

## **SUMMARY**

The present disclosure is directed to novel fusion proteins comprising a portion of an immunoglobulin (Ig) molecule, compositions thereof, and methods for making and using the disclosed fusion proteins. The disclosed fusion proteins are useful for extending the serum half-life of bioactive molecules in an organism.

In one example, the present disclosure describes a fusion protein comprising: a) a bioactive molecule selected from the group consisting of erythropoietin, Factor IX, IFN $\alpha$ , GCSF, and IFN $\beta$ ; and b) a single chain Fc (sFc) fragment of an IgG molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 62, 63, and 64. In one aspect, the present disclosure refers to a fusion protein comprising: a) a bioactive molecule selected from the group consisting of erythropoietin, Factor IX, IFN $\alpha$ , GCSF, and IFN $\beta$ ; and b) a single chain Fc (sFc) fragment of an IgG molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 62, 63, and 64, and c) a hinge region between the bioactive molecule and the sFc fragment, wherein the hinge region is mutated and does not form disulfide bonds so that the fusion protein is a monomer that is incapable of forming a dimer.

In another example, the present disclosure describes a fusion protein comprising: a) a single chain Fc fragment (sFc) of an IgG molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 62, 63, and 64; b) a hinge region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-60; and c) a bioactive molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 65, 67, 69, 71, and 73. In another aspect, the present disclosure refers to a fusion protein comprising: a) a single chain Fc fragment (sFc) of an IgG molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 62, 63, and 64; b) a hinge region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-60, wherein the hinge region does not form disulfide bonds; and c) a bioactive molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 65, 67, 69, 71, and 73, wherein the fusion protein is a monomer that is incapable of forming a dimer.

One example of the present disclosure relates to a fusion protein, hybrid, conjugate and compositions thereof. The fusion protein of the present disclosure generally comprises (a) bioactive molecule and (b) a portion of a constant heavy region (C<sub>H</sub>) derived from an Ig molecule (the Ig fragment). The Ig fragment can include any portion of the constant heavy region, including one or more constant heavy domains, a hinge region, an Fc region, and/or combinations thereof. The amino acid sequence of the (a) bioactive molecule and (b) Ig fragment of the fusion

protein are derived from the wild-type sequence of each fragment, as disclosed in the art. Derived sequences include the wild-type sequence as well as homologues, analogues, fragments, and other variants of the wild-type sequence.

In certain examples, the Ig fragment of the fusion protein comprises a single chain Fc (sFc or scFc), a monomer, that is incapable of forming a dimer. In some examples, the fusion protein includes a sequence corresponding to an immunoglobulin hinge region. In various examples, the hinge region contains a modification that prevents the fusion protein from forming a disulfide bond with another fusion protein or another immunoglobulin molecule. In some examples, the hinge region is modified by mutating and/or deleting one or more cysteine amino acids to prevent the formation of a disulfide bond. In some examples, the bioactive molecule is linked to the scFc through a hinge region. In specific examples, the fusion protein comprises the bioactive molecule at its N-terminus that is linked to a scFc through a mutated hinge region. In another example, the present disclosure relates to compositions, including pharmaceutical compositions, comprising the fusion protein and a pharmaceutically acceptable carrier or excipient. In yet another aspect, the present disclosure provides for a pharmaceutical composition comprising the fusion protein as disclosed herein and a pharmaceutically acceptable carrier or excipient.

Another example of the present disclosure relates to methods for making and using a fusion protein and compositions thereof. In yet another aspect, the present disclosure provides for a method for producing a fusion protein comprising: a) providing a bioactive molecule and an sFc fragment comprising a hinge region, b) mutating the hinge region by amino acid substitution and/or deletion to form a mutated sFc, wherein the hinge region does not form disulfide bonds, and c) combining the bioactive molecule and the mutated sFc. In some examples, the method for making the fusion protein comprises (i) providing a bioactive molecule, an Fc fragment, and a hinge region, (ii) modifying the hinge region to prevent it from forming a disulfide bond, and (iii) linking the bioactive molecule to the Fc fragment through the mutated hinge region to form the fusion protein, hybrid, conjugate, or composition thereof. The present disclosure also provides a method for purifying the fusion protein, comprising (i) providing a fusion protein, and (ii) purifying the fusion protein by Protein A or Protein G-based chromatography media.

Detailed description of the invention is given in the following embodiments with reference to the accompanying drawings.

**CLAIMS**

1. A fusion protein comprising:
  - a) a bioactive molecule selected from the group consisting of erythropoietin, Factor IX, IFN $\alpha$ , GCSF, and IFN $\beta$ ;
  - b) a single chain Fc (sFc) fragment of an IgG molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 62, 63, and 64, and
  - c) a hinge region between the bioactive molecule and the sFc fragment, wherein the hinge region is mutated and does not form disulfide bonds so that the fusion protein is a monomer that is incapable of forming a dimer.
2. The fusion protein according to claim 1, wherein the hinge region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-60.
3. The fusion protein according to claim 1, wherein the hinge region comprises an amino acid sequence of SEQ ID NO: 23 or 27.
4. The fusion protein according to claim 1, wherein the bioactive molecule has an amino acid sequence selected from the group consisting of SEQ ID NOs: 65, 67, 69, 71, and 73.
5. The fusion protein according to claim 1, wherein the bioactive molecule is erythropoietin.
6. The fusion protein according to claim 1, wherein the bioactive molecule is linked to the sFc fragment through a mutated hinge region.
7. The fusion protein according to claim 1, wherein the amino acid sequence of the fusion protein is selected from the group consisting of SEQ ID NOs: 66, 68, 70, 72, and 74.
8. A pharmaceutical composition comprising the fusion protein according to claim 1 and a pharmaceutically acceptable carrier or excipient.
9. A method for producing a fusion protein comprising:
  - a) providing a bioactive molecule and an sFc fragment comprising a hinge region,
  - b) mutating the hinge region by amino acid substitution and/or deletion to form a mutated sFc, wherein the hinge region does not form disulfide bonds, and

- c) combining the bioactive molecule and the mutated sFc,  
wherein the fusion protein is a monomer that is incapable of forming a dimer.
10. The method according to claim 9, wherein the hinge region is mutated by substitution and/or deletion of a cysteine residue.
11. The method according to claim 9, wherein the bioactive molecule is combined with the mutated sFc through the hinge region.
12. The method according to claim 9, wherein the bioactive molecule is a cytokine, a growth factor, a hormone, or a functional portion thereof.
13. The method according to claim 9, wherein the bioactive molecule is erythropoietin, Factor IX, IFN $\alpha$ , GCSF, or IFN $\beta$ .
14. A fusion protein comprising:
- a) a single chain Fc fragment (sFc) of an IgG molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 62, 63, and 64;
  - b) a hinge region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-60, wherein the hinge region does not form disulfide bonds; and
  - c) a bioactive molecule having an amino acid sequence selected from the group consisting of SEQ ID NOs: 65, 67, 69, 71, and 73,
- wherein the fusion protein is a monomer that is incapable of forming a dimer.
15. The fusion protein according to claim 14, wherein the amino acid sequence of the hinge region is SEQ ID NO: 23 or 27.
16. The fusion protein according to claim 14, wherein the amino acid sequence of the fusion protein is selected from the group consisting of SEQ ID NOs: 66, 68, 70, 72, and 74.