# Methods for producing molecules

This application claims priority from EP24165466.4 filed 22 March 2024, the contents and elements of which are herein incorporated by reference for all purposes.

## Technical Field

The present disclosure relates to the fields of molecular biology and recombinant polypeptide production technology.

## Background

Therapeutic pharmaceuticals often display microheterogeneities, necessitating detailed characterization to determine their impact on drug efficacy, safety, and the establishment of analytical control strategies to maintain consistent product quality (Word Health Organisation: WHO Guideline for the safe production and quality control of monoclonal antibodies for use in humans). In particular, polypeptides expression from cells in culture comprise enzymatic and abiotic, chemical-driven process-induced micro heterogeneities as a consequence of post-translational modifications, such as N- and O-type glycosylations, cysteine modifications, galactosylations, carbonylations, oxidations, glycation, aspartate isomerizations, and C-terminal lysine variations (Geist *et al.*, MAbs (2013) 5(1):150-161; Luo *et al.* Biotechnol Bioeng. (2012) 109(9):2306-2315; Raju *et al.*, MAbs (2012) 4(3):385-391; Gramer *et al.*, Adv Biochem Eng Biot. (2014) 139:123-166).

Lysine is one of the most chemically-reactive amino acids in biology and specialized cellular enzymes ensure spatial and temporal regulation of lysine modifications (Abbasov *et al.* Nat Chem. (2021) 13(11):1081-1092). Hydroxylation of lysine to hydroxylysine (HyL) is a well-known molecular modification of endogenous proteins, and is often required for essential downstream regulatory events and structural organizations (Markolovic *et al.*, Nat Chem Biol. (2018) 14(7):688-695; Zurlo *et al.*, Biochim Biophys Acta. (2016) 1866(2):208-220; Cockman *et al.*, Proc Natl Acad Sci U S A. (2022) 119(32):e2201483119).

Lysine hydroxylation modifications have been identified in recombinantly-expressed tissue plasminogen activator (rtPA), soluble and chimeric CD4 receptor variants, the De13a toxin of a marine cone snail, Somastostatin, and in IgG1 mAbs (Molony *et al.*, Tech Prot Chem. (1995) 6:91-98; Aguilar *et al.*, Biochemistry. (2005) 44(33):11130-11136; Andrews *et al.*, J Biol Chem. (1984) 259(24):5021-5024; Xie *et al.*, MAbs. (2016) 8(2): 371-378). Hydroxylation in the recombinant IgG1 mAb has been identified by a +16 Da mass shift, and is comparable to the other proteins comprising hydroxylation of the lysine of a Xaa-Lys-Gly (XKG; SEQ ID NO:2) consensus motif (Xie *et al.*, MAbs. (2016) 8(2): 371-378).

The presence of a HyL in proteins alters their chemicophysical properties, as the additional hydroxyl group can effect local changes in hydrophobicity and charge, as well as act as accessible active group for subsequent modifications like glycosylation (reviewed *e.g.* in de Giorgi *et al.*, Biochem Soc Trans. (2021) 49(2):855-866). Such alterations have the potential to act as signaling modulators in biological systems and may induce undesirable complexity in the technical development and production of therapeutic proteins. Moreover, since HyL residues are utilized for cross-linking in collagens, accidental aggregate

and/or neoepitope formation of HyL of modified therapeutic proteins could affect the stability and functionality of the protein, *e.g.* by modulating target protein binding efficacy and possibly also immunogenicity in recipient subjects. In addition, HyL can be targeted by lysyl oxidases to form hydroxyallysine by oxidative deamination, ammonia and the strong oxidizing reagent $H_2O_2$, or serve as motif for downstream O-glycosylation events (Abbasov *et al.* Nat Chem. (2021) 13(11):1081-1092).

WO 2023/232961 A1 describes an approach to reduce the level of HyL in polypeptides expressed from cells in culture, via genetic modification to prevent expression of PLOD enzymes, having lysyl hydroxylase activity. There remains a need in the art for techniques to control the hydroxylation of lysine residues in expressed polypeptides.

**Summary**

In a first aspect, the present disclosure provides a method for producing a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation, and/or including a proline residue susceptible to hydroxylation, wherein the method comprises culturing cells comprising nucleic acid for expressing the polypeptide for the majority of the period of culture in cell culture medium having an Fe ion concentration of less than 60 µM (*e.g.* less than 45 µM, *e.g.* less than 30 µM, *e.g.* less than 15 µM).

In some embodiments, the method comprises seeding the cells in cell culture medium comprising an Fe ion concentration of less than 60 µM (*e.g.* less than 45 µM, *e.g.* less than 30 µM, *e.g.* less than 15 µM).

In some embodiments, the method comprises culturing cells comprising nucleic acid for expressing the polypeptide in cell culture medium comprising an Fe ion concentration of less than 45 µM, for the majority of the period of culture. In some embodiments, the method comprises culturing cells comprising nucleic acid for expressing the polypeptide in cell culture medium comprising an Fe ion concentration of less than 30 µM, for the majority of the period of culture. In some embodiments, the method comprises culturing cells comprising nucleic acid for expressing the polypeptide in cell culture medium comprising an Fe ion concentration of less than 15 µM, for the majority of the period of culture. In some embodiments, the method comprises culturing cells comprising nucleic acid for expressing the polypeptide in cell culture medium comprising an Fe ion concentration of less than 10 µM, for the majority of the period of culture.

In some embodiments, the method comprises seeding the cells in cell culture medium comprising an Fe ion concentration of less than 45 µM. In some embodiments, the method comprises seeding the cells in cell culture medium comprising an Fe ion concentration of less than 30 µM. In some embodiments, the method comprises seeding the cells in cell culture medium comprising an Fe ion concentration of less than 15 µM. In some embodiments, the method comprises seeding the cells in cell culture medium comprising an Fe ion concentration of less than 10 µM.

In some embodiments, the polypeptide comprises an amino acid sequence including a lysine residue susceptible to hydroxylation, and the amino acid sequence including a lysine residue susceptible to hydroxylation comprises the amino acid sequence of SEQ ID NO:1.

In some embodiments, the polypeptide comprises an amino acid sequence including a lysine residue susceptible to hydroxylation, and the polypeptide comprises an amino acid sequence selected from SEQ ID NOs:5, 7, 9 and 11.

In some embodiments, the polypeptide comprises the VL-CH1 region of a crossFab moiety.

In some embodiments, the polypeptide comprises an amino acid sequence including a lysine residue susceptible to hydroxylation, and the polypeptide comprises an amino acid sequence selected from SEQ ID NOs:106, 107 and 108.

In some embodiments, the polypeptide is a constituent polypeptide of a multispecific antigen-binding molecule.

**Description**

**Lysine hydroxylation**

Aspects and embodiments of the present disclosure relate to the production of polypeptides comprising an amino acid sequence including a lysine residue susceptible to hydroxylation.

Hydroxylation of lysine to hydroxylysine (HyL) is an important step in the formation of extracellular matrices. Hydroxylysines of collagens and pro-collagens provide for their cross-linking by O-glycosylation (see *e.g.* Pornprasertsuk *et al.*, J Bone Miner Res. (2004) 19(8):1349-1355 and Schegg *et al.*, Mol Cell Biol. (2009) 29(4):943-952). Lysine hydroxylation is catalysed in the endoplasmic reticulum by procollagen-lysine 2-oxoglutarate 5-dioxygenases (PLODs).

In humans, three genes – *PLOD1*, *PLOD2* and *PLOD3* – encode the protein lysyl hydroxylases that catalyse 5R-hydroxylysine formation (from lysine) in pro-collagen and collagen-like proteins. For PLOD2, two splice variants exist; LH2a (PLOD2A) and LH2b (PLOD2B), which differ in that LH2b incorporates the small exon 13A (Valtavaara *et al.* J Biol Chem. (1997) 272(11):6831-6834). The variety of PLOD gene products and splice variants suggest different layers of regulation in (patho)physiological processes, but their roles are not yet fully understood (Qi *et al.*, Front Cell Dev Biol. (2018) 6:66). For example, in mammalian systems, a multiprotein complex comprising PLOD1 and the proline hydroxylases P3H3 and P3H4 is responsible for the hydroxylation of C5 of lysyl and C4 prolyl residues in pro-collagen α chains, which are required for accurate assembly and cross-linking of collagen fibrils.

PLODs 1-3 (EC 1.14.11.4) are members of the 2-oxoglutarate (2OG) dioxygenase enzyme class (EC 1.14.11.-). Other members of this class having lysyl hydroxylase activity include the Jumonji domain-

containing (JmjC) enzymes JMJD4, JMJD6 (EC 1.14.11.4) and JMJD7 (EC 1.14.11.63). PLOD1, PLOD2 and PLOD3 catalyse the modification of lysine to 5R-hydroxy-L-lysine. JMJD4 catalyses the modification of lysine to 4RS-hydroxy-L-lysine, while JMJD6 catalyses the modification of lysine to 5S-hydroxy-L-lysine, and JMJD7 catalyses the modification of lysine to 3S-hydroxy-L-lysine.

All of PLOD1, PLOD2, PLOD3, JMJD4, JMJD6 and JMJD7 require $Fe^{2+}$ as a co-factor. In a sequential binding mechanism, 2OG first binds to the substrate and followed by oxygen, and an active ferryl intermediate is formed by oxidative decarboxylation of 2OG. The ferryl intermediate then reacts with the substrate, leading to hydroxylation.

The lysine hydroxylation according to the present disclosure is enzyme-catalysed lysine hydroxylation, catalysed by an enzyme requiring $Fe^{2+}$ as a co-factor. In some embodiments, the lysine hydroxylation catalysed by a 2-oxoglutarate (2OG) dioxygenase (*i.e.* an enzyme of EC 1.14.11.-).

In some embodiments, the lysine hydroxylation is catalysed by a PLOD (*e.g.* PLOD1, PLOD2 or PLOD3), JMJD4, JMJD6 or JMJD7. In some embodiments, the lysine hydroxylation is hydroxylation of lysine to 5R-hydroxy-L-lysine, 4RS-hydroxy-L-lysine, 5S-hydroxy-L-lysine or 3S-hydroxy-L-lysine.

In some embodiments, the lysine hydroxylation is catalysed by a PLOD, *e.g.* selected from PLOD1, PLOD2 and PLOD3. In some embodiments, the lysine hydroxylation is hydroxylation of lysine to 5R-hydroxy-L-lysine.

**Proline hydroxylation**

Aspects and embodiments of the present disclosure pertain to polypeptides having amino acid sequences comprising a proline residue susceptible to hydroxylation.

Hydroxyproline is a major component of collagen; hydroxyproline hydroxyl groups participate in the formation of hydrogen bonds, and thus contribute to collagen's structural integrity. Post-translational modification of proline to hydroxyproline is catalysed in the endoplasmic reticulum by prolyl hydroxylases. Prolyl hydroxylases include procollagen-proline 4-dioxygenase (P3H4; EC 1.14.11.2) and procollagen-proline 3-dioxygenase (P3H3; EC 1.14.11.7). Prolyl hydroxylases are members of the 2-oxoglutarate (2OG) dioxygenase enzyme class (EC 1.14.11.-), and require $Fe^{2+}$ as a cofactor. Procollagen-proline 4-dioxygenase catalyses the modification of proline to (2S,4R)-4-hydroxyproline, and procollagen-proline 3-dioxygenase catalyses the modification of proline to (2S,3S)-3-hydroxyproline.

The proline hydroxylation according to the present disclosure is enzyme-catalysed proline hydroxylation, catalysed by an enzyme requiring $Fe^{2+}$ as a co-factor. In some embodiments, the proline hydroxylation is catalysed by a 2-oxoglutarate (2OG) dioxygenase (*i.e.* an enzyme of EC 1.14.11.-).

In some embodiments, the proline hydroxylation is catalysed by procollagen-proline 4-dioxygenase, procollagen-proline 3-dioxygenase or proline 3-hydroxylase. In some embodiments, the proline

hydroxylation is hydroxylation of proline to trans-4-hydroxyproline, trans-3-hydroxyproline or cis-3-hydroxyproline. In some embodiments, the proline hydroxylation is catalysed by procollagen-proline 4-dioxygenase. In some embodiments, the proline hydroxylation is hydroxylation of proline to trans-4-hydroxyproline.

**Polypeptides and polypeptide complexes**

As explained hereinabove, aspects and embodiments of the present disclosure relate to the production of polypeptides comprising: an amino acid sequence including a lysine residue susceptible to hydroxylation, and/or an amino acid sequence including a proline residue susceptible to hydroxylation.

As used herein, a 'peptide' refers to a chain of two or more amino acid monomers linked by peptide bonds. Peptides typically have a length in the region of about 2 to about 50 amino acids. A 'polypeptide' comprises more than one peptide bond, and comprises 3 or more amino acids.

The polypeptide according to the present disclosure may be any polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation (*e.g.* as described hereinabove), and/or an amino acid sequence including a proline residue susceptible to hydroxylation (*e.g.* as described hereinabove).

Aspects and embodiments of the present disclosure pertain to polypeptides having amino acid sequences comprising a lysine residue susceptible to hydroxylation.

Enzyme-catalysed hydroxylation of lysine requires a three-dimensional protein structure permitting access of the enzyme to the lysine residue. Xie *et al.*, MAbs. (2016) 8(2): 371-378 reports SEQ ID NO:2 as a consensus sequence for lysyl hydroxylase-catalysed hydroxylation of lysine to 5-hydroxylysine, and also describes hydroxylation of the lysine at position 24 of SEQ ID NO:16, at the beginning of the CH1 domain of IgG1 antibodies produced from Chinese hamster ovary (CHO) cells in culture.

Proteins that commonly contain hydroxylysine (HyL) residues include collagens and collagen-like domain containing proteins. Molony *et al.*, Tech Prot Chem. (1995) 6:91-98 reports hydroxylation of the lysine at position 2 of SEQ ID NO:12 in up to 20% of recombinant human plasminogen activator, and hydroxylation of the lysine at position 11 of SEQ ID NO:13 in up to 10% of recombinant antibody CD4/rCD4IgG produced from cells in culture. Aguilar *et al.*, Biochemistry. (2005) 44(33):11130-11136 reports that in up to 100% of De13a (marine cone snail toxin), the lysines at positions 18 and 25 of SEQ ID NO:14 are hydroxylated. Andrews *et al.*, J Biol Chem. (1984) 259(24):5021-5024 reports that up to 40% of somatostatin-28 (a peptide hormone from anglerfish), the lysine at position 24 of SEQ ID NO:15 is hydroxylated.

The experimental examples of the present disclosure also observe hydroxylation of the lysine residues at the following positions and polypeptides of three different bispecific antibodies provided in Fab-Fc(KiH)-crossFab-Fab format: position 13 of SEQ ID NO:17/SEQ ID NO:18 (position 12 of SEQ ID NO:24), within

the VH region of the light chain of the Fab moieties; position 14 of SEQ ID NO:19/SEQ ID NO:23, within the κ CL region of the light chain of the Fab and crossFab moieties; position 10 of SEQ ID NO:20 within the CH1 domains of the heavy chain of the crossFab moieties; position 21 of SEQ ID NO:26, within the CH1 domains of the heavy chain of the crossFab moieties; position 21 of SEQ ID NO:21 within the CH1 domains of the heavy chain of the Fab moieties; position 2 of SEQ ID NO:22, within the κ CL region of the light chain of the crossFab moiety; and position 10 of SEQ ID NO:25, within the VH region of the Fab moiety on the heavy chain 'hole' polypeptide of TCB2 (*i.e.* T-cell bispecific antibody 2, herein).

In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:1. In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:2. In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:3. In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:4. In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:5.

In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26. In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, an amino acid sequence selected from: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173 and SEQ ID NO:174. In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:9. In some embodiments, an amino acid sequence including a lysine residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:11.

Aspects and embodiments of the present disclosure pertain to polypeptides having amino acid sequences comprising a proline residue susceptible to hydroxylation.

Enzyme-catalysed hydroxylation of proline requires a three-dimensional protein structure permitting access of the enzyme to the proline residue. The consensus sequence for procollagen-proline 4-dioxygenase-catalysed hydroxylation of proline to trans-4-hydroxyproline is shown in SEQ ID NO:27. Proteins that typically contain hydroxyproline residues include collagens and collagen-like domain

containing proteins, the constituent polypeptides of the C1q complex, acetylcholinesterase, collectins, elastins, PrP, Argonaute 2 and conotoxins (see *e.g.* Gorres and Raines, Crit Rev Biochem Mol Biol. (2010) 45(2): 106-124).

