Trimer in the serum of all four animals at the 6-, 12-, and 24-hour time points post-dosing. Peptide TF-18-KtoR-WW C-Trimer was detectable at 24 hours post-dosing in the plasma of all four animals following oral administration.

Table 1. Peptide TF-18-KtoR-WW C-Trimer Serum Concentration Following Oral Administration

<u>Time</u>	<u>Male 1</u>	<u>Male 2</u>	<u>Female 1</u>	<u>Female 2</u>
6 h	0	0	0	0
12 h	0	0	15.9	0
24 h	77.5	149	15.9	149

[0463] Example 46. Oral dosing of Peptide TF-18-KtoR-WW C-Trimer

[0464] Peptide TF-18-KtoR-WW C-Trimer was administered intravenously (IV; 5 mg/kg), subcutaneously (SC; 50 mg/kg), and orally (po; 100 mg/kg) to C57Bl/6 mice (3 animals per group). The vehicle was 91 % sodium phosphate buffer (20 mM) and 9 % sucrose. Peptide TF-18-KtoR-WW C-Trimer concentration (nM) was measured in plasma or kidney (in the case of 50 mg/kg sc dosing) by mass spec. Briefly, tissues of the gut, liver and kidney were thoroughly flushed of luminal content prior to homogenization and analysis. Peptide TF-18-KtoR-WW C-Trimer was extracted from serum by precipitating serum components using organic solvent (acetonitrile crash), separated from remaining contaminating proteins by C18 reverse-phase HPLC at 40 °C, and the peptide identified by ESI-MS. Peptide TF-18-KtoR-WW C-Trimer levels were quantified by comparing signal in unknown samples to that a standard curve and quality control samples. All samples were spiked with a known amount of a “heavy” internal standard to control for extraction, injection, and ionization variabilities.

[0465] Referring to Table 2 and Figure 29, Peptide TF-18-KtoR-WW C-Trimer was detected in the plasma following administration of 5.0 mg/kg IV or 50 mg/kg SC. It was also detected in the kidney following 50 mg/kg SC dosing; ten percent of the initial exposure present in the kidney after 24 hours. The long terminal half-life of Peptide TF-18-KtoR-WW C-Trimer was 18 hours following intravenous administration.

[0466] Referring to Table 3 and Figure 30, Oral (PO) dosing (100 mg/kg) resulted in low amounts of Peptide TF-18-KtoR-WW C-Trimer being detectable in liver and kidney (in 2 of 3 animals), indicating that some Peptide TF-18-KtoR-WW C-Trimer entered systemic circulation. Plasma exposures were measurable in one animal per group. Peptide TF-18-KtoR-WW C-Trimer was detectable in the liver and kidney which is indicative of

systemic distribution following oral delivery. In addition, high concentrations of Peptide TF-18-KtoR-WW C-Trimer were observed in the tissues of the small and large intestine.

Table 2. Pharmacokinetics Following 5.0 mg/kg IV and 50 mg/mg SC Dosing

	50 mg/kg IV	50 mg/mg SC
Cl (mL/min/kg)	2.5	
V _{SS} (L/kg)	1.1	
Half-life (h)	18	
MRT (h)	7.5	
AUC _{LAST} (nM · h)	3,820	18,100
AUC _{TOTAL} (nM ·)	4,300	18,200
AUC extra (%)	11	0.9
C _{MAX} (nM)		6,680
T _{MAX} (h)		0.5
F _{SC} (%)		47

Table 3. Pharmacokinetics Following 100 mg/kg Oral Dosing

	100 mg/kg PO	
Time point	2h	6 h
Plasma (nM)	22.4 (1 mouse only)	27.4 (1 mouse only)
Liver (nM)	3,850 ± 5,100	187 ± 59
Kidney (nM)	90 ± 28	221 ± 86
Small intestine (nM)	10,100 ± 7920	1,010 (1 mouse only)
Large intestine (nM)	8,320 ± 5,170	868 ± 436

[0467] Example 47. LPS Challenge Model

[0468] Tg1278 (mTNF KO/hTNF KI) mice are a transgenic strain with a normally regulated and expressed human TNF α (hTNF α) in the absence of murine TNF α . To induce acute, TNF-mediated inflammation, groups of male and female mice received a single injection of 10 μ g/mouse of LPS. Six hours after the LPS treatment, mouse sera were collected for analysis of IL-6 production. IL-6 was detected by ELISA. Referring to Figure 31, subcutaneous administration of Peptide TF-18-KtoR-WW C-Trimer at 5, 15, and 50 mg/kg per mouse one hour prior to LPS treatment resulted in full inhibition of TNF-

mediated IL-6 production at all doses. The activity of Peptide TF-18-KtoR-WW C-Trimer was equal to a 10 mg/kg per mouse dose of research grade entanercept (labeled Enbrel in the figure) administered 16 hours prior to LPS treatment. Referring to Figure 32, Peptide TF-18-KtoR-WW C-Trimer was able to inhibit production of mouse KC (chemokine (C-X-C motif) ligand 1 (CXCL1)), in a similar manner.

