[0005] IL-2 can induce the expansion of T cells to enhance adoptive immunotherapy and is approved by the FDA for the treatment of melanoma and renal cell carcinoma, with complete remission in a subset of patients. However, IL-2 can also promote pathologic responses, and a therapeutic goal is to maintain the desired actions of this cytokine while blocking untoward deleterious responses.

[0006] We have previously used the structure of the high-affinity IL-2-IL-2R complex to develop IL-2 "superkines" with augmented action due to enhanced binding affinity for IL-2Rβ, which eliminates the functional requirement for IL-2Rα. The super-IL-2 platform was also used to generate variants that retain increased binding affinity for IL-2Rβ but that exhibited decreased binding to γc and thereby have defective IL-2Rβ-γc heterodimerization and signaling.

[0007] Continued refinement and development of human IL-2 signaling variants is of great interest for clinical purposes, including the treatment of cancer and autoimmune diseases. Variant proteins for this purpose are provided herein.

SUMMARY

[0008] Variant IL-2 proteins and uses thereof are provided. In some ~~embodiment~~examples, the IL-2 variant proteins have greater potency for activation of IL-2 signaling pathways in cells lacking CD25 expression, relative to wild-type IL-2. Such proteins may be referred to herein as IL-2 superagonists. The EC50 for a superagonist on CD25- cells may be reduced at least two-fold relative to the wild-type protein, at least 3-fold relative to the wild-type protein, at least 4-fold relative to the wild-type protein, at least 5-fold relative to the wild-type protein, for example an EC50 of less than about 2 nM, less than about 1.5 nM, less than about 1 nM, less than about 0.5 nM, etc.

[0009] In one ~~embodiment~~example, provided herein is an IL-2 superagonist variant having increased binding affinity for CD122 relative to wild-type IL-2, where the binding affinity has a Kd less than about 10-9, less than about 10-10, or less that about 10-12. Such variants are exemplified, for example, by the variants of SEQ ID NO: 1, SEQ ID NO: 7 and SEQ ID NO: 8 or alternatively in revertant sequences in which the residues at positions 69 and 115 are reverted back to wild-type valine residues. Other variants have a greater potency for activation of IL-2 signaling pathways in cells lacking CD25 expression, but do not have a substantially increased binding affinity relative to the wild type IL-2 protein. Such variants are exemplified, for example, by the variants of SEQ ID NO: 6, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 or alternatively in revertant sequences in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0010] In other ~~embodiment~~examples, the variant IL-2 proteins have greater selectivity for CD25 expressing cells, and have reduced potency for activation of IL-2 signaling pathways in cells lacking CD25 expression, relative to wild-type IL-2. Such proteins may be referred to herein as CD25 selective agonists.

[0011] IL-2 variants find use, for example, as IL-2 selective agonists and superagonists in applications where modulation of IL-2 signaling is desirable, for example by upregulation of IL-2 signaling for treatment of cancer; and for select activation of IL-2 signaling in autoimmune diseases, and the like. Also provided are nucleic acids encoding such IL-2 variants, methods of making such IL-2 variants, pharmaceutical compositions that include such IL-2 variants and methods of treatment using such IL-2 variants.

[0012] In some ~~embodiment~~examples, the IL-2 variant comprises one or more amino acid substitutions that increase IL-2 potency in CD25- cells, selected from substitutions at amino acid positions 27, 28, 31, 32, 35, 39, 52, 72, 74, 75, a region comprising residues 74-80, 85, 86, and 92, numbered in accordance with wild-type hIL-2.

[0013] In some ~~embodiment~~examples the IL-2 variant comprises one or more of the amino acid changes: G27M; I28L; Y31A; K32D/E/S; K35S; M39L or alternatively M39I; E52S or alternatively E52D, E52N, E52T; L72A/Q/D/L/H/T; loop: GDDPKTI, DSTDETV, DSTDERI, DSTDSRI, SKDQDKV, ADDKDTI, ADDQDKI, AQSKNFHL,SKDQKKV, SDDQDKV; L80V/I; R81Δ; P82Δ; L85V or alternatively L85M or L85A; I86V; and I92F.