In some embodiments, an amino acid sequence including a proline residue susceptible to hydroxylation comprises, or consists of, SEQ ID NO:27.

The skilled person is able to identify amino acid sequences including a lysine/proline residue susceptible to hydroxylation, and polypeptides comprising such sequences. For example, an amino acid sequence including a lysine/proline residue susceptible to hydroxylation may be an amino acid sequence which is known from the scientific literature to comprise a lysine/proline residue susceptible to hydroxylation. The skilled person is also able to identify amino acid sequences including a lysine/proline residue susceptible to hydroxylation, and polypeptides comprising such sequences, via *in silico* analysis of amino acid sequences as described in Hu *et al.*, PLoS One. (2010) 5(12): e15917, or using tools for identifying/predicting hydroxylysine/hydroxyproline sites such as HydLoc (described *e.g.* in Huang *et al.*, Chemometrics and Intelligent Laboratory Systems (2020) 202: 104035), iHyd-PseAAC (described *e.g.* in Xu *et al.*, Int. J. Mol. Sci. (2014) 15(5), 7594-7610) or OH-PRED (described *e.g.* in Jia *et al.*, J Biomol Struct Dyn. (2017) 35(4):829-835).

The skilled person is also able to identify amino acid sequences including a lysine/proline residue susceptible to hydroxylation, and polypeptides comprising such sequences via empirical determination of the presence of hydroxylysine/hydroxyproline in polypeptides expressed recombinantly in lysyl hydroxylase and/or prolyl hydroxylase-expressing cells.

For example, whether a given amino acid sequence includes a lysine residue susceptible to hydroxylation can be evaluated by expressing a polypeptide comprising or consisting of the amino acid sequence in lysyl hydroxylase-expressing cells, under conditions permitting lysine hydroxylation, and subsequent analysis of the expressed polypeptides to determine whether they contain hydroxylysine. Where the expressed polypeptides are determined to contain hydroxylysine, the amino acid sequence is determined to comprise an amino acid sequence including a lysine residue susceptible to hydroxylation. Similarly, whether a given amino acid sequence includes a proline residue susceptible to hydroxylation can be evaluated by expressing a polypeptide comprising or consisting of the amino acid sequence in prolyl hydroxylase-expressing cells, under conditions permitting proline hydroxylation, and subsequent analysis of the expressed polypeptides to determine whether they contain hydroxyproline. Where the expressed polypeptides are determined to contain hydroxyproline, the amino acid sequence is determined to comprise an amino acid sequence including a proline residue susceptible to hydroxylation.

The given polypeptide may be expressed by transcription from DNA encoding the polypeptide, and subsequent translation of the transcribed RNA. Expressed polypeptides may subsequently be isolated/purified prior to analysis to determine whether they comprise contain hydroxylysine/hydroxyproline. Evaluation to determine expressed polypeptides comprise

hydroxylysine/hydroxyproline may comprise analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Such analysis permits identification of hydroxylysine/hydroxyproline at specific positions within the amino acid sequence of the polypeptide.

By way of illustration, a given polypeptide can be analysed to determine whether it comprises an amino acid sequence including a lysine residue susceptible to hydroxylation essentially as described in Example 1 herein.

In some embodiments, an amino acid sequence including a lysine/proline residue susceptible to hydroxylation, or a polypeptide comprising such sequence, may be identified by *in silico* analysis for identifying/predicting hydroxylysine/hydroxyproline sites, and subsequent confirmation by empirical evaluation.

In some embodiments, a polypeptide according to the present disclosure is a constituent polypeptide of a polypeptide complex. In some embodiments, the methods of the present disclosure are methods for producing a polypeptide complex, wherein the polypeptide complex comprises a polypeptide according to the present disclosure. Such methods may comprise culturing cells comprising nucleic acid encoding the constituent polypeptides of a polypeptide complex comprising a polypeptide according to the present disclosure under conditions suitable for expression of the polypeptides, and for association of the polypeptides to form the polypeptide complex. 'Polypeptide complexes' are characterised by protein:protein interaction between their constituent polypeptides. In some embodiments, protein:protein interaction comprises non-covalent interaction, *e.g.* electrostatic interaction (*e.g.* ionic bonding, hydrogen bonding) and/or Van der Waals forces. In some embodiments, protein:protein interaction comprises covalent interaction (*e.g.* disulfide bonding, electron sharing). By way of illustration, the experimental examples of the present disclosure describe the production of polypeptide complexes comprising polypeptides comprising lysine residues susceptible to hydroxylation, formed by interaction between the CH3 regions of a first polypeptide comprising a CH3 region and a second polypeptide comprising a CH3 region.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide suitable for use in therapy or prophylaxis of a disease/condition. A polypeptide suitable for use in therapy or prophylaxis of a disease/condition may be any polypeptide whose administration is useful for the treatment or prevention of a disease/condition. In some embodiments, a polypeptide suitable for use in therapy or prophylaxis of a disease/condition may be a polypeptide for which deficiency thereof is positively associated with, or implicated in the pathology of, a disease or condition. In some embodiments, a polypeptide suitable for use in therapy or prophylaxis of a disease/condition may be a polypeptide which inhibits the expression and/or activity of a factor whose expression or activity is positively associated with, or implicated in the pathology of, a disease or condition.

In some embodiments, the polypeptide is: an antigen-binding polypeptide, an aptamer, a constituent polypeptide of an antigen-binding polypeptide complex, an Fc fusion protein, an anticoagulant, a blood

factor, a bone morphogenetic protein, a decoy receptor for a ligand, a decoy ligand for a receptor, an enzyme, a growth factor, a hormone, an interferon, an interleukin, a thrombolytic factor, a transcription factor, an epigenetic modifier, a constituent polypeptide of a site-specific nuclease nucleic acid editing system (*e.g.* a CRISPR/Cas9 system, a CRISPR/Cpf1 system, a CRISPR/C2c1 system, a CRISPR/C2c2 system, a CRISPR/C2c3 system, a ZFN system or a TALEN system), a constituent polypeptide of a ribonucleoprotein, or a viral protein (*e.g.* a capsid protein or a viral enzyme).

In some embodiments, a polypeptide according to the present disclosure is, or is derived from, an antigen-binding polypeptide. In some embodiments, a polypeptide according to the present disclosure is, or is derived from, a constituent polypeptide of an antigen-binding polypeptide complex.

Antigen-binding polypeptides and antigen-binding polypeptide complexes are capable of binding to a target antigen. Antigen-binding polypeptides and antigen-binding polypeptide complexes include antibodies (*i.e.* immunoglobulins (Igs)) and antigen-binding fragments and derivatives thereof. As used herein, 'antibodies' include monoclonal antibodies, polyclonal antibodies, monospecific and multispecific (*e.g.*, bispecific, trispecific, *etc.*) antibodies, and antibody-derived antigen-binding molecules such as scFv, scFab, diabodies, triabodies, scFv-Fc, minibodies, single domain antibodies (*e.g.* VhH), *etc.* Antigen-binding fragments of antibodies include *e.g.* Fv, Fab, $F(ab')_2$ and F(ab') fragments. In some embodiments, an antigen-binding molecule may be an antibody or an antigen-binding fragment thereof. Antigen-binding polypeptides may be *e.g.* a peptide aptamer, thioredoxin, monobody, anticalin, Kunitz domain, avimer, knottin, fynomer, atrimer, DARPin, affibody, nanobody (*i.e.* a single-domain antibody (sdAb)), affilin, armadillo repeat protein (ArmRP), OBody or fibronectin – reviewed *e.g.* in Reverdatto *et al.*, Curr Top Med Chem. 2015; 15(12): 1082–1101, which is hereby incorporated by reference in its entirety (see also *e.g.* Boersma *et al.*, J Biol Chem (2011) 286:41273-85 and Emanuel *et al.*, Mabs (2011) 3:38-48).

In some embodiments, a target antigen is a disease-associated antigen. A 'disease-associated antigen' refers to an antigen whose presence is indicative of a given disease/disease state, or an antigen for which an elevated level of the antigen is positively-correlated with a given disease/disease state. The disease-associated antigen may be an antigen whose expression is associated with the development, progression or severity of symptoms of a given disease. The disease-associated antigen may be associated with the cause or pathology of the disease, or may be expressed abnormally as a consequence of the disease. A disease-associated antigen may be an antigen of an infectious agent or pathogen, a cancer-associated antigen or an autoimmune disease-associated antigen.

In some embodiments, the disease-associated antigen is an antigen of a pathogen. The pathogen may be prokaryotic (bacteria), eukaryotic (*e.g.* protozoan, helminth, fungus), virus or prion. In some embodiments, the pathogen is an intracellular pathogen. In some embodiments the pathogen is a virus, *e.g.* a virus as described hereinabove. In some embodiments the pathogen is a bacterium.

In some embodiments, the target antigen is a cancer-associated antigen. A cancer-associated antigen is an antigen whose expression or overexpression is associated with cancer. In some embodiments, the cancer-associated antigen is a receptor molecule, *e.g.* a cell surface receptor. In some embodiments, the cancer-associated antigen is a cell signalling molecule, *e.g.* a cytokine, chemokine, interferon, interleukin or lymphokine. In some embodiments, the cancer-associated antigen is a growth factor or a hormone. In some embodiments, the cancer-associated antigen is a viral antigen. A cancer cell antigen may be abnormally expressed by a cancer cell (*e.g.* the cancer cell antigen may be expressed with abnormal localisation), or may be expressed with an abnormal structure by a cancer cell. A cancer cell antigen may be capable of eliciting an immune response. In some embodiments, the antigen is expressed at the cell surface of the cancer cell (*i.e.* the cancer cell antigen is a cancer cell surface antigen). In some embodiments, the part of the antigen which is bound by an antigen-binding molecule described herein is displayed on the external surface of the cancer cell (*i.e.* is extracellular). The cancer cell antigen may be a cancer-associated antigen. In some embodiments the cancer cell antigen is an antigen whose expression is associated with the development, progression or severity of symptoms of a cancer. The cancer-associated antigen may be associated with the cause or pathology of the cancer, or may be expressed abnormally as a consequence of the cancer. In some embodiments, the cancer cell antigen is an antigen whose expression is upregulated (*e.g.* at the RNA and/or protein level) by cells of a cancer, *e.g.* as compared to the level of expression by comparable non-cancerous cells (*e.g.* non-cancerous cells derived from the same tissue/cell type). In some embodiments, the cancer-associated antigen may be preferentially expressed by cancerous cells, and not expressed by comparable non-cancerous cells (*e.g.* non-cancerous cells derived from the same tissue/cell type). In some embodiments, the cancer-associated antigen may be the product of a mutated oncogene or mutated tumor suppressor gene. In some embodiments, the cancer-associated antigen may be the product of an overexpressed cellular protein, a cancer antigen produced by an oncogenic virus, an oncofetal antigen, or a cell surface glycolipid or glycoprotein.

Cancer-associated antigens are reviewed by Zarour HM, DeLeo A, Finn OJ, *et al.* Categories of Tumor Antigens. In: Kufe DW, Pollock RE, Weichselbaum RR, *et al.*, editors. Holland-Frei Cancer Medicine. 6th edition. Hamilton (ON): BC Decker; 2003. Cancer-associated antigens include oncofetal antigens: CEA, Immature laminin receptor, TAG-72; oncoviral antigens such as HPV E6 and E7; overexpressed proteins: fibroblast activation protein (FAP), B-cell maturation antigen (BCMA), CD19, HER2/neu, EGFR, TYRP1, GPRC5D, BING-4, calcium-activated chloride channel 2, cyclin-B1, 9D7, Ep-CAM, EphA3, telomerase, mesothelin, SAP-1, survivin, melanoma-associated chondroitin sulfate proteoglycan (MCSP); cancer-testis antigens: BAGE, CAGE, GAGE, MAGE, SAGE, XAGE, CT9, CT10, NY-ESO-1, PRAME, SSX-2; lineage restricted antigens: MART1, Gp100, tyrosinase, TRP-½, MC1R, prostate specific antigen; mutated antigens: β-catenin, BRCA½, CDK4, CML66, Fibronectin, MART-2, p53, Ras, TGF-βRII; post-translationally altered antigens: MUC1, idiotypic antigens: Ig, TCR. Other cancer cell antigens include heat-shock protein 70 (HSP70), heat-shock protein 90 (HSP90), glucose-regulated protein 78 (GRP78), vimentin, nucleolin, feto-acinar pancreatic protein (FAPP), alkaline phosphatase placental-like 2 (ALPPL-2), siglec-5, stress-induced phosphoprotein 1 (STIP1), protein tyrosine kinase 7 (PTK7), and cyclophilin

B. In some embodiments the cancer cell antigen is a cancer cell antigen described in Zhao and Cao, Front Immunol. (2019) 10:2250, which is hereby incorporated by reference in its entirety.

In some embodiments, the target antigen is selected from MCSP, EGFR, FAP, CEA, TYRP1, GPRC5D and CD19. In some embodiments, the target antigen is MCSP. In some embodiments, the target antigen is EGFR. In some embodiments, the target antigen is FAP. In some embodiments, the target antigen is CEA. In some embodiments, the target antigen is TYRP1. In some embodiments, the target antigen is GPRC5D. In some embodiments, the target antigen is CD19.

In some embodiments, the target antigen is an immune cell surface molecule. An immune cell surface molecule is any molecule which is expressed in or at the cell membrane of an immune cell. In some embodiments, the part of the immune cell surface molecule which is bound by the antigen-binding moiety is on the external surface of the immune cell (*i.e.* is extracellular). The immune cell surface molecule may be expressed at the cell surface of any immune cell. In some embodiments, the immune cell may be a cell of hematopoietic origin, *e.g.* a neutrophil, eosinophil, basophil, dendritic cell, lymphocyte, or monocyte. The lymphocyte may be *e.g.* a T cell, B cell, natural killer (NK) cell, NKT cell or innate lymphoid cell (ILC), or a precursor thereof (*e.g.* a thymocyte or pre-B cell). The immune cell may express a CD3 polypeptide (*e.g.* CD3γ CD3ε CD3ζ or CD3δ), a TCR polypeptide (TCRα or TCRβ), CD27, CD28, CD4 or CD8. In some embodiments, the immune cell is a T cell, *e.g.* a CD3+ T cell. In some embodiments, the T cell is a CD3+, CD4+ T cell. In some embodiments, the T cell is a CD3+, CD8+ T cell. In some embodiments, the T cell is a T helper cell (TH cell). In some embodiments, the T cell is a cytotoxic T cell (*e.g.* a cytotoxic T lymphocyte (CTL)). In some embodiments, the immune cell is a T cell or an NK cell.

In some embodiments, an immune cell surface molecule may be a CD3-TCR complex polypeptide, *e.g.* TCRα, TCRβ, TCRγ, TCRδ, TRAC, TRBC1, TRBC2, TRGC1, TRGC2, TRDC, CD3ε, CD3δ, CD3γ, CD3ζ or CD3η. In some embodiments, an immune cell surface molecule is CD3, CD8, CD4 or CD28. In some embodiments, an immune cell surface molecule is a checkpoint molecule (*e.g.* PD-1, CTLA-4, LAG-3, TIM-3, VISTA, TIGIT or BTLA), or a ligand for a checkpoint molecule (*e.g.* PD-L1, PD-L2, CD80, CD86, MHC class I, MHC Class II, Galectin 9, VSIG3, VSIG8, LRIG1, PSGL1, CD155 or HVEM). In some embodiments the immune cell surface molecule is a costimulatory molecule (*e.g.* CD28, OX40, 4-1BB, ICOS or CD27), or a ligand for a costimulatory molecule (*e.g.* CD86, CD80, OX40L 4-1BBL, ICOSL or CD70).

Immunoglobulins and their structures are described *e.g.* in Schroeder and Cavacini J Allergy Clin Immunol. (2010) 125(202):S41-S52, which is hereby incorporated by reference in its entirety. Immunoglobulins of type G (*i.e.* IgG) are ~150 kDa glycoproteins comprising two heavy chains and two light chains. From N- to C-terminus, immunoglobulin heavy chains comprise a heavy chain variable region (VH) followed by a heavy chain constant region comprising three constant regions (CH1, CH2, and CH3, with a CH1-CH2 hinge region provided between CH1 and CH2). Immunoglobulin light chains comprise a light chain variable region (VL) followed by a light chain constant region (CL). Depending on

the heavy chain, immunoglobulins may be classed as IgG (*e.g.* IgG1, IgG2, IgG3, IgG4), IgA (*e.g.* IgA1, IgA2), IgD, IgE, or IgM. The light chain may be kappa (κ) or lambda (λ).