[0469] Various publications, including patents, patent application publications, and scientific literature, are cited herein, the disclosures of which are incorporated by reference in their entireties for all purposes.

[0470] While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

TABLE 4 PEPTIDE SEQUENCES DISCLOSED

SEQ ID NO:1

C-X²-X³-X⁴-X⁵-X⁶-F-F-N-X¹⁰-X¹¹-C wherein each of X² through X⁶, X¹⁰ is a D-amino acid or a D- α -amino acid analog thereof; and

X² is the D form of any of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D- α - amino acid analog thereof;

X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L), or a D- α -amino acid analog thereof;

X⁴ is a Polar amino acid comprising the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D- α -amino acid analog thereof;

X⁵ is the D form of Pro, Trp, His, Gln, Gly, Arg, Ala, Val or Leu, or a D- α -amino acid thereof;

X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof;

X¹⁰ is the D form of Trp (W) or Tyr (Y), or a D- α -amino acid analog thereof;

X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H) or a D- α -amino acid analog thereof; and

C denotes the D form of Cysteine, F denotes the D form of phenylalanine, and N denotes the D form of asparagine, or a D- α -amino acid analog of C, F or N

SEQ ID NO:2

C*-X²-X³-X⁴-X⁵-X⁶-F-N-N-X¹⁰-X¹¹-C* (SEQ ID NO:2), wherein each of X¹ through X⁶, X¹⁰ and X¹¹ is a D-amino acid or a D α-amino acid analog thereof, and C, F, and N are the D forms of cysteine, phenylalanine, and asparagine, or a D-α-amino acid analogs thereof; and

X² is the D form of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D-α- amino acid analog thereof;

X³ is selected from the D forms of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L), or a D-α-amino acid analog thereof;

X⁴ is a Polar amino acid selected from the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D-α-amino acid analog thereof;

X⁵ is D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), or a D-α-amino acid analog thereof;

X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D-α-amino acid analog thereof;

X¹⁰ is the D form of Trp (W) or Tyr (Y), or a D-α-amino acid analog thereof;

X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H), or a D-α-amino acid analog thereof; and * denotes an optional intramolecular disulfide bond, C denotes the D form of Cysteine, or a D-α-amino acid analog thereof, F denotes the D form of phenylalanine, or a D-α-amino acid analog thereof; and N denotes the D form of asparagine, or a D-α-amino acid analog thereof.

SEQ ID NO:3

C*-X²-[W/F/Y]-X⁴-X⁵-X⁶-F-N-N-[W/Y]-W-C* (SEQ ID NO:3)

X² is the D form of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D-α- amino acid analog thereof;

X⁴ is a Polar amino acid selected from the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G), or a D-α-amino acid analog thereof;

X⁵ is D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), or a D-α-amino acid analog thereof;

X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof; and * denotes an optional intramolecular disulfide bond, C denotes the D form of Cysteine, or a D- α -amino acid analog thereof, F denotes the D form of phenylalanine, or a D- α -amino acid analog thereof; W denotes the D-form of tryptophan, or a D- α -amino acid analog thereof, F- denotes the D-form of phenylalanine, or a D- α -amino acid analog thereof, Y-denotes the D-form of tyrosine, or a D- α -amino acid analog thereof, and N denotes the D form of asparagine, or a D- α -amino acid analog thereof.

SEQ ID NO:4

CVWQPKFNYYWC

SEQ ID NO:5

CVWQPRFNYYWC

SEQ ID NO:6

CTFQPRFNYYWC

SEQ ID NO:7

CTFQPRFNNWWC

SEQ ID NO:8

CSFQPRFNYYWC

SEQ ID NO:9

CSFQPRFNNWWC

SEQ ID NO:10

CVFQPRFNYYWC

SEQ ID NO:11

CVFQPRFNNWWC

SEQ ID NO:12
CTFQWRFNNYWC

SEQ ID NO:13
CLYQPVFNNWWC

SEQ ID NO:14
CVFQAAFNNYWC

SEQ ID NO:15
CVFQHHFNNWWC

SEQ ID NO:16
CHFNPREFNNWWC

SEQ ID NO:17
CVWQPHFNNYWC

SEQ ID NO:18
CVFQGRFNNWWC

SEQ ID NO:19
CVFQHRFNNWWC

SEQ ID NO:20
CVFNPRFNNWWC

SEQ ID NO:21
CVFKPRFNNWWC

SEQ ID NO:22
CAYQRQFNNWWC

SEQ ID NO:23
CWFEHRFNNWHC

SEQ ID NO:24
CHFQHRFNNWWC

SEQ ID NO:25
CHFQPRFNNWWC

SEQ ID NO:26
CTYQPRFNNWWC

SEQ ID NO:27
CQFQPRFNNWQC

SEQ ID NO:28
CHFSQRFNNWWC

SEQ ID NO:29
VRSSRT PSDKPVAHV ANPQAEGQLQ WLNRRANALL ANGVELRDNQ
LVVPSEGLYLIYSQVLFKGQ GCPSTHVLLT HTISRIAVSY QTKVNLLSAI
KSPCQRETPE GAEAKPWYEPIYLGGVFQLE KGDRLSAEIN RPDYLDFAES
GQVYFGIIL