[0014] In one ~~embodiment~~example, the IL-2 variant having a greater binding affinity for CD122 as compared to wild-type human IL-2, comprises the amino acid changes: G27M; I28L; K32D; M39L; E52S; V69A; L72A/Q/D/L/H/T; loop: GDDPKTI; L80I; R81Δ; P82Δ; L85V; I86V; I92F; and V115I, for example as set forth in SEQ ID NO: 1 or alternatively in revertant sequence of SEQ ID NO: 1 in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0015] In one ~~embodiment~~example, the IL-2 variant having a greater binding affinity for CD122 as compared to wild-type human IL-2 comprises the amino acid changes: G27M; I28L; K32D; V69A; L72Q; loop: DSTDETV; L80V; R81Δ; P82Δ; and V115I, for example as set forth in SEQ ID NO: 7 or alternatively in revertant sequence of SEQ ID NO: 7 in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0016] In one ~~embodiment~~example, the IL-2 variant having a greater binding affinity for CD122 as compared to wild-type human IL-2, comprises the amino acid changes: G27M; I28L; K32D; M39L; E52S; V69A; L72A; loop: DSTDERI; L80I; R81Δ; P82Δ; L85V; I86V; I92F; and V115I,

for example as set forth in SEQ ID NO: 8 or alternatively in revertant sequence of SEQ ID NO: 8 in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0017] In some ~~embodiment~~examples, the IL-2 variant is CD25 selective, and comprises one or more of the amino acid changes: G27M; I28L; K32E; E52S; V69A; L72Q; loop: GDDPKTI; L80I; R81Δ; P82Δ; and V115I, for example as set forth in SEQ ID NO: 6 or alternatively in revertant sequence of SEQ ID NO: 6 in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0018] In some ~~embodiment~~examples, the IL-2 variant has greater potency on CD25- cells in the absence of greater binding affinity for CD122 and comprises one or more of the amino acid changes: G27M; I28L; Y31A; K32D/E/S; K35S; M39L; E52S; V69A; L72A/Q/D/L/H/T; loop: AQSKNFHL; and V115I, for example as set forth in SEQ ID NO: 15 or alternatively in revertant sequence of SEQ ID NO: 15 in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0019] In some ~~embodiment~~examples, the IL-2 variant has greater potency on CD25- cells in the absence of greater binding affinity for CD122 and comprises one or more of the amino acid changes: G27M; I28L; K32D; M39L; E52S; V69A; L72Q; loop: AQSKNFHL; and V115I, for example as set forth in SEQ ID NO: 16 or alternatively in revertant sequence of SEQ ID NO: 16 in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0020] In some ~~embodiment~~examples, the IL-2 variant has greater potency on CD25- cells in the absence of greater binding affinity for CD122 and comprises one or more of the amino acid changes: G27M; I28L; K32D; M39L; E52S; V69A; loop: AQSKNFHL; and V115I, for example as set forth in SEQ ID NO: 17 or alternatively in revertant sequence of SEQ ID NO: 17 in which the residues at positions 69 and 115 are reverted back to wild-type valine residues.

[0021] In certain ~~embodiment~~examples, the subject IL-2 variant has an enhanced capability to stimulate STAT5 phosphorylation in a CD122+ T cell as compared to wild-type hIL-2. In some ~~embodiment~~examples, the T cell is a CD8+ T cell. In some ~~embodiment~~examples, the subject IL-2 variant has an enhanced capability to stimulate the pERK1/ERK2 signaling in CD122+ T cell as compared to wild-type hIL-2.

[0022] In some ~~embodiment~~examples the amino acid substitutions described herein are further combined with amino acid substitutions F42A, E61R, R38D, Y45A, or combinations thereof that introduce mutations that affect CD25 binding, e.g. see Thanos et al. (2006) PNAS 103(42):15422-7 and Vazquez-Lombardi et al. (2017) 8:15373.

[0023] In another ~~aspect~~example, provided herein is an IL-2 variant fusion protein comprising any one of the IL-2 variants described herein linked to a human Fc antibody fragment.