Herein, a 'CH1 region' refers to an amino acid sequence corresponding to the region of an Ig heavy chain constant region (IGHG) formed by positions 118 to 215, according to the EU numbering system (which is described in Edelman *et al.*, Proc Natl Acad Sci USA. (1969) 63(1):78-85). A 'CH1-CH2 hinge region' refers to an amino acid sequence corresponding to the region of an Ig heavy chain constant region (IGHG) formed by positions 216 to 230, according to the EU numbering system. A 'CH2 region' refers to an amino acid sequence corresponding to the region of an Ig heavy chain constant region (IGHG) formed by positions 231 to 340, according to the EU numbering system. A 'CH2-CH3 region' refers to an amino acid sequence corresponding to the region of an Ig heavy chain constant region (IGHG) formed by positions 231 to 447, according to the EU numbering system. A 'CH3 region' refers to an amino acid sequence corresponding to the region of an Ig heavy chain constant region (IGHG) formed by positions 341 to 447, according to the EU numbering system. A 'CL region' refers to an amino acid sequence corresponding to: (i) the region of an Ig light chain kappa constant region (IGKC) formed by positions 108 to 214, according to the EU numbering system, or (ii) the region of an Ig light chain lambda constant region (IGLC) formed by positions 107A to 216, according to the Kabat numbering system (Kabat *et al.*, Sequences of proteins of immunological interest. 5th Edn., US Department of Health and Human Services, NIH publication No. 91-3242, p647 (1991)).

Antigen-binding polypeptides and antigen-binding polypeptide complexes according to the present disclosure also include molecules comprising an antigen-binding region/domain derived from an antibody. Antibody-derived antigen-binding molecules may comprise an antigen-binding region/domain that comprises, or consists of, the antigen-binding region of an antibody (*e.g.* an antigen-binding fragment of an antibody). In some embodiments, the antigen-binding region/domain of an antibody-derived antigen-binding molecule may be or comprise the Fv (*e.g.* provided as an scFv) or the Fab region of an antibody, or the whole antibody. For example, antigen-binding molecules according to the present disclosure include antibody-drug conjugates (ADCs) comprising a (cytotoxic) drug moiety.

Antigen-binding polypeptides and antigen-binding polypeptide complexes according to the present disclosure also include chimeric antigen receptors (CARs), which are recombinant receptors providing both antigen-binding and T cell activating functions (CAR structure, function and engineering is reviewed *e.g.* in Dotti *et al.*, Immunol Rev (2014) 257(1) and Jayaraman *et al.*, EBioMedicine (2020) 58:102931, both of which are hereby incorporated by reference in their entirety).

Antigen-binding polypeptides and antigen-binding polypeptide complexes according to the present disclosure include multispecific antigen-binding polypeptides and multispecific antigen-binding polypeptide complexes. By 'multispecific' it is meant that the antigen-binding polypeptide/polypeptide complex displays specific binding to more than one (*e.g.* 2, 3, 4, 5, 6, 7, 8, *etc.*) target antigen. In some embodiments, the antigen-binding polypeptide/polypeptide complex is a bispecific antigen-binding polypeptide/polypeptide complex. In some embodiments, the antigen-binding polypeptide/polypeptide

complex comprises at least two different antigen-binding domains (*i.e.* at least two antigen-binding domains, *e.g.* comprising non-identical VHs and VLs).

Multispecific antigen-binding polypeptides/polypeptide complexes according to the present disclosure may be provided in any suitable format, such as those formats described in described in Brinkmann and Kontermann, MAbs (2017) 9(2): 182-212, which is hereby incorporated by reference in its entirety. Suitable formats include those shown in Figure 2 of Brinkmann and Kontermann, MAbs (2017) 9(2): 182-212: antibody conjugates, *e.g.* $IgG_2$, $F(ab')_2$ or CovX-Body; IgG or IgG-like molecules, *e.g.* IgG, chimeric IgG, κλ-body common HC; CH1/CL fusion proteins, *e.g.* scFv2-CH1/CL, VHH2-CH1/CL; 'variable domain only' bispecific antigen-binding molecules, *e.g.* tandem scFv (taFV), triplebodies, diabodies (Db), dsDb, Db(kih), DART, scDB, dsFv-dsFv, tandAbs, triple heads, tandem dAb/VHH, tertravalent dAb.VHH; Non-Ig fusion proteins, *e.g.* $scFv_2$-albumin, scDb-albumin, taFv-albumin, taFv-toxin, miniantibody, DNL-$Fab_2$, DNL-$Fab_2$-scFv, DNL-$Fab_2$-IgG-$cytokine_2$, ImmTAC (TCR-scFv); modified Fc and CH3 fusion proteins, *e.g.* scFv-Fc(kih), scFv-Fc(CH3 charge pairs), scFv-Fc (EW-RVT), scFv-fc (HA-TF), scFv-Fc (SEEDbody), taFv-Fc(kih), scFv-Fc(kih)-Fv, Fab-Fc(kih)-scFv, Fab-scFv-Fc(kih), Fab-scFv-Fc(BEAT), Fab-scFv-Fc (SEEDbody), DART-Fc, scFv-CH3(kih), TriFabs; Fc fusions, *e.g.* Di-diabody, scDb-Fc, taFv-Fc, scFv-Fc-scFv, HCAb-VHH, Fab-scFv-Fc, $scFv_4$-Ig, $scFv_2$-Fcab; CH3 fusions, *e.g.* Dia-diabody, scDb-CH3; IgE/IgM CH2 fusions, *e.g.* scFv-EHD2-scFv, scFvMHD2-scFv; Fab fusion proteins, *e.g.* Fab-scFv (bibody), Fab-$scFv_2$ (tribody), Fab-Fv, Fab-dsFv, Fab-VHH, orthogonal Fab-Fab; non-Ig fusion proteins, *e.g.* DNL-$Fab_3$, DNL-$Fab_2$-scFv, DNL-$Fab_2$-IgG-$cytokine_2$; asymmetric IgG or IgG-like molecules, *e.g.* IgG(kih), IgG(kih) common LC, ZW1 IgG common LC, Biclonics common LC, CrossMab, CrossMab(kih), scFab-IgG(kih), Fab-scFab-IgG(kih), orthogonal Fab IgG(kih), DuetMab, CH3 charge pairs + CH1/CL charge pairs, hinge/CH3 charge pairs, SEED-body, Duobody, four-in-one-CrossMab(kih), LUZ-Y common LC; LUZ-Y scFab-IgG, FcFc*; appended and Fc-modified IgGs, *e.g.* IgG(kih)-Fv, IgG HA-TF-Fv, IgG(kih)scFab, scFab-Fc(kih)-scFv2, scFab-Fc(kih)-scFv, half DVD-Ig, DVI-Ig (four-in-one), CrossMab-Fab; modified Fc and CH3 fusion proteins, *e.g.* Fab-Fc(kih)-scFv, Fab-scFv-Fc(kih), Fab-scFv-Fc(BEAT), Fab-scFv-Fc-SEEDbody, TriFab; appended IgGs - HC fusions, *e.g.* IgG-HC, scFv, IgG-dAb, IgG-taFV, IgG-CrossFab, IgG-orthogonal Fab, IgG-(CαCβ) Fab, scFv-HC-IgG, tandem Fab-IgG (orthogonal Fab), Fab-IgG(CαCβ Fab), Fab-IgG(CR3), Fab-hinge-IgG(CR3); appended IgGs - LC fusions, *e.g.* IgG-scFv(LC), scFv(LC)-IgG, dAb-IgG; appended IgGs - HC and LC fusions, *e.g.* DVD-Ig, TVD-Ig, CODV-Ig, $scFv_4$-IgG, Zybody; Fc fusions, *e.g.* Fab-scFv-Fc, $scFv_4$-Ig; F(ab')2 fusions, *e.g.* $F(ab')_2$-$scFv_2$; CH1/CL fusion proteins *e.g.* $scFv_2$-CH1-hinge/CL; modified IgGs, *e.g.* DAF (two-in one-IgG), DutaMab, $Mab^2$; and non-Ig fusions, *e.g.* DNL-$Fab_4$-IgG. The skilled person is readily able to design and produce multispecific antigen-binding polypeptides/polypeptide complexes.

In some embodiments, the antigen-binding polypeptide/polypeptide complex is an immune cell engager. Immune cell engagers are reviewed *e.g.* in Goebeler and Bargou, Nat. Rev. Clin. Oncol. (2020) 17: 418–434 and Ellerman, Methods (2019) 154:102-117, both of which are hereby incorporated by reference in their entirety. Immune cell engager molecules comprise an antigen-binding region for a target antigen of interest, and an antigen-binding region for recruiting/engaging an immune cell of interest. Immune cell

engagers recruit/engage immune cells through an antigen-binding region specific for an immune cell surface molecule.

The best studied immune cells engagers are bispecific T cell engagers (BiTEs), which comprise: (i) a target antigen-binding domain, and (ii) a CD3 polypeptide (typically CD3ε)-binding domain, through which the BiTE recruits T cells. Binding of the BiTE to its target antigen (*i.e.* through domain (i)) and to the CD3 polypeptide expressed by the T cell (*i.e.* through domain (ii)) results in activation of the T cell, and ultimately directs T cell effector activity against cells expressing the target antigen. Other kinds of immune cell engagers are well known in the art, and include natural killer cell engagers such as bispecific killer engagers (BiKEs) and trispecific killer engagers (TriKEs), which recruit and activate NK cells.

In some embodiments, the polypeptide according to the present disclosure is a multispecific antigen-binding polypeptide, or a constituent polypeptide of a multispecific antigen-binding polypeptide complex. In some embodiments, the polypeptide is a bispecific antigen-binding polypeptide, or a constituent polypeptide of a bispecific antigen-binding polypeptide complex. In some embodiments, the polypeptide is a bispecific T cell engager, or a constituent polypeptide of a bispecific T cell engager.

Recombinant co-expression of constituent polypeptides of multispecific antigen-binding polypeptide complexes and their subsequent association can result in several possible combinations. It can be advantageous to introduce amino acid substitutions in the immunoglobulin constant regions to improve the yield of the desired combinations of polypeptides. Modifications may promote *e.g.* hydrophobic and/or electrostatic interaction between CH2 and/or CH3 regions of different polypeptide chains. Suitable modifications are described *e.g.* in Ha *et al.*, Front. Immunol (2016) 7:394 (which is hereby incorporated by reference in its entirety), and include the following formats, as shown in Table 1 of Ha *et al.*, Front. Immunol (2016) 7:394: KiH, KiHs-s, HA-TF, ZW1, 7.8.60, DD-KK, EW-RVT, EW-RVTs-s, SEED or A107.

CrossMab technology facilitates correct heavy and light chain pairings in multispecific antigen-binding polypeptide complexes. CrossMab technology is described *e.g.* in Klein *et al.*, MAbs. 2016 Aug-Sep; 8(6): 1010-1020, which is hereby incorporated by reference in its entirety. In CrossMab molecules, the VH/VL and/or CL/CH1 regions are swapped in an antigen-binding moiety, to minimise the chances of mispairing with polypeptides of another antigen-binding moiety. See, for example, Figure 1 of Klein *et al.*, MAbs. 2016 Aug-Sep; 8(6): 1010-1020. CrossFab refers to an embodiment of CrossMab technology in which a Fab moiety is formed by association between a first polypeptide comprising a VL domain and a CH1 domain, and a second polypeptide comprising a VH domain and a CL domain (see *e.g.* Fenn *et al.*, PLoS One. (2013) 8(4): e61953). Accordingly, a crossFab moiety according to the present disclosure refers to a moiety comprising (i) a polypeptide comprising a VL domain and a CH1 domain, and (ii) a polypeptide comprising a VH domain and a CL domain.

In the experimental examples of the present disclosure, the inventors determine that the lysine residue at the position corresponding to CH1 position 121 (EU numbering) is particularly susceptible to hydroxylation when provided in the context of a VL provided immediately N-terminal thereto (in the amino

acid sequence of a polypeptide). That is, in polypeptides comprising (from N-terminus to C-terminus) a VL, followed by CH1, the lysine at position 121 (EU numbering) of CH1 is found to be particularly susceptible to hydroxylation.

Accordingly, in some embodiments an amino acid sequence including a lysine residue susceptible to hydroxylation according to the present disclosure (*e.g.* an amino acid sequence according to SEQ ID NO:1, 2, 6, 7, 10 or 11) is comprised in a polypeptide which is a constituent polypeptide of a crossFab moiety.

In some embodiments, the polypeptide according to the present disclosure is a constituent polypeptide of a crossFab moiety. Accordingly, in some embodiments, the polypeptide according to the present disclosure comprises, or consists of, a VL-CH1 region. In some embodiments, the polypeptide comprises, or consists of, the VL-CH1 region of a crossFab moiety.

That is, in some embodiments, the polypeptide is a constituent polypeptide of a polypeptide complex forming a crossFab moiety. In accordance with such embodiments, in some embodiments the polypeptide complex forming a crossFab moiety does not comprise a further antigen-binding moiety (*i.e.* does not comprise an antigen-binding moiety other than the crossFab moiety).

Examples of polypeptides comprising a VL-CH1 region include polypeptides consisting of the amino acid sequence of SEQ ID NO:42, 45, 57, 62, 66, 70, 78, 81, 90, 93, 96, 97, 100, 101 or 104. Examples of polypeptides consisting of a VL-CH1 region include polypeptides consisting of the amino acid sequence of SEQ ID NO:62, 70 or 78. In some embodiments, the polypeptide according to the present disclosure comprises, or consists of, an amino acid sequence having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:62, 70 or 78.

In some embodiments, the polypeptide comprises the structure: N-term-[...]-[VL]-[CH1]-[...]-C-term; wherein 'N-term' represents the N-terminus of the polypeptide, 'C-term' represents the C-terminus of the polypeptide, '[...]' indicates the optional presence of further amino acid sequence(s)/protein domain(s), each '-' indicates an optional linker sequence, '[VL]' indicates the amino acid sequence of the light chain variable region (VL) of an antibody, and 'CH1' indicates amino acid sequence of the CH1 region of an Ig heavy chain constant region.

In some embodiments, the polypeptide comprises the structure: N-term-[...]-[LC-FR4]-[CH1]-[...]-C-term; wherein 'N-term' represents the N-terminus of the polypeptide, 'C-term' represents the C-terminus of the polypeptide, '[...]' indicates the optional presence of further amino acid sequence(s)/protein domain(s), each '-' indicates an optional linker sequence, '[LC-FR4]' indicates the amino acid sequence of framework region 4 of the light chain variable region (VL) of an antibody, and 'CH1' indicates amino acid sequence of the CH1 region of an Ig heavy chain constant region.

In some embodiments, the polypeptide comprising a VL-CH1 region further comprises a CH2 region, *e.g.* C-terminal to the CH1 region. In some embodiments, the polypeptide comprising a VL-CH1 region further comprises a CH3 region, *e.g.* C-terminal to the CH1 region. In some embodiments, the polypeptide comprising a VL-CH1 region further comprises a CH2-CH3 region, *e.g.* C-terminal to the CH1 region.

In some embodiments, the polypeptide comprises the structure: N-term-[…]-[VL]-[CH1]-[CH2]-[…]-C-term; wherein 'N-term' represents the N-terminus of the polypeptide, 'C-term' represents the C-terminus of the polypeptide, '[...]' indicates the optional presence of further amino acid sequence(s)/protein domain(s), each '-' indicates an optional linker sequence, '[VL]' indicates the amino acid sequence of the light chain variable region (VL) of an antibody, 'CH1' indicates amino acid sequence of the CH1 region of an Ig heavy chain constant region, and 'CH2' indicates amino acid sequence of the CH2 region of an Ig heavy chain constant region. In some embodiments, the polypeptide comprises the structure: N-term-[…]-[VL]-[CH1]-[CH2-CH3]-[…]-C-term; wherein 'N-term' represents the N-terminus of the polypeptide, 'C-term' represents the C-terminus of the polypeptide, '[...]' indicates the optional presence of further amino acid sequence(s)/protein domain(s), each '-' indicates an optional linker sequence, '[VL]' indicates the amino acid sequence of the light chain variable region (VL) of an antibody, 'CH1' indicates amino acid sequence of the CH1 region of an Ig heavy chain constant region, and 'CH2-CH3' indicates amino acid sequence of the CH2-CH3 region of an Ig heavy chain constant region.