SEQ ID NO:30
GGEEK

SEQ ID NO:31
GGRRRK

SEQ ID NO:32 – Peptide DD-18
DDCVWQPKFNYYWC

SEQ ID NO:33 – Peptide 18 V1

CVWQPKFNNYWCGGEEEEK

SEQ ID NO:34 – Peptide 18 V2

CVWQPKFNNYWCGGRRRK

SEQ ID NO:35 – Peptide DD-18 Mutant

DDCVWQPKFNNYGC

SEQ ID NO:36 – Peptide DD-18 Di-Mutant

DDCDWQDKFNNYWC

SEQ ID NO:37 – Peptide DD-18-ex

DDCVWQPKFNNYWCGGSAETVE

SEQ ID NO:38 – Peptide DGA-18

DGACVWQPKFNNYWC

SEQ ID NO:39 – Peptide DD-18-KtoR

DDCVWQPRFNNYWC

SEQ ID NO:40 – Peptide DD-18-KtoR-R3

DDCVWQPRFNNYWCGGRRRK

SEQ ID NO:41 –Peptide DD-Hit16

DDCHFNPFRNNWWC

SEQ ID NO:42 – Peptide DD-18-noVal

DDCWQPKFNNYWC

SEQ ID NO:43 – Peptide DD-18-plusAla

DDCAVWQPKFNNYWC

SEQ ID NO:44 – Peptide DD-18-KtoR-PEG12-K
DDCVWQPRFNNYWC-PEG12-K

SEQ ID NO:45 Peptide K-PEG12-DD-18-KtoR
K-PEG12-DDCVWQPRFNNYWC

SEQ ID NO:46 - IZ coiled-coil
IKKEIEAIKKEQEAIKKKIEAIEKEA

SEQ ID NO:47 – Peptide 018-WtoG
DDCVGQPKFNNYWC

SEQ ID NO:48 – Peptide 018-NtoG
DDCVWQPKFNGYWC

SEQ ID NO:49 – Peptide DDD-018
DDDCVWQPKFNNYWC

SEQ ID NO:50 – Peptide DD-hit19
DDCQFQPRFNNWQC

SEQ ID NO:51 – Peptide DD-hit33
DDCHFSQRFNNWWC

SEQ ID NO:52 – Peptide DD-hit 92
DDCAYQRQFNNWWC

SEQ ID NO:53 - Peptide DD-hit 193
DDCWFEHRFNNWHC

SEQ ID NO:54 – PEG4-DD-18
PEG4-DDCVWQPKFNNYWC-amide

SEQ ID NO:55 - DD-PEG4-018

DD-PEG4-CVWQPKFNYYWC

SEQ ID NO:56 – Peptide D-018

DCVWQPKFNYYWC

SEQ ID NO:57 – Peptide DD-018-NtoQ

DDCVWQPKFNQYYWC

SEQ ID NO:58 – Peptide DD-TF-018-KtoR

DDCTFQPRFNYYWC

SEQ ID NO:59 – Peptide DD-Hit14

DDCVFQHHFNWWC

SEQ ID NO:60 – Peptide DDD-018-KtoR

DDDCVWQPRFNYYWC

SEQ ID NO:61 – Peptide DD-TF-018-KtoR-WW

DDCTFQPRFNWWC

SEQ ID NO:62 – Peptide DD-VF-018-KtoR

DDCVFQPRFNYYWC

SEQ ID NO:63 – Peptide DD-SF-018-KtoR

DDCSFQPRFNYYWC

SEQ ID NO: 64 – VF-018-KtoR-WW

DDCVFQPRFNWWC

SEQ ID NO:65 - DtoE-VF-018-KtoR-WW

EDCVFQPRFNWWC

SEQ ID NO:66 - VF-018-QtoK-KtoR-WW

DDCVFKPRFNNWWC

SEQ ID NO:67 - VF-018-QtoKbio-KtoR-WW

DDCVFX⁶PRFNNWWC; wherein X⁶ is K(biotin)