[0024] In another ~~aspect~~example, provided herein is a pharmaceutical composition comprising any one of the IL-2 variants or the IL-2 variant fusion protein described herein and a pharmaceutically acceptable carrier.

[0025] In some ~~embodiment~~examples, methods are provided for the use of an IL-2 variant provided herein for the treatment of disease. The superagonist IL-2 proteins induce a superior expansion of cytotoxic T cells, leading to improved responses in vivo with less expansion of T regulatory cells and reduced pulmonary edema. Treatment modalities include, without limitation, treatment of cancer, treatment of immunodeficiency, treatment of infectious disease (particularly chronic or difficult to treat infections), use as a vaccine adjuvant, and the like. In such ~~embodiment~~examples, an effective dose of the IL-2 variant is administered to an individual.

[0026] In some ~~embodiment~~examples, methods are provided for the in silico design of cytokine ligands based on crystallographic structures. The design principles are based on the topology of the ligand-receptor interaction. By preferentially stabilizing a cytokine according to its high resolution crystal structure with the receptor, the cytokine can be turned into superagonists. The method identifies locations where packing can be improved in order to increase binding. Core positions are subjected to redesign and tested in a computer model to identify positions where improvements can be introduced. The resulting sequences are analyzed and compared. The computer model is then used to generate backbone structure ensembles while allowing the entire core to redesign while monitoring the residue changes in the identified regions. A subset of residues that improve packing are visually selected. The identities of the subset are held and the redesign performed iteratively until the sequences converge. On identification of candidate sequences, the coding sequence can be generated and expressed for testing.

[0027] In some ~~embodiment~~examples, the present disclosure provides a variant human IL-2 protein comprising one or more amino acid changes. In some ~~embodiment~~examples, the present disclosure provides a variant human IL-2 protein having greater potency for activation of IL-2 signaling pathways in cells lacking CD25 expression relative to wild-type IL-2, comprising one or more amino acid substitutions relative to wild-type human IL-2.

[0028] In some ~~embodiment~~examples, the one or more amino acid changes is selected from the group consisting of: an amino acid substitution to methionine at position 27 (G27M), an amino acid substitution at position 28, an amino acid substitution to alanine at position 31 (Y31A), an amino acid substitution at position 32, an amino acid substitution to serine at position 35 (K35S), an amino acid substitution at position 39, an amino acid substitution at position 52, an amino acid deletion at position 82 (P82Δ), and an amino acid substitution to isoleucine at position 115

(V115I), relative to the amino acid sequence of wild-type human IL-2 protein represented by SEQ ID NO: 19.

[0028a] In one aspect, the present disclosure provides a variant human IL-2 protein comprising one or more amino acid changes, wherein the one or more amino acid changes is selected from the group consisting of:
(i) an amino acid substitution to methionine at position 27 (G27M);
(ii) an amino acid substitution at position 28;
(iii) an amino acid substitution to alanine at position 31 (Y31A);
(iv) an amino acid substitution at position 32 selected from the group consisting of a substitution to aspartic acid (K32D), a substitution to glutamic acid (K32E), and a substitution to serine (K32S);
(v) an amino acid substitution to serine at position 35 (K35S);
(vi) an amino acid deletion at position 82 (P82Δ); and
(vii) an amino acid substitution to isoleucine at position 115 (V115I),
relative to the amino acid sequence of wild-type human IL-2 protein represented by SEQ ID NO: 19.

[0029] In some ~~embodiment~~examples, the one or more amino acid substitutions relative to wild-type human IL-2 is selected from the group consisting of: an amino acid substitution to methionine at position 27 (G27M), an amino acid substitution at position 28, an amino acid substitution to alanine at position 31 (Y31A), an amino acid substitution at position 32, an amino acid substitution to serine at position 35 (K35S), an amino acid substitution at position 39, an amino acid substitution at position 52, and an amino acid substitution to isoleucine at position 115 (V115I).

[0030] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid deletion at position 81 (R81Δ) or a substitution at position 81.