In some embodiments, the polypeptide comprising a VL-CH1 region lacks an amino acid sequence forming all or part of an antigen-binding moiety N-terminal to the VL region. In some embodiments, the polypeptide comprises the structure: N-term-[VL]-[CH1]-[…]-C-term; wherein 'N-term' represents the N-terminus of the polypeptide, 'C-term' represents the C-terminus of the polypeptide, '[...]' indicates the optional presence of further amino acid sequence(s)/protein domain(s), each '-' indicates an optional linker sequence, '[VL]' indicates the amino acid sequence of the light chain variable region (VL) of an antibody, and 'CH1' indicates amino acid sequence of the CH1 region of an Ig heavy chain constant region. In some embodiments, the polypeptide comprising a VL-CH1 region lacks an amino acid sequence forming all or part of another antigen-binding moiety (*i.e.* an antigen-binding moiety other than the antigen-binding moiety in which the VL-CH1 region is/is to be comprised).

Linker sequences are known to the skilled person, and are described, for example in Chen *et al.*, Adv Drug Deliv Rev (2013) 65(10): 1357-1369, which is hereby incorporated by reference in its entirety. In some embodiments, a linker sequence may be a flexible linker sequence. Flexible linker sequences allow for relative movement of the amino acid sequences which are linked by the linker sequence. Flexible linkers are known to the skilled person, and several are identified in Chen *et al.*, Adv Drug Deliv Rev (2013) 65(10): 1357-1369. Flexible linker sequences often comprise high proportions of glycine and/or serine residues. In some embodiments, a linker between a VL region (*e.g.* the LC-FR4 thereof) and a CH1 region consists of two serine residues.

In some embodiments, the polypeptide according to the present disclosure comprises, or consists of, an amino acid sequence having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%,

≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:106, 107 or 108.

**Particular exemplary polypeptides and polypeptide complexes**

In some embodiments, a polypeptide is a constituent polypeptide of a polypeptide complex according to the present disclosure.

In some embodiments, a polypeptide or polypeptide complex according to the present disclosure comprises a VH region comprising the heavy chain CDRs, and a VL region comprising the light chain CDRs, of an antigen-binding molecule that binds to CD3, *e.g.* as shown in Table A herein. In some embodiments, a polypeptide or polypeptide complex according to the present disclosure comprises the VH region and the VL region of an antigen-binding molecule that binds to CD3, *e.g.* as shown in Table A herein.

In some embodiments, the polypeptide or polypeptide complex comprises: (i) a VH region comprising HC-CDR1, HC-CDR2 and HC-CDR3 as indicated in column A of Table A, and (ii) a VL region comprising LC-CDR1, LC-CDR2 and LC-CDR3 as indicated in column B of Table A, wherein the sequences of columns A and B are selected from the same row of Table A. By way of illustration, in some embodiments the polypeptide or polypeptide complex comprises a VH region comprising HC-CDR1 = SEQ ID NO:124, HC-CDR2 = SEQ ID NO:125, and HC-CDR3 = SEQ ID NO:126; and a VL region comprising LC-CDR1 = SEQ ID NO:128, LC-CDR2 = SEQ ID NO:129, and LC-CDR3 = SEQ ID NO:130 (*i.e.* VH comprising HC-CDR1-3 and VL comprising LC-CDR1-3 of CH2527, as shown in row 1 of Table A).

In some embodiments, the polypeptide or polypeptide complex comprises: (i) a VH region comprising or consisting of an amino acid sequence having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to an amino acid sequence indicated in column A of Table B, and (ii) a VL region comprising or consisting of an amino acid sequence having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to an amino acid sequence indicated in column B of Table B; wherein the sequences of columns A and B are selected from the same row of Table B. In some embodiments, the polypeptide or polypeptide complex comprises: (i) a VH region comprising or consisting of an amino acid sequence indicated in column A of Table B, and (ii) a VL region comprising or consisting of an amino acid sequence indicated in column B of Table B; wherein the sequences of columns A and B are selected from the same row of Table B. By way of illustration, in some embodiments the polypeptide or polypeptide complex comprises a VH region comprising SEQ ID NO:120; and a VL region comprising SEQ ID NO:127 (*i.e.* the VH and VL of CH2527, as shown in row 1 of Table B).

*Table A*

| Row | Clone | Column A | | | Column B | | |
|---|---|---|---|---|---|---|---|
| | | VH | | | VL | | |
| | | HC-CDR1 | HC-CDR2 | HC-CDR3 | LC-CDR1 | LC-CDR2 | LC-CDR3 |
| 1 | CH2527 | SEQ ID NO:124 | SEQ ID NO:125 | SEQ ID NO:126 | SEQ ID NO:128 | SEQ ID NO:129 | SEQ ID NO:130 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **2** | Clone 22 | SEQ ID NO:132 | SEQ ID NO:125 | SEQ ID NO:133 | SEQ ID NO:128 | SEQ ID NO:129 | SEQ ID NO:130 |
| **3** | P035.093 | SEQ ID NO:135 | SEQ ID NO:125 | SEQ ID NO:136 | SEQ ID NO:128 | SEQ ID NO:129 | SEQ ID NO:130 |
| **4** | P035.093 (Kabat) | SEQ ID NO:164 | SEQ ID NO:165 | SEQ ID NO:166 | SEQ ID NO:167 | SEQ ID NO:168 | SEQ ID NO:130 |
| **5** | 40G5c | SEQ ID NO:138 | SEQ ID NO:139 | SEQ ID NO:140 | SEQ ID NO:142 | SEQ ID NO:143 | SEQ ID NO:144 |
| **6** | V9 | SEQ ID NO:146 | SEQ ID NO:147 | SEQ ID NO:148 | SEQ ID NO:150 | SEQ ID NO:151 | SEQ ID NO:152 |

*Table B*

| | | **Column A** | **Column B** |
|---|---|---|---|
| **Row** | **Clone** | **VH** | **VL** |
| **1** | CH2527 | SEQ ID NO:120 | SEQ ID NO:127 |
| **2** | Clone 22 | SEQ ID NO:131 | SEQ ID NO:127 |
| **3** | P035.093 | SEQ ID NO:134 | SEQ ID NO:127 |
| **4** | 40G5c | SEQ ID NO:137 | SEQ ID NO:141 |
| **5** | V9 | SEQ ID NO:145 | SEQ ID NO:149 |

In some embodiments, a polypeptide according to the present disclosure is a polypeptide described in WO 2013/026833 A1, which is hereby incorporated by reference in its entirety. In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex described in WO 2013/026833 A1. In some embodiments, a polypeptide is a constituent polypeptide of an antigen-binding polypeptide complex described in WO 2013/026833 A1.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to one of SEQ ID NOs:28 to 80.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO: 42, 45, 57, 62, 66, 70 or 78.

In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex comprising:

(i) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:29; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:42; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:44;

(ii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence

identity to SEQ ID NO:29; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:45; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:46;

(iii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:38; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:39;

(iv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:36; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:37;

(v) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:40; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:41;

(vi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:34; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:35;

(vii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:38; and a polypeptide having at least 70% (*e.g.*

one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:77;

(viii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:28; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:29; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30;

(ix) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:31; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:32; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:33;

(x) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:42; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:43; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:44;

(xi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:29; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:42; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:60;

(xii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:42; a polypeptide having at least 70% (*e.g.* one of

≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:44; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:61;

(xiii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:39; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:62; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:63;

(xiv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:39; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:62; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:64;

(xv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:44; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:65; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:66; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:67;

(xvi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:62; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:65; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%,

≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:67; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:68;

(xvii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:65; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:67; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:70; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:71;

(xviii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:39; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:78; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:79;

(xix) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:30; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:39; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:78; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:80;

(xx) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:48; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:49; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:52;

(xxi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:49; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:52; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:77;

(xxii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:47; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:48; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:49;

(xxiii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:33; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:50; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:51;

(xxiv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:54; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:55; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:56;

(xxv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:54; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:56; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:77;

(xxvi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:33; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:51; and a polypeptide having at least 70% (*e.g.*

one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:53;

(xxvii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:44; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:57; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:58; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:59;

(xxviii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:58; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:59; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:62; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:69;

(xxix) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:62; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:72; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:73; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:74; or

(xxx) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:62; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:75; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:76; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%,

≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:77.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide described in WO 2021/255142 A1, which is hereby incorporated by reference in its entirety. In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex described in WO 2021/255142 A1. In some embodiments, a polypeptide is a constituent polypeptide of an antigen-binding polypeptide complex described in WO 2021/255142 A1.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to one of SEQ ID NOs:81 to 105, or 153 to 155.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:81, 90, 93, 96, 97, 100, 101, 104 or 153.

In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex comprising:
(i) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:81; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:82; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:85;
(ii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:81; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:82; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:86;

(iii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:81; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:82; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:87;

(iv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:81; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:82; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:88;

(v) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:81; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:82; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:89;

(vi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:85; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:90; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:91; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:92;

(vii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:86; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:90; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:91; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:92;

(viii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:89; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:90; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:91; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:92;

(ix) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:85; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:93; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:94; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:95;

(x) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:86; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:93; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:94; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:95;

(xi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:89; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:93; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:94; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:95;

(xii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:86; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:96; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:98; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:99;

(xiii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:86; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:100; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:102; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:103;

(xiv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:84; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:97; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:98; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:99;

(xv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:84; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:101; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:102; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:103;

(xvi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:85; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:104; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:105;

(xvii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:86; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:104; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:105; or

(xviii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:89; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:104; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:105; or

(xix) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:97; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:98; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:99; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:116; or

(xx) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:93; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:94; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:95; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:116; or

(xxi) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:153; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:154; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:155.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide described in WO 2014/131712 A1, which is hereby incorporated by reference in its entirety. In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex described in WO 2014/131712 A1. In some embodiments, a polypeptide is a constituent polypeptide of an antigen-binding polypeptide complex described in WO 2014/131712 A1.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to one of SEQ ID NOs:58 or 109 to 114.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:110.

In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex comprising:

(i) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:109; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%,

≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:110; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:111; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:112; or

(ii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:113; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:110; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:114; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:58.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide described in WO 2020/127619 A1, which is hereby incorporated by reference in its entirety. In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex described in WO 2020/127619 A1. In some embodiments, a polypeptide is a constituent polypeptide of an antigen-binding polypeptide complex described in WO 2020/127619 A1.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to one of SEQ ID NOs:82 to 84 or 115 to 119.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:115 or 117.

In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex comprising:

(i) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:115; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:82; a polypeptide having at least 70% (*e.g.* one of

≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:116;

(ii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:115; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:82; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:83; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:84;

(iii) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:117; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:118; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:119; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:116; or

(iv) a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:117; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:118; a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:119; and a polypeptide having at least 70% (*e.g.* one of ≥70%, ≥75%, ≥80%, ≥85%, ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100%) amino acid sequence identity to SEQ ID NO:84.

In some embodiments, a polypeptide according to the present disclosure is a polypeptide described in WO 2015/095392 A1, which is hereby incorporated by reference in its entirety. In some embodiments, a polypeptide complex according to the present disclosure is a polypeptide complex described in WO 2015/095392 A1. In some embodiments, a polypeptide is a constituent polypeptide of an antigen-binding polypeptide complex described in WO 2015/095392 A1.

In some embodiments, a polypeptide complex according to the present disclosure is forimtamig. Forimtamig (also known as RG6234 or RO7425781) is a GPRC5DxCD3 T-cell-engaging bispecific antibody formed by association between (i) a polypeptide having the amino acid sequence of SEQ ID NO:93, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:94, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:95, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:116.

**Fe concentrations**

Aspects and embodiments of the present disclosure the production of a polypeptide by culturing cells comprising nucleic acid encoding the polypeptide in the presence of certain concentrations of iron (Fe).

The present disclosure is based in part on the inventors' discovery that culturing cells comprising nucleic acid encoding polypeptides comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation in cell culture medium comprising relatively low Fe concentration favours the production of polypeptides comprising the non-hydroxylated form.

By way of illustration, the experimental examples of the present disclosure demonstrate that culturing cells comprising nucleic acid encoding polypeptides comprising an amino acid sequence having a lysine residue susceptible to hydroxylation, while maintaining a relatively low concentration of Fe in the cell culture, increases the proportion of polypeptides comprising lysine and decreases the proportion of polypeptides comprising hydroxylysine and at the relevant position, among the expressed polypeptides. Without wishing to be bound by any particular theory, the inventors consider that the relative increase in the proportion of polypeptides comprising lysine (rather than hydroxylysine) at the relevant position may arise as a consequence of the reduced availability of $Fe^{2+}$ to serve as a cofactor for the lysyl hydroxylases that catalyse the hydroxylation of lysine to hydroxylysine. The inventors reason that reduced availability of $Fe^{2+}$ would affect prolyl hydroxylases in the same way, as they are similarly 2OG dioxygenases that require $Fe^{2+}$ as a cofactor.

Accordingly, the present disclosure provides the production of a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation, and/or including a proline residue susceptible to hydroxylation, from cells comprising nucleic acid encoding the polypeptide in cell culture, wherein the concentration of Fe ions (*e.g.* $Fe^{2+}$, $Fe^{3+}$) in the cell culture medium is provided and/or maintained at low levels (*e.g.* <60 μM, <40 μM, <15 μM).

Herein, where reference is made to a concentration of Fe ions, it is to be understood that the concentration takes into account all Fe ions – including ferrous ($Fe^{2+}$) and ferric ($Fe^{3+}$) ions – in the relevant solution (*i.e.* cell culture medium).

Techniques for determining the iron concentration of a given solution (*e.g.* cell culture medium) are known to the skilled person, and are described *e.g.* in Mohite, Asian J. Research Chem. (2011) 4(3): 348-361.

They include colorimetric methods, atomic absorption spectroscopy (AAS), inductively-coupled plasma mass spectrometry (ICP-MS), inductively-coupled plasma optical emission spectrometry (ICP-OES), flame emission spectroscopy, X-ray fluorescence and electrochemical methods. Colorimetric methods include analysis by ferrozine assay (see *e.g.* Viollier et al., Applied Geochemistry (2000) 15(6): 785-790), bathophenanthroline assay and triazine assay (see *e.g.* Verschoor and Molot, Limnol. Oceanogr.: Methods (2013) 11: 113-112). Ferrozine, bathophenanthroline and triazine each react with Fe ions to form coloured complexes, which can be detected spectrophotometrically. The Iron Bio test kit (Roche, Cat #06990045001) provides reagents and instructions for evaluating the iron concentration of a solution in a ferrozine assay, using a Cedex Bio or Cedex Bio HT Analyzer. AAS-based methods include flame AAS (see *e.g.* Wu *et al.*, Applied Spectroscopy Reviews (2009) 44(5):411-437) and graphite furnace AAS (see *e.g.* Borges, "Graphite Furnace Atomic Absorption Spectrometry", in "Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation"; Wiley, 2022), both of which involve atomizing the sample and measuring the absorbance of light at the characteristic wavelength for iron. ICP-MS is described *e.g.* in Wilschefski and Baxter, Clin Biochem Rev. (2019) 40(3): 115-133 and Michalke *et al.*, Front. Chem. (2019) 7: 136, and can measure trace levels of iron in a sample by ionizing the sample and measuring the mass-to-charge ratio of the ions. ICP-OES is described *e.g.* in Oral *et al.*, Atomic Spectroscopy 37(4):142-149 and measures the light emitted at specific wavelengths by atoms or ions within a plasma source, which is indicative of the concentration of iron. Flame emission spectroscopy involves nebulization of a sample into a flame, and measurement of the intensity of the emitted light at a specific wavelength corresponding to iron. X-ray fluorescence is described *e.g.* in Marguí *et al.*, Chemosphere. (2022) 303(Pt 1):135006. Electrochemical techniques for the evaluation of iron concentrations are described in Min, Electroanalysis (2012) 24(8): 1693-1702 and Ahour, Scientific Reports (2023) 13:1557, and include amperometry- (hydrodynamic amperometry) and voltammetry- (*e.g.* adsorptive stripping voltammetry (AdSV), cyclic voltammetry, square wave voltammetry) based methods. Such methods involve applying a potential to a sample and measuring the resulting current, which is related to the concentration of iron in the sample.

In some aspects and embodiments of the present disclosure, the iron concentration of a given cell culture may be expressed in terms of the available amount of Fe per cell, which is calculated as the amount of Fe in the cell culture medium divided by the number of cells in the culture.