SEQ ID NO:68 - VF-018-PtoG-KtoR-WW

DDCVFQGRFNNWWC

SEQ ID NO:69 - VF-018-PtoH-KtoR-WW

DDCVFQHRFNNWWC

SEQ ID NO:70 - VF-018-QtoN-KtoR-WW

DDCVFNPRFNNWWC

Seq ID NO:71 - sucD-018-KtoR

suc-DCVWQPRFNNYWC, where suc is succinic acid

SEQ ID NO:72 - Pen-TF-018-KtoR-WW

DDXTFQPRFNNWWC, where X is D-Penicillamine

SEQ ID NO:73 - TF-018-KtoR-WW-Pen

DDCTFQPRFNNWWX, where X is D-Penicillamine

SEQ ID NO:74 - homoLeu-F-018-KtoR-WW

DDCXFQPRFNNWWC, where X is D-homoleucine

SEQ ID NO:75 - norLeu-F-018-KtoR-WW

DDCXFQPRFNNWWC, where X is D-norleucine

SEQ ID NO:76 – DD-018-WtoG2

DDCVGQPKFNNYGC

SEQ ID NO:77
CIFQQQFNYYWC

SEQ ID NO:78
CMHQQRFNWWC
SEQ ID NO:79
CTFRVRFNYYWC

SEQ ID NO:80
CIFQWRFNYYWC

SEQ ID NO:81
CTFQWHFNYYWC

SEQ ID NO:82
CVFQHLLFNWWC

SEQ ID NO:83
CTFQWLFNYYWC

SEQ ID NO:84
CAFQWRFNYYWC

SEQ ID NO:85
CTFRWRFNYYWC

SEQ ID NO:86
CTSQWRFNYYWC

SEQ ID NO:87
CTFQLRFNYYWC

SEQ ID NO:88

CTFQVRFNNYWC

SEQ ID NO:89

CIWQPKFNNYWC

SEQ ID NO:90

CTFQRRFNNYWC

SEQ ID NO:91

CTFQWSFNNYWC

SEQ ID NO:92

CVFQRHFNNWWC

SEQ ID NO:93

CVSTHHFNNWWC

SEQ ID NO:94

CAFQHHFNNWWC

SEQ ID NO:95

CAYQRHFNNWWC

SEQ ID NO:96

CHFNPWFNNWWC

SEQ ID NO:97

CHFNRRFNNWWC

SEQ ID NO:98

CHFSQLFNNWWC

SEQ ID NO:99

CLYQLVFNNWWC

SEQ ID NO:100
CQFRPRFNNWQC

SEQ ID NO:101
CTLQQQFNNYWC

SEQ ID NO:102
CTSRWRFNNYWC

SEQ ID NO:103
CVFQASFNNYWC

SEQ ID NO:104
CVFQHSFNNWWC

SEQ ID NO:105
CVFRHHFNNWWC

SEQ ID NO:106
CVFTHHFNNWWC

SEQ ID NO:107
CVSQHHFNNWWC

SEQ ID NO:108
CVWQPEFNNYWC

SEQ ID NO:109
CVWQQKFNNYWC

SEQ ID NO:110

CVWRPKFNYYWC

SEQ ID NO: 111

C-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C, wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) or Tyr (Y); X¹¹ is the D form of Trp (W), Gln (Q), Tyr, or His (H); and C denotes the D form of Cysteine.

SEQ ID NO: 112

C*-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C*, wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) and Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), and Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) and Tyr (Y); X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H); C denotes the D form of Cysteine; and * denotes an optional intramolecular bond.

SEQ ID NO: 113

C*-X²-[W/F/Y]-[Polar]-X⁵-X⁶-F-N-N-[W/Y]-W-C* (SEQ ID NO:3), wherein X² is the D form of any of the canonical L-amino acids other than Cys, X⁵ is the D form of any of the canonical L-amino acids other than cysteine, X⁶ is the D form of any of the canonical L-amino acids other than cysteine, Polar represents a D-amino acid comprising one of R, K, H, E, D, Q, N, T, S, P, A, or G, and the * indicate an optional intramolecular disulfide bond.

SEQ ID NO:114

X*TFQPRFNNWWC*, where * denotes an optional intramolecular disulfide bond and X is D-Penicillamine

SEQ ID NO:115

C*TFQPRFNNWX*, where * denotes an optional intramolecular disulfide bond and X is D-Penicillamine

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C-X²-X³-X⁴-X⁵-X⁶-F-N-N-X¹⁰-X¹¹-C (SEQ ID NO:1), wherein each of X¹ through X⁶, X¹⁰ and X¹¹ is a D-amino acid or a D- α -amino acid analog thereof; and

a. X² is the D form of Thr (T), Val (V), His (H), Leu (L), Gln (Q), Ala (A), Ile (I), Met (M), or Trp (W), or a D- α - amino acid analog thereof;

b. X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) and Leu (L), or a D- α -amino acid analog thereof;

c. X⁴ is a Polar amino acid comprising the D form of Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Lys, Ala (A), and Gly (G), or a D- α -amino acid analog thereof;

d. X⁵ is the D form of Pro (P), Trp (W), His (H), Gln (Q), Arg (R), Ala (A), Val (V), Leu (L), or Gly (G), or a D- α -amino acid analog thereof;

e. X⁶ is the D form of Arg (R), His (H), Lys (K), Glu (E), Gln (Q), Val (V), Leu (L), Ser (S), or Ala (A), or a D- α -amino acid analog thereof;

f. X¹⁰ is the D form of Trp (W) or Tyr (Y), or a D- α -amino acid analog thereof;

g. X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H), or a D- α -amino acid analog thereof; and

k. C denotes the D form of Cysteine, or a D- α -amino acid analog thereof; N denotes the D form of asparagine, or a D- α -amino acid analog thereof; and F denotes the D form of phenylalanine, or a D- α -amino acid analog thereof.

2. The D-peptide or a salt thereof of claim 1, wherein the core TNF α binding domain has the following amino acid sequence: C-X²-[W/F/Y]-X⁴-X⁵-X⁶-F-N-N-[W/Y]-W-C (SEQ ID NO:3).