[0031] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises one or more amino acid substitutions at positions selected from the group consisting of: 69, 72, a region encompassing amino acid residues 74-80, 85, 86, and 92.

[0032] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 28. In some ~~embodiment~~examples, the amino acid substitution at position 28 comprises a substitution to leucine (I28L).

[0033] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 32. In some ~~embodiment~~examples, the amino acid substitution at position 32 is selected from the group consisting of: a substitution to aspartic acid (K32D), a substitution to glutamic acid (K32E), and a substitution to serine (K32S).

[0034] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 39. In some ~~embodiment~~examples, the amino acid substitution at position 39 is selected from the group consisting of: a substitution to leucine (M39L) and a substitution to isoleucine (M39I).

[0035] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 52. In some ~~embodiment~~examples, the amino acid substitution at position 52 is selected from the group consisting of: a substitution to serine (E52S), a substitution to aspartic acid (E52D), a substitution to asparagine (E52N), and a substitution to threonine (E52T).

[0036] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 69. In some ~~embodiment~~examples, the amino acid substitution at position 69 comprises a substitution to alanine (V69A).

[0037] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 72. In some ~~embodiment~~examples, the amino acid substitution at

position 72 is selected from the group consisting of: a substitution to alanine (L72A), a substitution to glutamine (L72Q), a substitution to aspartic acid (L72D), a substitution to histidine (L72H), and a substitution to threonine (L72T).

[0038] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 81. In some ~~embodiment~~examples, the amino acid substitution at position 81 comprises a substitution to aspartic acid (R81D).

[0039] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 85. In some ~~embodiment~~examples, the amino acid substitution at position 85 is selected from the group consisting of: a substitution to valine (L85V), a substitution to methionine (L85M), and a substitution to alanine (L85A).

[0040] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 86. In some ~~embodiment~~examples, the amino acid substitution at position 86 comprises a substitution to valine (I86V).

[0041] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at position 92. In some ~~embodiment~~examples, the amino acid substitution at position 92 comprises a substitution to phenylalanine (I92F).

[0042] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises an amino acid substitution at the region encompassing amino acid residues 74-80. In some ~~embodiment~~examples, the amino acid substitution at the region encompassing amino acid residues 74-80 comprises an amino acid sequence of GDDPKTI, DSTDETV, DSTDERI, DSTDSRI, SKDQDKV, SKDQKKV, SDDQDKV, ADDKDTI, or ADDQDKI.

[0043] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes G27M, I28L, K32D, M39L, E52S, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

[0044] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, L85V, and V115I.

[0045] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and (V115I).

[0046] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D,

M39L, E52S, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

[0047] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

[0048] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72D, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

[0049] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32E, E52S, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

[0050] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with DSTDETV, R81Δ, P82Δ, and V115I.

[0051] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with DSTDERI, R81Δ, P82Δ, L85V, I86V, I92F, and V115I.

[0052] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with DSTDSRI, R81Δ, P82Δ, and V115I.

[0053] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, substitution of the region encompassing amino acid residues 74-80 with SKDQKKV, R81Δ, P82Δ, L85V, I86V, I92F, and V115I.

[0054] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32S, M39L, E52S, V69A, substitution of the region encompassing amino acid residues 74-80 with SDDQDKV, R81Δ, P82Δ, L85V, I86V, I92F, and V115I.

[0055] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, L72H, substitution of the region encompassing amino acid residues 74-80 with ADDKDTI, R81Δ, P82Δ, and V115I.

[0056] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, Y31A, K32S, K35S, V69A, L72T, substitution of the region encompassing amino acid residues 74-80 with ADDQDKI, R81Δ, P82Δ, and V115I.

[0057] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, R81D, and V115I.

[0058] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, L72Q, R81D, and V115I.

[0059] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, R81D, and V115I.

[0060] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72Q, R81D, and V115I.

[0061] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure comprises amino acid changes, wherein the amino acid changes consist of G27M, I28L, K32D, L72Q, and R81D.