Techniques for determining the iron content of cells are described *e.g.* in Abbasi *et al.*, Scientific Reports (2021) 11: 6008, Kakhlon and Cabantchik, Free Radic Biol Med. (2002) 33(8):1037-1046 and Pourcelot *et al.*, Biochim Biophys Acta. (2015) 1853(7):1596-1605 and Amor *et al.*, Environ Microbiol. (2020) 22(3): 823–831. The mean concentration of Fe in a given population of cells (*e.g.* cells of a given cell culture) can be determined by measuring the concentration of Fe in a plurality of cells sampled from the population, and determining the average (mean) concentration of Fe of the sampled cells.

In aspects and embodiments of the present disclosure, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation in cell culture

medium having an Fe ion concentration of less than 60 μM, *e.g.* less than 40 μM. In some aspects and embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation in cell culture medium having an Fe ion concentration of less than 45 μM, *e.g.* less than 15 μM. In some embodiments, the cell culture medium has an Fe ion concentration of one of ≤55 μM, ≤50 μM, ≤45 μM, ≤40 μM, ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM. In some embodiments, the cell culture medium has an Fe ion concentration of one of ≤45 μM, ≤30 μM, or ≤15 μM. In some embodiments, the cell culture medium has an Fe ion concentration of ≤10 μM. In some embodiments, the cell culture medium has an Fe ion concentration of ≤15 μM. In some embodiments, the cell culture medium has an Fe ion concentration of >0 μM. In some embodiments, the cell culture medium has an Fe ion concentration of one of >0 μM and ≤55 μM, >0 μM and ≤50 μM, >0 μM and ≤45 μM, >0 μM and ≤40 μM, >0 μM and ≤37.5 μM, >0 μM and ≤35 μM, >0 μM and ≤32.5 μM, >0 μM and ≤30 μM, >0 μM and ≤27.5 μM, >0 μM and ≤25 μM, >0 μM and ≤22.5 μM, >0 μM and ≤20 μM, >0 μM and ≤17.5 μM, >0 μM and ≤15 μM, >0 μM and ≤12.5 μM, >0 μM and ≤10 μM, >0 μM and ≤7.5 μM, >0 μM and ≤5 μM, or >0 μM and ≤2.5 μM. In some embodiments, the cell culture medium has an Fe ion concentration of one of>0 μM and ≤45 μM, >0 μM and ≤30 μM, or >0 μM and ≤15 μM.

In aspects and embodiments of the present disclosure, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation under conditions such that the concentration of Fe in the cell culture is <1.5 fmol/cell. In some aspects and embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation under conditions such that the concentration of Fe in the cell culture is ≤1.125 fmol/cell, *e.g.* ≤0.375 fmol/cell. In some embodiments, the concentration of Fe in the cell culture is one of ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell. In some embodiments, the concentration of Fe in the cell culture is one of ≤1.125 fmol/cell, ≤0.75 fmol/cell or ≤0.375 fmol/cell. In some embodiments, the concentration of Fe in the cell culture is ≤1 fmol/cell. In some embodiments, the concentration of Fe in the cell culture is ≤0.375 fmol/cell. In some embodiments, the concentration of Fe in the cell culture is >0 fmol/cell. In some embodiments, the concentration of Fe in the cell culture is one of >0 fmol/cell and ≤1.4 fmol/cell, >0 fmol/cell and ≤1.3 fmol/cell, >0 fmol/cell and ≤1.2 fmol/cell, >0 fmol/cell and ≤1.1 fmol/cell, >0 fmol/cell and ≤1 fmol/cell, >0 fmol/cell and ≤0.9 fmol/cell, >0 fmol/cell and ≤0.8 fmol/cell, >0 fmol/cell and ≤0.7 fmol/cell, >0 fmol/cell and ≤0.6 fmol/cell, >0 fmol/cell and ≤0.5 fmol/cell, >0 fmol/cell and ≤0.4 fmol/cell, >0 fmol/cell and ≤0.3 fmol/cell, >0 fmol/cell and ≤0.2 fmol/cell, or >0 fmol/cell and ≤0.1 fmol/cell. In some embodiments, the concentration of Fe in the cell culture is one of >0 fmol/cell and ≤1.125 fmol/cell, >0 fmol/cell and ≤0.75 fmol/cell, or >0 fmol/cell and ≤0.375 fmol/cell.

In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation

and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration of less than 60 μM, *e.g.* less than 40 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration equal to or less than 45 μM, *e.g.* less than 15 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration of one of ≤55 μM, ≤50 μM, ≤45 μM, ≤40 μM, ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration of one of ≤45 μM, ≤30 μM, or ≤15 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration of ≤10 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration of ≤15 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration of one of >0 μM and ≤55 μM, >0 μM and ≤50 μM, >0 μM and ≤45 μM, >0 μM and ≤40 μM, >0 μM and ≤37.5 μM, >0 μM and ≤35 μM, >0 μM and ≤32.5 μM, >0 μM and ≤30 μM, >0 μM and ≤27.5 μM, >0 μM and ≤25 μM, >0 μM and ≤22.5 μM, >0 μM and ≤20 μM, >0 μM and ≤17.5 μM, >0 μM and ≤15 μM, >0 μM and ≤12.5 μM, >0 μM and ≤10 μM, >0 μM and ≤7.5 μM, >0 μM and ≤5 μM, or >0 μM and ≤2.5 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture in cell culture medium having an Fe ion concentration of one of >0 μM and ≤45 μM, >0 μM and ≤30 μM, or >0 μM and ≤15 μM.

In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is <1.5 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or

including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is ≤1.125 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is one of ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is one of ≤1.125 fmol/cell, ≤0.75 fmol/cell or ≤0.375 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is ≤1 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is ≤0.375 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is one of >0 fmol/cell and ≤1.4 fmol/cell, >0 fmol/cell and ≤1.3 fmol/cell, >0 fmol/cell and ≤1.2 fmol/cell, >0 fmol/cell and ≤1.1 fmol/cell, >0 fmol/cell and ≤1 fmol/cell, >0 fmol/cell and ≤0.9 fmol/cell, >0 fmol/cell and ≤0.8 fmol/cell, >0 fmol/cell and ≤0.7 fmol/cell, >0 fmol/cell and ≤0.6 fmol/cell, >0 fmol/cell and ≤0.5 fmol/cell, >0 fmol/cell and ≤0.4 fmol/cell, >0 fmol/cell and ≤0.3 fmol/cell, >0 fmol/cell and ≤0.2 fmol/cell, or >0 fmol/cell and ≤0.1 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is one of >0 fmol/cell and ≤1.125 fmol/cell, >0 fmol/cell and ≤0.75 fmol/cell, or >0 fmol/cell and ≤0.375 fmol/cell.

Herein, 'the majority of the period of culture' is >50% of the period of culture. By way of illustration, where the period of culture is 14 days, the majority of the period of culture is >7 days out of the 14 day period. In some embodiments, the Fe ion concentration in the culture is maintained at the relevant concentration for the majority of period of culture.

In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%,

≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of less than 60 μM, *e.g.* less than 40 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of equal to or less than 45 μM, *e.g.* less than 15 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of one of ≤55 μM, ≤50 μM, ≤45 μM, ≤40 μM, ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of one of ≤45 μM, ≤30 μM, or ≤15 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of ≤10 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of ≤15 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of one of >0 μM and ≤55 μM, >0 μM and ≤50 μM, >0 μM and ≤45 μM, >0 μM and ≤40 μM, >0 μM and ≤37.5 μM, >0 μM and ≤35 μM, >0 μM and ≤32.5 μM, >0 μM and ≤30 μM, >0 μM and ≤27.5 μM, >0 μM and ≤25 μM, >0 μM and ≤22.5 μM, >0 μM and ≤20 μM, >0 μM and ≤17.5 μM, >0 μM and ≤15 μM, >0 μM and ≤12.5 μM, >0 μM and ≤10 μM, >0 μM and ≤7.5 μM, >0 μM and ≤5 μM, or >0 μM and ≤2.5 μM. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, in cell culture medium having an Fe ion concentration of one of >0 μM and ≤45 μM, >0 μM and ≤30 μM, or >0 μM and ≤15 μM.

In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is <1.5 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is ≤1.125 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is one of ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is one of ≤1.125 fmol/cell, ≤0.75 fmol/cell or ≤0.375 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is ≤1 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is ≤0.375 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is one of >0 fmol/cell and ≤1.4 fmol/cell, >0 fmol/cell and ≤1.3 fmol/cell, >0 fmol/cell and ≤1.2 fmol/cell, >0 fmol/cell and ≤1.1 fmol/cell, >0 fmol/cell and ≤1 fmol/cell, >0 fmol/cell and ≤0.9 fmol/cell, >0 fmol/cell and ≤0.8 fmol/cell, >0 fmol/cell and ≤0.7 fmol/cell, >0 fmol/cell and ≤0.6 fmol/cell, >0 fmol/cell and ≤0.5 fmol/cell, >0 fmol/cell and ≤0.4 fmol/cell, >0 fmol/cell and ≤0.3 fmol/cell, >0 fmol/cell and ≤0.2 fmol/cell, or >0 fmol/cell and ≤0.1 fmol/cell. In some embodiments, the methods comprise culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or

including a proline residue susceptible to hydroxylation for >50%, *e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture, under conditions such that the concentration of Fe in the cell culture is one of >>0 fmol/cell and ≤1.125 fmol/cell, >0 fmol/cell and ≤0.75 fmol/cell, or >0 fmol/cell and ≤0.375 fmol/cell.

In some aspects and embodiments of the present disclosure, the cell culture may be established (*i.e.* 'set up') in cell culture medium having an Fe ion concentration of less than 60 μM, *e.g.* less than 40 μM. In some aspects and embodiments of the present disclosure, the cell culture may be established in cell culture medium such that the concentration of Fe in the cell culture is <1.5 fmol/cell. The methods may comprise 'seeding' the cells in cell culture medium having a specified Fe ion concentration. It will be appreciated that 'establishing' a cell culture or 'seeding' cells comprises contacting cells with cell culture medium, *e.g.* within an appropriate cell culture vessel.

Accordingly, in some aspects and embodiments of the present disclosure, the methods comprise seeding cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation in cell culture medium having an Fe ion concentration of less than 60 μM, *e.g.* less than 40 μM. In some aspects and embodiments, the methods comprise seeding cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation in cell culture medium having an Fe ion concentration of less than 45 μM, *e.g.* less than 15 μM. In some embodiments, the cells are seeded in cell culture medium having an Fe ion concentration of one of ≤55 μM, ≤50 μM, ≤45 μM, ≤40 μM, ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM. In some embodiments, the cells are seeded in cell culture medium having an Fe ion concentration of one of ≤45 μM, ≤30 μM, or ≤15 μM. In some embodiments, the cells are seeded in cell culture medium having an Fe ion concentration of ≤10 μM. In some embodiments, the cells are seeded in cell culture medium having an Fe ion concentration of ≤15 μM. In some embodiments, the cells are seeded in cell culture medium having an Fe ion concentration of one of >0 μM and ≤55 μM, >0 μM and ≤50 μM, >0 μM and ≤45 μM, >0 μM and ≤40 μM, >0 μM and ≤37.5 μM, >0 μM and ≤35 μM, >0 μM and ≤32.5 μM, >0 μM and ≤30 μM, >0 μM and ≤27.5 μM, >0 μM and ≤25 μM, >0 μM and ≤22.5 μM, >0 μM and ≤20 μM, >0 μM and ≤17.5 μM, >0 μM and ≤15 μM, >0 μM and ≤12.5 μM, >0 μM and ≤10 μM, >0 μM and ≤7.5 μM, >0 μM and ≤5 μM, or >0 μM and ≤2.5 μM. In some embodiments, the cells are seeded in cell culture medium having an Fe ion concentration of one of >0 μM and ≤45 μM, >0 μM and ≤30 μM, or >0 μM and ≤15 μM.

In some aspects and embodiments according to the present disclosure, the methods comprise maintaining the Fe ion concentration within the cell culture medium at less than 60 μM (*e.g.* less than 40 μM, *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM) for the majority of the period of culture (*e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture). In some aspects and embodiments, the methods comprise maintaining the Fe ion

concentration within the cell culture medium at less than 45 μM (*e.g.* ≤30 μM, *e.g.* ≤15 μM) for the majority of the period of culture (*e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture).

In some aspects and embodiment present disclosure, the methods comprise seeding cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation in cell culture medium such that the concentration of Fe in the cell culture is <1.5 fmol/cell. In some aspects and embodiments, the methods comprise seeding cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation and/or including a proline residue susceptible to hydroxylation in cell culture medium such that the concentration of Fe in the cell culture is ≤1.125 fmol/cell. In some embodiments, the cells are seeded in cell culture medium such that the concentration of Fe in the cell culture is one of ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell. In some embodiments, the cells are seeded in cell culture medium such that the concentration of Fe in the cell culture is one of ≤1.125 fmol/cell, ≤0.75 fmol/cell or ≤0.375 fmol/cell. In some embodiments, the cells are seeded in cell culture medium such that the concentration of Fe in the cell culture is <1 fmol/cell. In some embodiments, the cells are seeded in cell culture medium such that the concentration of Fe in the cell culture is ≤0.375 fmol/cell. In some embodiments, the cells are seeded in cell culture medium such that the concentration of Fe in the cell culture is one of >0 fmol/cell and ≤1.4 fmol/cell, >0 fmol/cell and ≤1.3 fmol/cell, >0 fmol/cell and ≤1.2 fmol/cell, >0 fmol/cell and ≤1.1 fmol/cell, >0 fmol/cell and ≤1 fmol/cell, >0 fmol/cell and ≤0.9 fmol/cell, >0 fmol/cell and ≤0.8 fmol/cell, >0 fmol/cell and ≤0.7 fmol/cell, >0 fmol/cell and ≤0.6 fmol/cell, >0 fmol/cell and ≤0.5 fmol/cell, >0 fmol/cell and ≤0.4 fmol/cell, >0 fmol/cell and ≤0.3 fmol/cell, >0 fmol/cell and ≤0.2 fmol/cell, or >0 fmol/cell and ≤0.1 fmol/cell. In some embodiments, the cells are seeded in cell culture medium such that the concentration of Fe in the cell culture is one of >0 fmol/cell and ≤1.125 fmol/cell, >0 fmol/cell and ≤0.75 fmol/cell, or >0 fmol/cell and ≤0.375 fmol/cell.

In some aspects and embodiments according to the present disclosure, the methods comprise maintaining the Fe ion concentration within the cell culture medium such that the concentration of Fe in the cell culture is <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell) for the majority of the period of culture (*e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture). In some aspects and embodiments, the methods comprise maintaining the Fe ion concentration within the cell culture medium such that the concentration of Fe in the cell culture is ≤1.125 fmol/cell (*e.g.* ≤0.75 fmol/cell, *e.g.* ≤0.375 fmol/cell) for the majority of the period of culture (*e.g.* one of ≥60%, ≥70%, ≥80%, ≥90% or ≥95% of the period of culture, or for the duration of the period of culture).

The Fe ion concentration in the culture medium of a given cell culture naturally decreases over time (*i.e.* where further Fe is not added to the culture), as a consequence of uptake and utilisation by the cells in culture.

The Fe ion concentration in the cell culture medium of a given cell culture may be maintained below a certain threshold (*e.g.* <60 μM; *e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM), and/or the concentration of Fe in the cell culture may be maintained below a certain threshold (*e.g.* <1.5 fmol/cell; *e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell) either by not adding Fe or Fe-containing compound(s) to the cell culture during the period of culture, or by only introducing Fe/Fe-containing compound(s) to the cell culture during the period of culture such that the Fe ion concentration in the cell culture medium/ concentration of Fe in the cell culture does not exceed the relevant threshold. For example, in embodiments wherein the culture is a fed-batch or continuous culture, cell culture medium added to the cell culture during the period of culture may lack Fe/Fe-containing compound(s) (*i.e.* may be Fe-free), or may comprise Fe/Fe-containing compound(s) in an amount such that following addition of the cell culture medium to the culture, the Fe ion concentration in the cell culture medium of the cell culture does not exceed the relevant threshold (*i.e.* <60 μM; <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM) and/or such that following addition of the cell culture medium to the culture, the concentration of Fe in the cell culture does not exceed the relevant threshold (*i.e.* is <1.5 fmol/cell; *e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

Conversely, the Fe ion concentration in the cell culture medium of a given cell culture may be maintained at or above a certain threshold value (*e.g.* >0 μM) and/or the concentration of Fe in the cell culture may be maintained above a certain threshold value (*e.g.* >0 fmol/cell) by adding Fe/Fe-containing compound(s) to the cell culture during the period of culture at a concentration such that following addition, the Fe ion concentration in the cell culture medium of the cell culture/the concentration of Fe in the cell culture is at or above the relevant threshold value. For example, in embodiments wherein the culture is a fed-batch or continuous culture, cell culture medium added to the cell culture during the period of culture may comprise Fe/Fe-containing compound(s) at a concentration such that following addition of the cell culture medium to the culture, the Fe ion concentration in the cell culture medium of the cell culture is >0 μM, and/or such that following addition of the cell culture medium to the culture, the concentration of Fe in the cell culture is >0 fmol/cell.