3. The D-peptide or a salt thereof of any one of claims 1 to 2, further comprising an intramolecular disulfide bond between the cysteine residues 1 and 12 of the core TNF α binding domain.
4. The D-peptide or a salt thereof of any one of claims 1 to 3, wherein:
 - a. X² is the D form of Thr, Val, His, Leu, Gln, Ala, Ile, Met or Trp, or a D- α -amino acid analog thereof;
 - b. X² is the D form of Thr, Val, His, Leu, or Gln, or a D- α -amino acid analog thereof;
 - c. X² is the D form of Thr, Val, His or Leu, or a D- α -amino acid analog thereof;
 - d. X² is the D form of Thr, Val or His, or a D- α -amino acid analog thereof;
 - e. X² is the D form of Thr or Val, or a D- α -amino acid analog thereof;
 - f. X² is the D form of Thr, or a D- α -amino acid analog thereof; or
 - g. X² is the D form of Val, or a D- α -amino acid analog thereof.
5. The D-peptide or a salt thereof of any one of claims 1 or 3 to 4, wherein:
 - a. X³ is the D form of Trp, Phe, Tyr, or Ser, or a D- α -amino acid analog thereof;
 - b. X³ is the D form of Trp, Phe, or Tyr, or a D- α -amino acid analog thereof;
 - c. X³ is the D form of Trp or Phe, or a D- α -amino acid analog thereof;
 - d. X³ is the D form of Trp or a D- α -amino acid analog thereof; or
 - e. X³ is the D form of Phe, or a D- α -amino acid analog thereof.
6. The D-peptide or a salt thereof of any one of claims 1 to 5, wherein:
 - a. X⁴ is the D form of Arg, His, Gln, Asn, Lys, Thr, or Ser, or a D- α -amino acid analog thereof;
 - b. X⁴ is the D form of Arg, His, Gln or Asn, or a D- α -amino acid analog thereof;
 - c. X⁴ is the D form of Arg, His or Gln, or a D- α -amino acid analog thereof;

- d. X⁴ is the D forms of Arg, Gln or Asn, or a D- α -amino acid analog thereof;
- or
- e. X⁴ is the D form of Gln, or a D- α -amino acid analog thereof.
- 7. The D-peptide or a salt thereof of any one of claims 1 to 6, wherein:
 - a. X⁵ is the D form of Pro, Trp, His, Gln, Gly, Arg, Ala, Val or Leu, or a D- α -amino acid analog thereof;
 - b. X⁵ is the D form of Pro, Trp, His, Gln, Gly, Arg, or Val, or a D- α -amino acid analog thereof;
 - c. X⁵ is the D form of Pro, Trp or His, or a D- α -amino acid analog thereof;
 - d. X⁵ is the D form of Pro or Trp, or a D- α -amino acid analog thereof; or
 - e. X⁵ is the D form of Pro, or a D- α -amino acid analog thereof.
- 8. The D-peptide or a salt thereof of any one of claims 1 to 7, wherein:
 - a. X⁶ is the D-form of Arg, His, Lys, Glu, Gln, Val, Leu, Ser or Ala, or a D- α -amino acid analog thereof;
 - b. X⁶ is the D-form of Arg, His, Lys, Glu, Gln, Val or Leu, or a D- α -amino acid analog thereof;
 - c. X⁶ is the D-form of Arg, His, Lys, Glu, or Gln, or a D- α -amino acid analog thereof;
 - d. X⁶ is the D-forms of Arg, His, Lys, or Glu, or a D- α -amino acid analog thereof;
 - e. X⁶ is the D-form of Arg, Lys, or His, or a D- α -amino acid analog thereof;
 - f. X⁶ is the D-form of Arg, or a D- α -amino acid analog thereof;
 - g. X⁶ is the D-form of Lys, or a D- α -amino acid analog thereof, or
 - h. X⁶ is the D-form of His, or a D- α -amino acid analog thereof.
- 9. The D-peptide or a salt thereof of any one of claims 1 and 3 to 8, wherein:
 - a. X¹⁰ is the D form of Trp, or a D- α -amino acid analog thereof; or
 - b. X¹⁰ is the D form of Tyr, or a D- α -amino acid analog thereof.

10. The D-peptide or a salt thereof of any one of claims 1 and 3 to 9, wherein:
- X^{11} is the D form of His (H), Trp (W), Tyr (Y), or Gln (Q), or a D- α -amino acid analog thereof;
 - X^{11} is the D-form of Tyr (Y), or a D- α -amino acid analog thereof, or
 - X^{11} is the D form of Trp (W), or a D- α -amino acid analog thereof.
11. The D-peptide or a salt thereof of any one of claims 1 to 10, wherein each of X^1 through X^6 , X^{10} , and X^{11} is a D-amino acid.
12. The D-peptide or a salt thereof of any one of the claims 1 to 11, wherein the core TNF α binding domain has an amino acid sequence:
- CVWQPKFNYYWC (SEQ ID NO:4);
 - CVWQPRFNYYWC (SEQ ID NO:5);
 - CTFQPRFNYYWC (SEQ ID NO:6);
 - CTFQPRFNNWWC (SEQ ID NO:7);
 - CSFQPRFNYYWC (SEQ ID NO:8);
 - CSFQPRFNNWWC (SEQ ID NO:9);
 - CVFQPRFNYYWC (SEQ ID NO:10);
 - CVFQPRFNNWWC (SEQ ID NO:11);
 - CTFQWRFNYYWC (SEQ ID NO:12);
 - CLYQPVFNNWWC (SEQ ID NO:13);
 - CVFQAAFNYYWC (SEQ ID NO:14);
 - CVFQHNFNWWC (SEQ ID NO:15);
 - CHFNPRFNNWWC (SEQ ID NO:16);
 - CVWQPHFNYYWC (SEQ ID NO:17);
 - CVFQGRFNNWWC (SEQ ID NO:18);
 - CVFQHNFNWWC (SEQ ID NO:19);
 - CVFNPRFNNWWC (SEQ ID NO:20);
 - CVFKPRFNNWWC (SEQ ID NO:21);