[0062] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure has a greater potency for activation of IL-2 signaling pathways in cells lacking CD25 expression. In some ~~embodiment~~examples, the variant IL-2 protein has an EC50 for activation of IL-2 signaling pathways in cells lacking CD25 expression at least two-fold lower than the wild-type protein. In some ~~embodiment~~examples, the variant IL-2 protein has increased binding affinity for human CD122. In some ~~embodiment~~examples, the variant IL-2 protein has a greater potency for activation of IL-2 signaling pathways in cells lacking CD25 expression, but does not have a substantially increased binding affinity relative to the wild type IL-2 protein.

[0063] In some ~~embodiment~~examples, the variant human IL-2 protein of the present disclosure is fused to a human IgG Fc domain. In some ~~embodiment~~examples, the IgG Fc domain is an IgG1, IgG2, IgG3, or IgG4 Fc domain.

[0064] In ~~some embodiments~~one aspect, the present disclosure provides a nucleic acid encoding a variant human IL-2 protein of the present disclosure. In some ~~embodiment~~examples, the present disclosure provides a vector comprising a nucleic acid, wherein the nucleic acid encodes a variant human IL-2 protein of the present disclosure. In one aspect, the present disclosure provides a vector comprising a nucleic acid encoding the variant human IL-2 protein of the present disclosure. In some ~~embodiment~~examples, the present disclosure provides a recombinant cell comprising a nucleic acid, wherein the nucleic acid encodes a variant human IL-2 protein of the present disclosure. In some ~~embodiment~~examples, the present disclosure provides a recombinant cell comprising a vector disclosed herein. In one aspect, the present disclosure provides a recombinant cell comprising the nucleic acid of the present disclosure or the vector of the present disclosure.

[0065] In ~~some embodiments~~one aspect, the present disclosure provides a pharmaceutical composition comprising a variant human IL-2 protein as disclosed herein, and a pharmaceutically acceptable carrier.

[0065a] In one aspect, the present disclosure refers to a pharmaceutical composition of the present disclosure for use in therapy.

[0066] In one aspect, the present disclosure provides use of the pharmaceutical composition of the present disclosure in the manufacture of a medicament for treating a disease in a subject in need thereof. In some ~~embodiment~~examples, the present disclosure provides a method of treating disease in a subject in need thereof, the method comprising administering to the patient a variant human IL-2 protein disclosed herein, or a pharmaceutical composition disclosed herein.

[0067] In one aspect, the present disclosure provides use of the pharmaceutical composition of the present disclosure in the manufacture of a medicament for treating a disease in a subject in need of expansion of T cells. In some ~~embodiment~~examples, the present disclosure provides a method of treating a disease in need of expansion of T cells, the method comprising administering an effective amount of a variant human IL-2 protein disclosed herein or a pharmaceutical composition disclosed herein. In some ~~embodiment~~examples, the disease is a hyperproliferative disease. In some ~~embodiment~~examples, the disease is cancer.

[0068] In ~~some embodiments~~one aspect, the present disclosure provides a method for in silico design of a cytokine based on crystallographic topology of the ligand receptor interaction, the method comprising: identifying locations where packing can be improved in order to increase binding; redesigning core positions and testing in a computer model to identify positions where improvements can be introduced: generating backbone structure ensembles while allowing the entire core to redesign while monitoring the residue changes in the identified regions; visually selecting a subset of resides that improve packing; holding identities of the subset and iteratively performing the redesign until the sequences converge; generating a coding sequence for the in silico designed cytokine.

BRIEF DESCRIPTION OF THE DRAWINGS

[0069] FIG. 1A illustrates exemplary IL-2 variants expressed as fusion proteins to mouse serum albumin (MSA). FIG. 1B shows a representative gel filtration elution profile of MSA-IL-2 wild-type (WT) fusion protein. FIG. 1C shows a representative gel filtration elution profile of MSA-H9-superagonist IL-2 (super-2) fusion protein. FIG. 1D shows a representative gel filtration elution profile of IL-2 variant protein SEQ ID NO: 3 (variant seq-3). FIG. 1E shows a Coomassie-