Where Fe or Fe-containing compound(s) are provided to a cell culture in accordance with the present disclosure, they may be provided in the form of cell culture medium comprising Fe (*e.g.* in the basal

formulation) or Fe-containing compound(s), or in the form of a cell culture medium supplement or additive comprising Fe or Fe-containing compound(s). For example, Fe may be provided in the form of ferric nitrate, ferrous sulfate and/or ferric citrate. Cell culture medium comprising Fe in the basal formulation is described herein.

Ferric nitrate may be provided in cell culture medium comprising ferric nitrate. Cell culture media comprising ferric nitrate include Dulbecco's Modified Eagle's Medium (DMEM), Glascow Modified Eagle's Medium (GMEM), H-Y Medium, Medium 199, Williams Medium E and DMEM/Ham's Nutrient Mixture F-12 (50:50). Ferrous sulfate may be provided in cell culture medium comprising ferrous sulfate. Cell culture media comprising ferrous sulfate include F-12 Coon's Modification, Ham's F-10, Ham's F-12, Ham's F-12 Kaighn's Modification (F12K), MCDB Media and DMEM/Ham's Nutrient Mixture F-12 (50:50).

**Cell comprising nucleic acid encoding polypeptides according to the present disclosure**

Methods for producing a polypeptide according to the present disclosure (*i.e.* a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation) comprise culturing cells comprising nucleic acid encoding the polypeptide. Methods for producing a polypeptide complex according to the present disclosure (*i.e.* comprising a constituent polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation) comprise culturing cells comprising nucleic acid encoding the constituent polypeptides of the polypeptide complex.

The present disclosure provides methods comprising culturing cells comprising a nucleic acid, or a plurality of nucleic acids, encoding a polypeptide according to the present disclosure. In some embodiments, the nucleic acid(s) comprise or consist of DNA and/or RNA.

The polypeptides of the present disclosure may be produced within a cell by translation of RNA encoding the polypeptides. The polypeptides of the present disclosure may be produced within a cell by transcription from nucleic acid encoding the polypeptides, and subsequent translation of the transcribed RNA.

In some embodiments, the cell comprises a nucleic acid, or a plurality of nucleic acids, encoding a polypeptide according to the present disclosure, and one or more additional polypeptides with which the polypeptide according to the present disclosure associates to form a polypeptide complex. That is, in some embodiments, the cell comprises a nucleic acid, or a plurality of nucleic acids, encoding the constituent polypeptides of a polypeptide complex, wherein one or more of the constituent polypeptides of the polypeptide complex is a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation. For example, in some embodiments, the cell comprises a nucleic acid, or a plurality of nucleic acids, encoding an antigen-binding polypeptide complex, wherein one or more of the constituent polypeptides of the antigen-binding polypeptide complex is a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation.

In some embodiments, the nucleic acid(s) may be, or may be comprised/contained in, a vector, or a plurality of vectors. A 'vector' as used herein is a nucleic acid molecule used as a vehicle to transfer exogenous nucleic acid into a cell. Thus a cell according to the present disclosure may comprise a vector, or plurality of vectors, comprising the nucleic acid or plurality of nucleic acids according to the present disclosure.

A polypeptide complex according to the present disclosure may be encoded by nucleic acid provided in non-identical vectors. For example, one or more of the constituent polypeptides of the polypeptide complex may be encoded by nucleic acid of a first vector, and one or more of the constituent polypeptides of the polypeptide complex may be encoded by nucleic acid of a second vector. In some embodiments, a vector may comprise multiple, non-overlapping copies of nucleic acid encoding a constituent polypeptide of a polypeptide complex according to the present disclosure.

A vector may facilitate delivery of nucleic acid(s) encoding the polypeptides according to the present disclosure to a cell. A vector may be an expression vector, comprising elements required for expressing the polypeptides according to the present disclosure. A vector may comprise elements facilitating integration of nucleic acid(s) into the genomic DNA of cell into which the vector is introduced.

A vector may be a vector for expression of the nucleic acid in the cell (*i.e.* an expression vector). Such vectors may include a promoter sequence operably linked to a nucleotide sequence encoding a polypeptide according to the present disclosure. A vector may also include a termination codon (*i.e.* 3' in the nucleotide sequence of the vector to the nucleotide sequence encoding a polypeptide) and expression enhancers. Any suitable vectors, promoters, enhancers and termination codons known in the art may be used to express a peptide or polypeptide from a vector according to the present disclosure.

The term 'operably linked' may include the situation where nucleic acid encoding a polypeptide according to the present disclosure and regulatory nucleic acid sequence(s) (*e.g.* a promoter and/or enhancers) are covalently linked in such a way as to place the expression of the nucleic acid encoding a polypeptide under the influence or control of the regulatory nucleic acid sequence(s) (thereby forming an expression cassette). Thus, a regulatory sequence is operably linked to the selected nucleic acid sequence if the regulatory sequence is capable of effecting transcription of the nucleic acid sequence. The resulting transcript(s) may then be translated into the desired polypeptide(s).

Vectors contemplated in connection with the present disclosure include DNA vectors, RNA vectors, plasmids (*e.g.* conjugative plasmids (*e.g.* F plasmids), non-conjugative plasmids, R plasmids, col plasmids, episomes), viral vectors (*e.g.* retroviral vectors, *e.g.* gammaretroviral vectors (*e.g.* murine Leukemia virus (MLV)-derived vectors, *e.g.* SFG vector), lentiviral vectors, adenovirus vectors, adeno-associated virus vectors, vaccinia virus vectors and herpesvirus vectors), transposon-based vectors, and artificial chromosomes (*e.g.* yeast artificial chromosomes), *e.g.* as described in Maus *et al.*, Annu Rev Immunol. (2014) 32:189-225 and Morgan and Boyerinas, Biomedicines (2016) 4:9, which are both hereby

incorporated by reference in their entirety. In some embodiments, a vector according to the present disclosure is a lentiviral vector.

In some embodiments, the vector may be a eukaryotic vector, *i.e.* a vector comprising the elements necessary for expression of protein from the vector in a eukaryotic cell. In some embodiments, the vector may be a mammalian vector, *e.g.* comprising a cytomegalovirus (CMV) or SV40 promoter to drive protein expression.

It will be appreciated that where cells are referred to herein in the singular (*i.e.* 'a/the cell'), pluralities/populations of such cells are also contemplated.

A cell according to the present disclosure may be a eukaryotic cell, *e.g.* a mammalian cell. The mammal may be a primate (rhesus, cynomolgous, non-human primate or human) or a non-human mammal (*e.g.* rabbit, guinea pig, rat, mouse or other rodent (including any animal in the order Rodentia), cat, dog, pig, sheep, goat, cattle (including cows, *e.g.* dairy cows, or any animal in the order Bos), horse (including any animal in the order Equidae), donkey, and non-human primate). In preferred embodiments, the cell is a human cell.

In some embodiments, the cell is, or is derived from, a cell type commonly used for the expression of polypeptides for use in therapy in humans. Exemplary cells are described *e.g.* in Kunert and Reinhart, Appl Microbiol Biotechnol. (2016) 100:3451–3461 (hereby incorporated by reference in its entirety), and include *e.g.* CHO (CVCL_0213), HEK 293 (CVCL_0045), PER.C6 (CVCL_G704), NS0 (CVCL_3940) and BHK (CVCL_1914) cells. In preferred embodiments, the cell is, or is derived from, a CHO cell.

It will be appreciated that the cell is preferably a lysyl hydroxylase- and/or prolyl hydroxylase-expressing cell. In some embodiments, the cell expresses a PLOD (*e.g.* PLOD1, PLOD2 and/or PLOD3), JMJD4, JMJD6 and/or JMJD7. In some embodiments, the cell expresses a PLOD (*e.g.* PLOD1, PLOD2 and/or PLOD3). In some embodiments, the cell expresses procollagen-proline 4-dioxygenase, procollagen-proline 3-dioxygenase and/or proline 3-hydroxylase.

Any suitable method may be employed to produce a cell to be employed in the methods of the present disclosure. Such methods may comprise nucleic acid transfer for permanent (*i.e.* stable) or transient expression of the transferred nucleic acid. In some embodiments, following introduction into a cell, nucleic acid(s) encoding the polypeptide(s) of interest may be integrated into or form part of the genomic DNA of the cell. In some embodiments, following introduction into a cell, nucleic acid(s) encoding the polypeptide(s) of interest may be maintained extrachromosomally.

Any suitable genetic engineering platform may be used, and include gammaretroviral vectors, lentiviral vectors, adenovirus vectors, DNA transfection, transposon-based gene delivery and RNA transfection, for example as described in Maus *et al.*, Annu Rev Immunol. (2014) 32:189-225, hereby incorporated by reference in its entirety. Methods also include those described *e.g.* in Wang and Rivière Mol Ther

Oncolytics. (2016) 3:16015, which is hereby incorporated by reference in its entirety. Suitable methods for introducing nucleic acid(s)/vector(s) into cells include transduction, transfection and electroporation.

**Methods for producing polypeptides**

Aspects and embodiments of the present disclosure relate to methods for producing a polypeptide, and to methods for producing a polypeptide complex.

The methods comprise culturing cells comprising nucleic acid encoding the polypeptide(s), under conditions suitable for expression of the polypeptide(s). In embodiments relating to the production of polypeptide complexes, the methods may comprise culturing cells under conditions suitable for association of the constituent polypeptides of a polypeptide complex with one another, to form the polypeptide complex.

Suitable culture conditions will be apparent to a person skilled in the art. Suitable culture conditions include conditions for the expression of antibodies from mammalian cells, as described *e.g.* in Birch and Racher, Adv Drug Deliv Rev. (2006) 58(5-6):671-85 and Li *et al.*, MAbs (2010) 2(5):466-477, both of which are hereby incorporated by reference in their entirety. Suitable culture conditions also include conditions suitable for the maintenance of cells of the CHO-K1 cell line (ATCC, Cat. No. CCL-61; CVCL_0214)) in *in vitro* culture.

The cells are cultured in cell culture medium comprising amino acids, vitamins, inorganic salts and sugars. In some embodiments, the cell culture medium comprises amino acids selected from: L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cystine/L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. In some embodiments, the cell culture medium comprises vitamins and/or vitaminoids selected from: D-biotin, choline chloride, D-calcium pantothenate, folic acid, *myo*-inositol, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, and vitamin B12. In some embodiments, the cell culture medium comprises further components selected from: calcium chloride, hypoxanthine, linoleic acid, putrescine hydrochloride, pyruvic acid, magnesium sulfate, potassium chloride, sodium bicarbonate, sodium chloride, sodium phosphate monobasic, thioctic acid and thymidine. In some embodiments, the cell culture medium comprises D-glucose.

In some embodiments, the cell culture medium comprises ferric nitrate and/or ferrous sulfate. In some embodiments, the cell culture medium lacks ferric nitrate and/or ferrous sulfate.

In some embodiments, the cell culture medium is serum-free. In some embodiments, the cell culture medium is protein-free. In some embodiments, the cell culture medium is DMEM/F12 (*e.g.* DMEM/F12 having the composition of ThermoFisher Scientific Cat No. 11320033) or a functional equivalent thereof. A functional equivalent of DMEM within the meaning for this invention refers to a medium that has a composition similar to DMEM/F12 and supports the growth of an exemplary culture of recombinant CHO cells expressing a protein of interest. In some embodiments, the cell culture medium has a composition

similar to DMEM/F12. In some embodiments, the cell culture medium comprises: (i) amino acids, *e.g.* glycine, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-cystine, L-glutamic acid, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine; (ii) vitamins and/or vitaminoids, *e.g.* biotin, choline chloride, D-calcium pantothenate, folic acid, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, vitamin B12 and i-inositol; calcium chloride, cupric sulfate, ferric nitrate, ferric sulfate, magnesium chloride, magnesium sulfate, potassium chloride, sodium bicarbonate, sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic, and zinc sulfate; and D-glucose (dextrose), hypoxanthine Na, linoleic acid, lipoic acid, phenol red, putrescine 2HCl, sodium pyruvate and thymidine.

In some embodiments, the cell culture medium is a cell culture medium suitable for the culture of mammalian cells. Such cell culture media include Roswell Park Memorial Institute (RPMI) 1640 medium (*e.g.* having the composition of ThermoFisher Scientific Cat No. 11875093), Dulbecco's Modified Eagle Medium (DMEM; *e.g.* having the composition of ThermoFisher Scientific Cat No. 11965084), F-12 medium (*e.g.* having the composition of ThermoFisher Scientific Cat No. 11765047), DMEM/F12 (*e.g.* having the composition of ThermoFisher Scientific Cat No. 11320033), CD-CHO medium (*e.g.* having the composition of ThermoFisher Scientific Cat No. 10743011), PowerCHO medium (*e.g.* having the composition of Lonza Cat No. BELN12-771Q) and HyClone™ ActiPro™ cell culture medium (*e.g.* having the composition of Cytiva Cat No. SH31039.01).

In preferred embodiments, the cell culture medium is suitable cell culture medium for the culture of cells for the production of molecules to be employed in therapy in humans. Such culture medium includes *e.g.* EX-CELL Advanced CHO Fed-Batch Medium (*e.g.* having the composition of Sigma-Aldrich Cat No. 14366C).

In some embodiments, the cell culture medium is a cell culture medium lacking iron in the basal formulation. Such cell culture media include alpha-MEM medium (also known as MEMα; *e.g.* having the composition of ThermoFisher Scientific Cat No. 21010046) Ames' Medium (*e.g.* having the composition of Sigma-Aldrich Cat No. A1420), Basal Medium Eagle (BME; *e.g.* having the composition of ThermoFisher Scientific Cat No. 21010046), BGJb Medium Fitton-Jackson Modification (*e.g.* having the composition of ThermoFisher Scientific Cat No. 12591038), Click's Medium (*e.g.* having the composition of Sigma-Aldrich Cat No. C5572), CMRL-1066 Medium (*e.g.* having the composition of Sigma-Aldrich Cat No. C0422), Fischer's Medium (*e.g.* having the composition of ThermoFisher Scientific Cat No. 21475025), Iscove's Modified Dulbecco's Medium (IMDM; *e.g.* having the composition of Sigma-Aldrich Cat No. I3390), L-15 (*e.g.* having the composition of ThermoFisher Scientific Cat No. 11415064), McCoy's 5A Modified Medium (*e.g.* having the composition of ThermoFisher Scientific Cat No. 16600082), RPMI-1640 (*e.g.* having the composition of ThermoFisher Scientific Cat No. 11875093), Swim's S-77 Medium (*e.g.* having the composition of Sigma-Aldrich Cat No. S2513) and Waymouth Medium MB (*e.g.* having the composition of ThermoFisher Scientific Cat No. 11220035).