- s. CAYQRQFNNWWC (SEQ ID NO:22);
- t. CWFEHRFNNWHC (SEQ ID NO:23);
- u. CHFQHRFNNWWC (SEQ ID NO:24);
- v. CHFQPRFNNWWC (SEQ ID NO:25);
- w. CTYQPRFNNWWC (SEQ ID NO:26);
- x. CQFQPRFNNWQC (SEQ ID NO:27); or
- y. CHFSQRFNNWWC (SEQ ID NO:28).

13. The D-peptide or a salt thereof of any of the preceding claims, wherein the core TNF α binding domain has an amino acid sequence set forth in SEQ ID NO:77 to 110.

14. The D-peptide or a salt thereof of any one of the preceding claims, further comprising a tag sequence attached to the N-terminus of the peptide.

15. The D-peptide or a salt thereof of claim 14, wherein the tag comprises the amino acid sequence D-Asp or D-AspAsp (DD).

16. The D-peptide or a salt thereof of any one of the preceding claims, further comprising a tag sequence attached to the C-terminus of the peptide.

17. The D-peptide or a salt thereof of claim 16, wherein the tag comprises the amino acid sequence D-GGEEEK (SEQ ID NO:30) or D-GGRRRK (SEQ ID NO:31).

18. The D-peptide or a salt thereof of any one of the preceding claims, wherein the N-terminus of the peptide comprises a cap.

19. The D-peptide or a salt thereof of claim 18, wherein the cap comprises an acetyl group or a protecting group.

20. The D-peptide or a salt thereof of any one of the preceding claims, wherein the C-terminus of the peptide comprises a cap.

21. The D-peptide or a salt thereof of claim 20, wherein the cap comprises an amide group or a protecting group.
22. The D-peptide or a salt thereof of any one of the preceding claims, further comprising a polyethylene glycol (PEG) group.
23. The D-peptide or a salt thereof of any one of the preceding claims, further comprising a Linker.
24. The D-peptide or a salt thereof of claim 23, wherein the Linker comprises a PEG group.
25. The D-peptide or a salt thereof of any one of claims 22 or 24, wherein the PEG group is attached to the N-terminus of the D-peptide.
26. The D-peptide or a salt thereof of any one of claims 22 or 24, wherein the PEG group is attached to the C-terminus of the D-peptide.
27. The D-peptide or a salt thereof of any one of claims 22 and 24 to 26, wherein each PEG group is a PEG group having from 1 to 48 subunits, 1 to 30 subunits, 1 to 24 subunits or 1 to 12 subunits.
28. The D-peptide or a salt thereof of claim 27, wherein each PEG group is a PEG group having from 6 subunits, 8 subunits, 10 subunits, or 12 subunits.
29. A multimer of the D-peptide of any one of the preceding claims, or a salt thereof.
30. The multimer or a salt thereof of claim 29, wherein the multimer is a dimer.
31. The multimer or a salt thereof of claim 29, wherein the multimer is a trimer.
32. The multimer or a salt thereof of any one of claims 29 to 31, further comprising a multimer scaffold attached to the D-peptides, optionally via a Linker.

33. The multimer or a salt thereof of claim 32, wherein the multimer scaffold is trimeric.

34. The multimer or a salt thereof of claim 32, wherein the multimer scaffold is a tetramer.

35. The multimer or a salt thereof of any one of claims 33 or 34, wherein the trifunctional cross-linker is tris(succinimidyl) aminotriacetate (TSAT), Tris-succinimidyl (6-aminocaproyl)aminotriacetate (LC-TSAT), an Fmoc scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)heptanedioate, an Fmoc scaffold having a PEG27 chain, a cyclohex scaffold (tris(2,5-dioxopyrrolidin-1-yl) cyclohexane-1,3,5-tricarboxylate), a nitro scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)-4-nitroheptane dioate), amine scaffold (bis(2,5-dioxopyrrolidin-1-yl) 4-amino-4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)heptanedioate), an amine-PEG27 scaffold, a cholesterol scaffold, a heterotetrameric PEG scaffold based on 3-{2-Amino-3-(2-carboxyethoxy)-2-[(2-carboxyethoxy)methyl]propoxy}propionic acid scaffold, a multimeric scaffold based on 4-amino-4-(2-carboxyethyl)heptanedioic acid, or 3-{2-Amino-3-(2-carboxyethoxy)-2-[(2-carboxyethoxy)methyl]propoxy}propionic acid.

36. The multimer or a salt thereof of claim 35, wherein the multimeric peptide construct has a structure of:

- a. Fmoc-[Peptide-PEG12-K-amide]₃;
- b. Fmoc-[Peptide-PEG4-K-amide]₃;
- c. Fmoc-[Peptide -PEG8-K-amide]₃;
- d. Fmoc-[Ac-K-PEG12-Peptide-amide]₃;
- e. [Peptide-PEG6-K-amide]₃-Fmoc;
- f. [Peptide-PEG12-K-amide]₃-PEG27-Fmoc;
- g. [Peptide-PEG12-K-amide]₃-Fmoc;
- h. [Peptide-PEG12-K-amide]₃-cyclohex;

- i. [Peptide-PEG12-K-amide]₃-nitro;
- j. [Peptide-PEG12-K-amide]₃-PEG27-amine;
- k. [Peptide-PEG12-K-amide]₃-amine;
- l. [Peptide-PEG12-K-amide]₃-PEG27-biotin; or
- m. [Peptide-PEG12-K-amide]₃-PEG27-cholesterol; and wherein Peptide is a D-peptide.

37. The D-peptide or a salt thereof of any one of claims 1 to 28 having an amino acid sequence of at least one of the following:

- a. DDCVWQPKFNYYWC (SEQ ID N:32);
- b. DGACVWQPKFNYYWC (SEQ ID NO:38);
- c. DDCVWQPRFNYYWC (SEQ ID NO:39);
- d. DDCVWQPRFNYYWCGGRRRK (SEQ ID NO:40);
- e. DCVWQPKFNYYWC (SEQ ID NO:56);
- f. DDDCVWQPRFNYYWC (SEQ ID NO:60);
- g. DDCTFQPRFNNWWC (SEQ ID NO:61);
- h. DDCVFQPRFNYYWC (SEQ ID NO:62);
- i. DDCSFQPRFNYYWC (SEQ ID NO:63); or
- j. DDCVFQPRFNNWWC (SEQ ID NO:64).

38. The multimer or a salt thereof of any one of claims 29 to 36, comprising D-peptides having an amino acid sequence of at least one of the following:

- a. DDCVWQPKFNYYWC (SEQ ID N:32);
- b. DGACVWQPKFNYYWC (SEQ ID NO:38);
- c. DDCVWQPRFNYYWC (SEQ ID NO:39);
- d. DDCVWQPRFNYYWCGGRRRK (SEQ ID NO:40);
- e. DCVWQPKFNYYWC (SEQ ID NO:56);
- f. DDDCVWQPRFNYYWC (SEQ ID NO:60);
- g. DDCTFQPRFNNWWC (SEQ ID NOL61);

- h. DDCVFQPRFNYYWC (SEQ ID NO:62);
- i. DDCSFQPRFNYYWC (SEQ ID NO:63); or
- j. DDCVFQPRFNNWWC (SEQ ID NO:64).

39. A pharmaceutical composition comprising at least one D-peptide or multimeric peptide construct, or pharmaceutically acceptable salt thereof, as set forth in any of the preceding claims, and at least one pharmaceutically acceptable excipient or carrier.

40. The pharmaceutical composition of claim 39, which is formulated for parenteral administration.

41. The pharmaceutical composition of claim 40, which is formulated for intravenous, intramuscular, or subcutaneous administration.

42. The pharmaceutical composition of claim 39, which is formulated for oral administration.

43. The pharmaceutical composition of claim 39, which is formulated for topical administration.

44. The pharmaceutical composition of claim 43, which is formulated for topical administration to the skin (dermal) or to the eye.

45. The pharmaceutical composition of claim 39, which is formulated for rectal administration.

46. A lyophilized composition comprising at least one D-peptide or multimeric peptide construct, or a pharmaceutically acceptable salt thereof, of any one of claims 1 to 38, and a stabilizing agent.

47. A lyophilized composition of the pharmaceutical composition of any one of claims 39 to 45 and a stabilizing agent.

48. A re-hydrated solution of the lyophilized composition of any one of claims 46 or 47.

49. A method of treating a TNF α -mediated disease, comprising administering an effective amount of the D-peptides of any of claims 1 to 28 or 37, the multimeric peptide construct of any one of claims 29 to 36 and 38 or the pharmaceutical composition of any one of claims 39 to 45, or pharmaceutically acceptable salt thereof.

50. The method of claim 49, wherein the TNF α -mediated disease is adult Crohn's Disease, juvenile Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile Idiopathic Arthritis, Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or Non-radiographic Spondyloarthritis.

51. The method of claim 50, wherein the TNF α -mediated disease is an inflammatory bowel disease.

52. The method of claim 51, wherein the inflammatory bowel disease is adult Crohn's Disease, pediatric Crohn's Disease, or Ulcerative Colitis.