In some embodiments, the methods comprise:

culturing cells comprising nucleic acid for expressing a polypeptide according to the present disclosure in cell culture medium comprising iron (*e.g.* comprising iron in the basal formulation), or in cell culture medium having an Fe ion concentration ≥40 μM, *e.g.* ≥45 μM, ≥60 μM; and

subsequently culturing the cells in cell culture medium lacking iron (*e.g.* lacking iron in the basal formulation), or in cell culture medium having an Fe ion concentration <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

In some embodiments, the methods comprise:

culturing cells comprising nucleic acid for expressing a polypeptide according to the present disclosure in cell culture medium such that the concentration of Fe in the cell culture is ≥1.5 fmol/cell; and

subsequently culturing the cells in cell culture medium such that the concentration of Fe in the cell culture is <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

In accordance with such embodiments, the methods may comprise removing the cell culture medium comprising iron, or cell culture medium having an Fe ion concentration ≥40 μM (*e.g.* ≥45 μM, ≥60 μM), from the cell culture, and replacing it with cell culture medium lacking iron, or cell culture medium having an Fe ion concentration <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

In some embodiments, cell culture medium having an Fe ion concentration ≥40 μM may be adjusted to have an Fe ion concentration 60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM) by addition of cell culture medium lacking iron, or cell culture medium having an Fe ion concentration <60 μM (*e.g.* <45 μM, <40 μM), thereby reducing the Fe ion concentration of the cell culture medium of the cell culture to below the relevant threshold. That is, cell culture medium having an Fe ion concentration ≥40 μM (*e.g.* ≥45 μM, ≥60 μM) may be diluted with cell culture medium lacking iron, or cell culture medium having an Fe ion concentration <60 μM (*e.g.* <45 μM, <40 μM), to achieve have an Fe ion concentration <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

In some embodiments, cell culture medium lacking iron, or cell culture medium having an Fe ion concentration <60 μM (*e.g.* <45 μM, <40 μM), may be added to the culture of cells in culture having a mean Fe concentration ≥1.5 fmol/cell, in order to achieve a concentration of Fe in the cell culture of <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8

fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

Culture of the cells for a period of time in cell culture medium comprising iron, or cell culture medium having an Fe ion concentration ≥40 μM (*e.g.* ≥45 μM, ≥60 μM), may provide the cells with sufficient iron for required for essential cellular processes, during subsequent culture in cell culture medium lacking iron, or cell culture medium having an Fe ion concentration <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM). That is, an initial period of culture in cell culture medium comprising iron, or cell culture medium having an Fe ion concentration ≥40 μM, may provide the cells in culture with sufficient iron, such that essential cellular processes are not substantially impaired during a subsequent period of culture in cell culture medium lacking iron, or cell culture medium having an Fe ion concentration <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

In some embodiments, the methods comprise culturing cells comprising nucleic acid for expressing a polypeptide according to the present disclosure in cell culture medium selected from Dulbecco's Modified Eagle's Medium (DMEM), Glascow Modified Eagle's Medium (GMEM), H-Y Medium, Medium 199, Williams Medium E, DMEM/Ham's Nutrient Mixture F-12 (50:50), F-12 Coon's Modification, Ham's F-10, Ham's F-12, Ham's F-12 Kaighn's Modification (F12K) and MCDB Media; and then subsequently culturing the cells in cell culture medium selected from alpha-MEM medium, Ames' Medium, Basal Medium Eagle (BME), BGJb Medium Fitton-Jackson Modification, Click's Medium, CMRL-1066 Medium: Fischer's Medium, Iscove's Modified Dulbecco's Medium (IMDM), L-15, McCoy's 5A Modified Medium, RPMI-1640, Swim's S-77 Medium, Waymouth Medium MB and HyClone™ ActiPro™ cell culture medium.

It will be appreciated that the cells are cultured under suitable environmental conditions.

The cells may be cultured at 29°C to 38°C, *e.g.* 34.5°C to 37.5°C, *e.g.* at one of about 34.5°C ,about 35°C, about 35.5°C, about 36°C, about 36.5°C, about 37°C, or about 37.5°C. In some embodiments, the cells may be cultured at 35°C (±1°C).

The cells may be cultured in 4% to 10% $CO_2$, *e.g.* 5% to 8% $CO_2$.

The cells may be cultured *e.g.* at ≥90% humidity, *e.g.* about 95% humidity.

The cells may be cultured without agitation, or with agitation. Agitation may be at 75 rpm to 175 rpm, *e.g.* 90 rpm to 130 rpm, *e.g.* about 110 rpm.

The pH of the cell culture may be between 6.8 to 7.4, *e.g.* one of about 6.8, 6.9, 7.0, 7.1, 7.2, 7.3 or 7.4. In some embodiments, the pH of the cell culture is about 7.0. In some embodiments, the pH of the cell culture is about 7.2.

Cell culture may be performed in a bioreactor provided with an appropriate supply of nutrients, air/oxygen and/or growth factors. Bioreactors may monitor and control environmental conditions such as pH, oxygen, flow rates into and out of, and agitation within the vessel such that optimum conditions are provided for the cells being cultured.

The culture may be a continuous culture, with a continuous flow of cell culture medium into, and a continuous flow of cultured cells from, the cell culture vessel. In some embodiments, the culture may be a batch culture, employing a closed system and a finite amount of cell culture medium. In some embodiments, the culture may be a fed-batch culture, in which cell culture medium is supplied to the cell culture vessel during the culture, but wherein unlike a continuous culture, material is not removed from the cell culture vessel during the course of cell culture.

In some embodiments, the methods comprise adding one or more components to the cell culture during the period of culture. In some embodiments, the methods comprise adding cell culture medium to the cell culture during the period of culture. In some embodiments, the methods comprise adding nutrients to the cell culture during the period of culture. In some embodiments, the methods comprise adding amino acids to the cell culture during the period of culture.

In some embodiments, components added to the cell culture during the period of culture as described in the preceding paragraph do not comprise ferric nitrate or ferrous sulfate. In some embodiments, components added to the cell culture during the period of culture as described in the preceding paragraph do not comprise Fe or Fe-containing compound(s). That is, in some embodiments, cell culture medium, nutrients and/or amino acids added to the cell culture during the period of culture do not comprise Fe or Fe-containing compound(s) (*i.e.* are Fe-free).

Following a period of cell culture, expressed polypeptides or polypeptide complexes of interest may be separated/isolated/purified, *e.g.* from the cell culture or cell culture medium.

In some embodiments, polypeptides or polypeptide complexes of interest are separated/isolated/purified from cell culture medium having an Fe ion concentration <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM). In some embodiments, polypeptides or polypeptide complexes of interest are not separated/isolated/purified from cell culture medium having an Fe ion concentration ≥40 μM. In some embodiments, the method comprises separating/isolating/purifying expressed polypeptides or polypeptide complexes of interest from cells cultured in cell culture medium lacking iron, or cell culture medium having an Fe ion concentration <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20

μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM, *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

In some embodiments, polypeptides or polypeptide complexes of interest are separated/isolated/purified from cell culture medium of cells in a cell culture having a concentration of Fe <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell). In some embodiments, polypeptides or polypeptide complexes of interest are not separated/isolated/purified from cell culture medium of cells in a cell culture having a concentration of Fe ≥1.5 fmol/cell. In some embodiments, the method comprises separating/isolating/purifying expressed polypeptides or polypeptide complexes of interest from cells in a cell culture having a concentration of Fe <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

The expressed polypeptides or polypeptide complexes of interest may be secreted into the cell culture medium from the cells in culture. Secreted polypeptides/polypeptide complexes can be collected by partitioning culture medium from the cells (*e.g.* by centrifugation), and subsequently isolating/purifying the secreted polypeptides/polypeptide complexes from the cell culture medium.

Techniques for the isolation/purification of expressed polypeptides/polypeptide complexes of interest from compositions comprising heterogeneous populations of proteins are well known to those of skill in the art, and are described, for example, in Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th Edition), Birch and Racher, Adv Drug Deliv Rev. (2006) 58(5-6):671-85, and Murphy *et al.*, Antibody Technology Journal (2016) 6:17-32, all of which are hereby incorporated by reference in their entirety.

Large-scale purification of polypeptides/polypeptide complexes is commonly based on affinity chromatography, *e.g.* using agents that bind specifically to a moiety of the polypeptides of interest/constituent polypeptide(s) of the polypeptide complexes. Purification may alternatively, or additionally, comprise purification by anion/cation exchange chromatography, hydrophobic interaction chromatography and/or size exclusion chromatography, which are well known to the person skilled in the art.

The various purification steps are designed to remove contaminant proteins from the cells or culture media to ppm levels, and to reduce DNA to ppb levels. Depending on the processes used, there may be additional specific contaminants to be removed (*e.g.* leached protein A/G). Purification may comprise filtration (*e.g.* using a 0.22 μm filter) to remove potential biological contaminants.

In addition to contaminants, it may also be desirable or necessary to remove undesired products such as aggregates and degradation products.

In some embodiments, isolating or purifying a polypeptide/polypeptide complex according to the present disclosure comprises isolation/purification by one or more of: affinity chromatography, (*e.g.* Protein G chromatography or Protein A chromatography), size exclusion chromatography, high-performance liquid chromatography, ultra-performance liquid chromatography, and ion-exchange chromatography. In some embodiments, the methods of the present disclosure comprise separating/isolating/purifying polypeptides/polypeptide complexes by size-exclusion chromatography and/or capillary electrophoresis.

Following isolation/purification, polypeptides/polypeptide complexes may be provided in a suitable buffer, *e.g.* for storage. As used herein, a 'buffer' refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. A composition comprising a polypeptide according to the present disclosure may comprise the polypeptide (*e.g.* in the form of a polypeptide complex comprising the polypeptide) in a buffer.

Examples of suitable buffers include acetate, histidine, histidine-arginine, histidine-methionine and other organic acid buffers. Polypeptides may be buffer exchanged into a buffer of interest by buffer dialysis.

In some embodiments, methods of the present disclosure comprise formulating a polypeptide/polypeptide complex according to the present disclosure to a composition, *e.g.* a pharmaceutical composition. In some embodiments the methods comprise mixing a polypeptide/polypeptide complex, or mixing a composition comprising a polypeptide/polypeptide complex, with a pharmaceutically-acceptable carrier, diluent, excipient or adjuvant.

**Further methods and uses**

The present disclosure provides further methods relating to the production of polypeptides comprising an amino acid sequence including a lysine residue susceptible to hydroxylation, and/or an amino acid sequence including a proline residue susceptible to hydroxylation, and the production of polypeptide complexes comprising such polypeptides.

A method for increasing the yield of a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or increasing the yield of a polypeptide complex comprising such a polypeptide) produced from cells in culture is provided, wherein the method comprises culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, and wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium to <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

Also provided is a method for increasing the yield of a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or increasing the yield of a polypeptide complex comprising such a polypeptide) produced from cells in culture, wherein

the method comprises culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, and wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium such that the concentration of Fe in the cell culture is <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

Also provided is a method for increasing the proportion of polypeptides comprising a lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or increasing the proportion of polypeptide complexes comprising such a polypeptide) among polypeptides (or polypeptide complexes) produced by culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium to <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

Also provided is a method for increasing the proportion of polypeptides comprising a lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or increasing the proportion of polypeptide complexes comprising such a polypeptide) among polypeptides (or polypeptide complexes) produced by culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium such that the concentration of Fe in the cell culture is <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

Also provided is a method for decreasing the yield of a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or decreasing the yield of a polypeptide complex comprising such a polypeptide) produced from cells in culture, wherein the method comprises culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, and wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium to <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

Also provided is a method for decreasing the yield of a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or decreasing the yield of a polypeptide complex comprising such a polypeptide) produced from cells in culture, wherein the method comprises culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, and wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium such that the concentration of Fe in the cell culture is

<1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

Also provided is a method for decreasing the proportion of polypeptides comprising a lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or decreasing the proportion of polypeptide complexes comprising such a polypeptide) among polypeptides (or polypeptide complexes) produced by culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium to <60 μM (*e.g.* <40 μM; *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM).

Also provided is a method for decreasing the proportion of polypeptides comprising a lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or decreasing the proportion of polypeptide complexes comprising such a polypeptide) among polypeptides (or polypeptide complexes) produced by culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex) in cell culture medium, wherein the method comprises maintaining or adjusting the Fe ion concentration in the cell culture medium such that the concentration of Fe in the cell culture is <1.5 fmol/cell (*e.g.* ≤1.4 fmol/cell, ≤1.3 fmol/cell, ≤1.2 fmol/cell, ≤1.1 fmol/cell, ≤1 fmol/cell, ≤0.9 fmol/cell, ≤0.8 fmol/cell, ≤0.7 fmol/cell, ≤0.6 fmol/cell, ≤0.5 fmol/cell, ≤0.4 fmol/cell, ≤0.3 fmol/cell, ≤0.2 fmol/cell or ≤0.1 fmol/cell; *e.g.* ≤1 fmol/cell; *e.g.* ≤1.125 fmol/cell; *e.g.* ≤0.75 fmol/cell; *e.g.* ≤0.375 fmol/cell).

In some embodiments, 'maintaining or adjusting the Fe ion concentration in the cell culture medium' may comprise reducing the Fe ion concentration in the cell culture medium. In such embodiments, the methods may comprise adding cell culture medium having an Fe ion concentration that is lower than the Fe ion concentration of the cell culture medium to which it is added, or adding cell culture medium (or an appropriate buffer, or water) lacking Fe to the cell culture (thereby diluting the Fe ions in the cell culture medium of the cell culture, and consequently reducing the Fe ion concentration).

The present disclosure further provides the use of cell culture medium comprising certain concentrations of Fe ions in methods relating to the production of polypeptides comprising an amino acid sequence including a lysine residue susceptible to hydroxylation, and/or an amino acid sequence including a proline residue susceptible to hydroxylation, and the production of polypeptide complexes comprising such polypeptides.

Also provided is the use of cell culture medium having an Fe ion concentration of less than 60 μM (*e.g.* less than 40 μM, *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM) to increase the yield of a polypeptide comprising an amino acid sequence including

a lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or to increase the yield of a polypeptide complex comprising such a polypeptide) produced from cells in culture.

Also provided is the use of cell culture medium having an Fe ion concentration of less than 60 μM (*e.g.* less than 40 μM, *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM) to increase the proportion of polypeptides comprising a lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or to increase the proportion of polypeptide complexes comprising such a polypeptide) among polypeptides (or polypeptide complexes) produced by culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex).

Also provided is the use of cell culture medium having an Fe ion concentration of less than 60 μM (*e.g.* less than 40 μM, *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM) to decrease the yield of a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or to decrease the yield of a polypeptide complex comprising such a polypeptide) produced from cells in culture.

Also provided is the use of cell culture medium having an Fe ion concentration of less than 60 μM (*e.g.* less than 40 μM, *e.g.* one of ≤37.5 μM, ≤35 μM, ≤32.5 μM, ≤30 μM, ≤27.5 μM, ≤25 μM, ≤22.5 μM, ≤20 μM, ≤17.5 μM, ≤15 μM, ≤12.5 μM, ≤10 μM, ≤7.5 μM, ≤5 μM or ≤2.5 μM; *e.g.* ≤10 μM; *e.g.* ≤45 μM; *e.g.* ≤30 μM; *e.g.* ≤15 μM) to decrease the proportion of polypeptides comprising a lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or to decrease the proportion of polypeptide complexes comprising such a polypeptide) among polypeptides (or polypeptide complexes) produced by culturing cells comprising nucleic acid for expressing the polypeptide (or the polypeptide complex).

**Increased yields and proportions of polypeptides/polypeptide complexes comprising the non-hydroxylated form of lysine/proline residues susceptible to hydroxylation, and reduced yields and proportions of polypeptides/polypeptide complexes comprising the hydroxylated form of lysine/proline residues susceptible to hydroxylation**

As explained herein, culturing cells comprising nucleic acid encoding a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation, and/or an amino acid sequence including a proline residue susceptible to hydroxylation in accordance with the culture conditions described herein increases the yield of the polypeptide comprising the lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or increases the yield of the polypeptide complex comprising such a polypeptide); and/or increases the proportion of polypeptides comprising the lysine/proline residue susceptible to hydroxylation in its non-hydroxylated form (or increases the proportion of polypeptide complexes comprising such polypeptides) among the polypeptides/polypeptide complexes produced by the cells in culture; and/or decreases the yield of the polypeptide comprising the lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or decreases the yield of the polypeptide complex comprising such a polypeptide); and/or decreases the proportion of polypeptides

comprising the lysine/proline residue susceptible to hydroxylation in its hydroxylated form (or decreases the proportion of polypeptide complexes comprising such polypeptides) among the polypeptides/polypeptide complexes produced by the cells in culture.

It will be appreciated that the 'yield' of a given form (*e.g.* a form comprising a lysine/proline residue susceptible to hydroxylation in its hydroxylated or non-hydroxylated form) of a given polypeptide/polypeptide complex refers to the amount/quantity of the given polypeptide/polypeptide complex produced. It will similarly be appreciated that the 'proportion' of a given form of a given polypeptide/polypeptide complex among different forms of a given polypeptide/polypeptide complex refers to the fraction/percentage of the given form of the given polypeptide/polypeptide complex within the total, summed amount/quantity of the different forms of the given polypeptide/polypeptide complex produced.

By way of illustration, within a population of polypeptide complexes comprising a polypeptide comprising an amino acid sequence including a lysine/proline residue susceptible to hydroxylation, the proportion of polypeptide complexes comprising the polypeptide having the lysine/proline residue in its non-hydroxylated form is the fraction/percentage of the total amount/quantity of polypeptide complexes of the population of polypeptide complexes that are polypeptide complexes comprising the polypeptide having the lysine/proline residue in its non-hydroxylated form.