53. The method of claim 51 or 52, wherein the administration is oral.

54. The method of claim 51 or 52, wherein the administration is rectal.

55. The method of claim 51 or 52, wherein the administration is parenteral.

56. The method of claim 49, wherein the TNF α -mediated disease is an inflammatory skin disease.

57. The method of claim 56, wherein the inflammatory skin disease is Plaque Psoriasis or Cutaneous Lupus.

58. The method of claim 56 or 57, wherein the administration is topical.

59. The method of claim 56 or 57, wherein the administration is parenteral.

60. The method of claim 49, wherein the TNF α -mediated disease is an inflammatory disease, wherein the inflammatory disease is Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile Idiopathic Arthritis, or Hidradenitis suppurativa.

61. The method of claim 60, wherein the administration is parenteral.

62. The method of claim 55, 59, or 61, wherein the parenteral administration is selected from intravenous, subcutaneous, and intramuscular.

63. A method of reducing TNF α -mediated inflammation, comprising administering a D-peptide or multimeric peptide construct thereof, or pharmaceutically acceptable salt thereof, of any one of claims 1 to 38, or a pharmaceutical composition of any one of claims 39 to 45, to a subject.

64. A method of inhibiting TNF α , comprising administering a D-peptide or multimeric peptide construct thereof, or pharmaceutically acceptable salt thereof, of any one of claims 1 to 38, or a pharmaceutical composition of any one of claims 39 to 45, to a subject.

65. A method of reducing an inflammatory response mediated by TNF α , comprising administering a D-peptide or multimeric peptide construct thereof, or pharmaceutically acceptable salt thereof, of any one of claims 1 to 38, or a pharmaceutical composition of any one of claims 39 to 45, to a subject.

66. The method of any one of claims 63 to 65, wherein the administering is by oral administration, by parenteral administration, by topical (dermal), or by rectal administration.

67. The method of any one of claims 63 to 65, wherein the D-peptide or a multimeric peptide construct thereof, or a pharmaceutically acceptable salt thereof is administered locally to reduce TNF α activity or inflammation or an inflammatory response.

68. The method of any one of claims 63 to 67, wherein the subject has a TNF α -mediated disease.

69. The method of claim 68, wherein the TNF α -mediated disease is adult Crohn's Disease, juvenile Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile Idiopathic Arthritis, Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or Non-radiographic Spondyloarthritis.

70. The D-peptide or a multimeric peptide construct thereof, or pharmaceutically acceptable salt thereof, according to any one of claims 1 to 38 for use as a medicament.

71. The D-peptide or a multimeric peptide construct thereof, or pharmaceutically acceptable salt thereof, according to any one of claims 1 to 38 for use in a method of treating a subject by therapy.

72. The D-peptide of claim 71, wherein the subject has a TNF α -mediated disease.

73. The D-peptide of claim 72, wherein the TNF α -mediated disease is adult Crohn's Disease, juvenile Crohn's Disease, Ulcerative Colitis, Plaque Psoriasis, Cutaneous Lupus, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis, or Juvenile Idiopathic Arthritis, Hidradenitis suppurativa, Uveitis (intermediate, posterior, or panuveitis), or Non-radiographic Spondyloarthritis.

74. A D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C-X²-

X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C (SEQ ID NO:111), wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) or Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), or Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) or Tyr (Y); X¹¹ is the D form of Trp (W), Gln (Q), Tyr, or His (H); and C denotes the D form of Cysteine.

75. The D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C*-X²-X³-X⁴-X⁵-X⁶-X⁷-X⁸-X⁹-X¹⁰-X¹¹-C* (SEQ ID NO:112), wherein each of X² through X¹¹ is a D-amino acid; and X² is the D form of any of the canonical L-amino acids other than cysteine; X³ is the D form of Trp (W), Phe (F), Tyr (Y), Ser (S), His (H) and Leu (L); X⁴ is a Polar amino acid comprising Arg (R), Lys (K), His (H), Glu (E), Asp (D), Gln (Q), Asn (N), Thr (T), Ser (S), Pro (P), Ala (A), and Gly (G); X⁵ is the D form of any of the canonical L-amino acids other than cysteine; X⁶ is the D form of any of the canonical L-amino acids other than cysteine; X⁷ is the D-form of Phe (F); X⁸ is the D form of Asn (N), X⁹ is the D form of Asn (N), X¹⁰ is the D form of Trp (W) and Tyr (Y); X¹¹ is the D form of Trp (W), Tyr (Y), Gln (Q) or His (H); C denotes the D form of Cysteine; and * denotes an optional intramolecular bond.

76. A D-peptide or a salt thereof, wherein the peptide comprises a core TNF α binding domain of D-amino acids and having the following amino acid sequence, C*-X²-[W/F/Y]-[Polar]-X⁵-X⁶-F-N-N-[W/Y]-W-C* (SEQ ID NO:113), wherein X² is the D form of any of the canonical L-amino acids other than Cys, X⁵ is the D form of any of the canonical L-amino acids other than cysteine, X⁶ is the D form of any of the canonical L-amino acids other than cysteine, Polar represents a D-amino acid comprising one of R,

K, H, E, D, Q, N, T, S, P, A, or G, and the * indicate an optional intramolecular disulfide bond.