An 'increased' or 'reduced' yield/proportion of a given form of a given polypeptide, or of a polypeptide complex comprising a given form of a given polypeptide, may be increased/reduced relative to the yield/proportion of such polypeptides/polypeptide complexes obtained by culture of cells of the same kind, according to a reference method for expressing polypeptides/polypeptide complexes from cells in culture.

A reference method for expressing polypeptides/polypeptide complexes from cells in culture may be a method in which the cells are not cultured in cell culture medium having an Fe ion concentration of less than 40 μM for the majority of the period of culture. For example, in the reference method the cells may be cultured in cell culture medium having an Fe ion concentration of ≥40 μM for the majority of the period of culture.

A reference method for expressing polypeptides/polypeptide complexes from cells in culture may be a method in which the cells are not cultured for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is <1.5 fmol/cell. For example, in the reference method the cells may be cultured for the majority of the period of culture under conditions such that the concentration of Fe in the cell culture is ≥1.5 fmol/cell.

An 'increased' yield/proportion of a given form of a given polypeptide, or of a polypeptide complex comprising a given form of a given polypeptide, refers to a yield/proportion that is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times,

≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the yield/proportion obtained by culture according to a reference method for expressing polypeptides/polypeptide complexes from cells in culture.

A 'reduced' yield/proportion of a given form of a given polypeptide, or of a polypeptide complex comprising a given form of a given polypeptide, refers to a yield/proportion that is less than 1 times, *e.g.* one of ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the yield/proportion obtained by culture according to a reference method for expressing polypeptides/polypeptide complexes from cells in culture.

In some embodiments, the methods of the present disclosure yield populations of polypeptides comprising an amino acid sequence comprising a lysine/proline residue susceptible to hydroxylation (or yield populations of polypeptide complexes comprising such polypeptides) in which ≤25% of polypeptides of polypeptides comprising the amino acid sequence comprising a lysine/proline residue susceptible to hydroxylation comprise the hydroxylated form of the residue at the relevant position. In some embodiments, ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1% of the polypeptides comprise the hydroxylated form of the residue at the relevant position. In some embodiments, ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10% of the polypeptides comprise the hydroxylated form of the residue at the relevant position. In some embodiments, ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10% of the polypeptides comprise the hydroxylated form of the residue at the relevant position.

In some embodiments, a polypeptide according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:1, and a method for producing the polypeptide (or a method of producing a polypeptide complex comprising the polypeptide) in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1. In some embodiments, a polypeptide according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:2, and a method for producing the polypeptide (or a method of producing a polypeptide complex comprising the polypeptide) in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:2 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:2.

In some embodiments, a polypeptide according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:5, and a method for producing the polypeptide (or a method of producing a polypeptide complex comprising the polypeptide) in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:5 comprise hydroxylysine at the position corresponding to position 3 of SEQ ID NO:5. In some embodiments, a polypeptide according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:7, and a method for producing the polypeptide (or a method of producing a polypeptide complex comprising the polypeptide) in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:7 comprise hydroxylysine at the position corresponding to position 6 of SEQ ID NO:7. In some embodiments, a polypeptide according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:9, and a method for producing the polypeptide (or a method of producing a polypeptide complex comprising the polypeptide) in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:9 comprise hydroxylysine at the position corresponding to position 11 of SEQ ID NO:9. In some embodiments, a polypeptide according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:11, and a method for producing the polypeptide (or a method of producing a polypeptide complex comprising the polypeptide) in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:11 comprise hydroxylysine at the position corresponding to position 10 of SEQ ID NO:11.

In some embodiments, a polypeptide/polypeptide complex according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:1, and comprises (i) a VH region comprising HC-CDR1, HC-CDR2 and HC-CDR3 as indicated in column A of Table A, and (ii) a VL region comprising LC-CDR1, LC-CDR2 and LC-CDR3 as indicated in column B of Table A, wherein the sequences of columns A and B are selected from the same row of Table A; and a method for producing the polypeptide/polypeptide complex in accordance with the present disclosure yields a population of

polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

In some embodiments, a polypeptide/polypeptide complex according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:1, and comprises (i) a VH region comprising SEQ ID NO:132, SEQ ID NO:125 and SEQ ID NO:133, and (ii) a VL region comprising SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130; and a method for producing the polypeptide/polypeptide complex in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

In some embodiments, a polypeptide/polypeptide complex according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:1, and comprises (i) a VH region comprising or consisting of an amino acid sequence indicated in column A of Table B, and (ii) a VL region comprising or consisting of an amino acid sequence indicated in column B of Table B, wherein the sequences of columns A and B are selected from the same row of Table B; and a method for producing the polypeptide/polypeptide complex in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

In some embodiments, a polypeptide/polypeptide complex according to the present disclosure comprises an amino acid sequence according to SEQ ID NO:1, and comprises (i) a VH region comprising or consisting of SEQ ID NO:131, and (ii) a VL region comprising or consisting of SEQ ID NO:127; and a method for producing the polypeptide/polypeptide complex in accordance with the present disclosure yields a population of polypeptides/polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

In some embodiments, a polypeptide complex according to the present disclosure comprises (i) a polypeptide having the amino acid sequence of SEQ ID NO:93, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:94, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:95, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:116; and a method for producing the polypeptide complex in accordance with the present disclosure yields a population of polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:93 comprise hydroxylysine at the position corresponding to position 346 of SEQ ID NO:93.

In some embodiments, a polypeptide complex according to the present disclosure comprises (i) a polypeptide having the amino acid sequence of SEQ ID NO:97, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:98, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:99, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:116; and a method for producing the polypeptide complex in accordance with the present disclosure yields a population of polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:97 comprise hydroxylysine at the position corresponding to position 350 of SEQ ID NO:97.

In some embodiments, a polypeptide complex according to the present disclosure comprises (i) a polypeptide having the amino acid sequence of SEQ ID NO:86, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:153, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:154, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:155; and a method for producing the polypeptide complex in accordance with the present disclosure yields a population of polypeptide complexes in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:153 comprise hydroxylysine at the position corresponding to position 350 of SEQ ID NO:153.

**Populations of polypeptides and polypeptide complexes**

The present disclosure provides populations of polypeptides according to the present disclosure, and populations of polypeptide complexes according to the present disclosure. It will be appreciated that such populations of polypeptides/polypeptide complexes comprise a plurality of the relevant polypeptide/polypeptide complex.

By way of illustration, a population of polypeptides according to the present disclosure having an amino acid sequence according to SEQ ID NO:1 comprises a plurality of (*i.e.* two or more) polypeptides, each having an amino acid sequence according to SEQ ID NO:1. In preferred embodiments, the polypeptides of the plurality of polypeptides have the same amino acid sequence. In accordance with the preceding sentence, 'the same amino acid sequence' ignores the hydroxylation status of amino acids of the amino acid sequences of the polypeptide. Thus, two polypeptides that are identical with the exception that one of the polypeptides comprises a hydroxylysine residue at a given position, and the other polypeptide comprises a lysine residue at the given position, are nevertheless considered to have 'the same amino acid sequence'.

By way of further illustration, a population of polypeptide complexes according to the present disclosure comprising a polypeptide having an amino acid sequence according to SEQ ID NO:1 comprises a plurality of (*i.e.* two or more) polypeptide complexes, each comprising a polypeptide having an amino acid sequence according to SEQ ID NO:1. In preferred embodiments, the plurality of polypeptide complexes comprise the same polypeptides; that is, each polypeptide complex comprises the same combination of polypeptides, having the same amino sequence. For example, in a population of polypeptide complexes formed by association between (i) a polypeptide having the amino acid sequence of SEQ ID NO:93, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:94, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:95, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:116; each polypeptide complex comprises polypeptides (i) to (iv).

The present disclosure provides populations of polypeptides, and populations of polypeptide complexes, that are obtained or obtainable by a method according to the present disclosure.

The present disclosure provides a population of polypeptides comprising an amino acid sequence comprising a lysine/proline residue susceptible to hydroxylation, or a population of polypeptide complexes comprising such a polypeptide, in which ≤25% of the polypeptides comprising an amino acid sequence comprising a lysine/proline residue susceptible to hydroxylation comprise the hydroxylated form of the residue at the relevant position. In some embodiments, ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1% of the polypeptides comprise the hydroxylated form of the residue at the relevant position. In some embodiments, ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10% of the polypeptides comprise the hydroxylated form of the residue at the relevant position. In some embodiments, ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10% of the polypeptides comprise the hydroxylated form of the residue at the relevant position.

In accordance with the preceding paragraph, a polypeptide of 'a population of polypeptides comprising an amino acid sequence comprising a lysine/proline residue susceptible to hydroxylation' may be selected from any embodiment of a polypeptide described herein, *e.g.* a polypeptide described in the section entitled 'Polypeptides and polypeptide complexes' or the section entitled 'Particular exemplary polypeptides and polypeptide complexes' hereinabove. Similarly, a polypeptide complex referred to in the

preceding paragraph may be selected from any embodiment of a polypeptide complex described herein, *e.g.* a polypeptide complex described in the section entitled 'Polypeptides and polypeptide complexes' or the section entitled 'Particular exemplary polypeptides and polypeptide complexes' hereinabove.

The present disclosure provides a population of polypeptides comprising an amino acid sequence according to SEQ ID NO:1, or a population of polypeptide complexes comprising such a polypeptide, in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides of the population comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1. The present disclosure provides a population of polypeptides comprising an amino acid sequence according to SEQ ID NO:2, or a population of polypeptide complexes comprising such a polypeptide, in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides of the population comprising an amino acid sequence according to SEQ ID NO:2 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:2.

The present disclosure provides a population of polypeptides comprising an amino acid sequence according to SEQ ID NO:5, or a population of polypeptide complexes comprising such a polypeptide, in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides of the population comprising an amino acid sequence according to SEQ ID NO:5 comprise hydroxylysine at the position corresponding to position 4 of SEQ ID NO:5. The present disclosure provides a population of polypeptides comprising an amino acid sequence according to SEQ ID NO:7, or a population of polypeptide complexes comprising such a polypeptide, in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides of the population comprising an amino acid sequence according to SEQ ID NO:7 comprise hydroxylysine at the position corresponding to position 6 of SEQ ID NO:7. The present disclosure provides a population of polypeptides comprising an amino acid sequence according to SEQ ID NO:9, or a population of polypeptide complexes comprising such a polypeptide, in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides of the population comprising an amino acid sequence according to SEQ ID NO:9 comprise hydroxylysine at the position corresponding to position 11 of SEQ ID NO:9. The present disclosure provides a population of polypeptides comprising an amino acid sequence according to SEQ ID NO:11, or a population of

polypeptide complexes comprising such a polypeptide, in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides of the population comprising an amino acid sequence according to SEQ ID NO:11 comprise hydroxylysine at the position corresponding to position 10 of SEQ ID NO:11.

Also provided is a population of polypeptides/polypeptide complexes comprising an amino acid sequence according to SEQ ID NO:1, and comprising (i) a VH region comprising HC-CDR1, HC-CDR2 and HC-CDR3 as indicated in column A of Table A, and (ii) a VL region comprising LC-CDR1, LC-CDR2 and LC-CDR3 as indicated in column B of Table A, wherein the sequences of columns A and B are selected from the same row of Table A; in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

Also provided is a population of polypeptides/polypeptide complexes comprising an amino acid sequence according to SEQ ID NO:1, and comprising (i) a VH region comprising SEQ ID NO:132, SEQ ID NO:125 and SEQ ID NO:133, and (ii) a VL region comprising SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130; in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

Also provided is a population of polypeptides/polypeptide complexes comprising an amino acid sequence according to SEQ ID NO:1, and comprising (i) a VH region comprising or consisting of an amino acid sequence indicated in column A of Table B, and (ii) a VL region comprising or consisting of an amino acid sequence indicated in column B of Table B, wherein the sequences of columns A and B are selected from the same row of Table B; in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

Also provided is a population of polypeptides/polypeptide complexes comprising an amino acid sequence according to SEQ ID NO:1, and comprising (i) a VH region comprising or consisting of SEQ ID NO:131, and (ii) a VL region comprising or consisting of SEQ ID NO:127; in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18%

and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:1 comprise hydroxylysine at the position corresponding to position 2 of SEQ ID NO:1.

Also provided is a population of polypeptide complexes, the polypeptide complexes comprising (i) a polypeptide having the amino acid sequence of SEQ ID NO:93, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:94, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:95, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:116; in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:93 comprise hydroxylysine at the position corresponding to position 346 of SEQ ID NO:93.

Also provided is a population of polypeptide complexes, the polypeptide complexes comprising (i) a polypeptide having the amino acid sequence of SEQ ID NO:97, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:98, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:99, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:116; in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:97 comprise hydroxylysine at the position corresponding to position 350 of SEQ ID NO:97.

Also provided is a population of polypeptide complexes, the polypeptide complexes comprising (i) a polypeptide having the amino acid sequence of SEQ ID NO:86, (ii) a polypeptide having the amino acid sequence of SEQ ID NO:153, (iii) a polypeptide having the amino acid sequence of SEQ ID NO:154, and (iv) a polypeptide having the amino acid sequence of SEQ ID NO:155; in which ≤25% (*e.g.* ≤20%, ≤19%, ≤18%, ≤17%, ≤16%, ≤15%, ≤14%, ≤13%, ≤12%, ≤11%, ≤10%, ≤9%, ≤8%, ≤7%, ≤6%, ≤5%, ≤4%, ≤3%, ≤2% or ≤1%; *e.g.* ≤15%, ≤14%, ≤13%, ≤12%, ≤11% or ≤10%; ≤20% and ≥5%, *e.g.* one of ≤19% and ≥6%, ≤18% and ≥7%, ≤17% and ≥8%, ≤16% and ≥9%, or ≤15% and ≥10%) of the polypeptides comprising an amino acid sequence according to SEQ ID NO:153 comprise hydroxylysine at the position corresponding to position 350 of SEQ ID NO:153.

A population of polypeptides, or a population of polypeptide complexes, according to the present disclosure may be provided in a composition. Such compositions may comprise the polypeptides/polypeptide complexes in a formulation suitable for clinical use. Such compositions may be referred to as 'pharmaceutical compositions' or 'medicaments'. Compositions according to the present disclosure may comprise one or more pharmaceutically-acceptable carriers (*e.g.* liposomes, micelles, microspheres, nanoparticles), diluents/excipients (*e.g.* starch, cellulose, a cellulose derivative, a polyol, dextrose, maltodextrin, magnesium stearate), adjuvants, fillers, buffers, preservatives (*e.g.* vitamin A,

vitamin E, vitamin C, retinyl palmitate, selenium, cysteine, methionine, citric acid, sodium citrate, methyl paraben, propyl paraben), anti-oxidants (*e.g.* vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium), lubricants (*e.g.* magnesium stearate, talc, silica, stearic acid, vegetable stearin), binders (*e.g.* sucrose, lactose, starch, cellulose, gelatin, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), xylitol, sorbitol, mannitol), stabilizers, solubilizers, surfactants (*e.g.*, wetting agents), masking agents or coloring agents (*e.g.* titanium oxide).

The term 'pharmaceutically-acceptable' as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, *etc.*, which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (*e.g.* a human subject) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, diluent, excipient, adjuvant, filler, buffer, preservative, anti-oxidant, lubricant, binder, stabilizer, solubilizer, surfactant, masking agent, coloring agent, flavoring agent or sweetening agent of a composition according to the present disclosure must also be 'acceptable' in the sense of being compatible with the other ingredients of the formulation. Suitable carriers, diluents, excipients, adjuvants, fillers, buffers, preservatives, anti-oxidants, lubricants, binders, stabilizers, solubilizers, surfactants, masking agents, coloring agents, flavoring agents or sweetening agents can be found in standard pharmaceutical texts, for example, Remington's 'The Science and Practice of Pharmacy' (Ed. A. Adejare), 23rd Edition (2020), Academic Press.

The compositions according to the present disclosure may be formulated for administration to a subject, *e.g.* administration via a route of administration as appropriate for the nature of the composition and the disease/condition to be treated/prevented. In some embodiments, a composition may be formulated for parenteral, systemic, topical, intracavitary, intravascular, intravenous, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, intratumoral, subcutaneous, intradermal, oral or transdermal administration. In some embodiments, a composition may be formulated for administration by injection or infusion, or administration by ingestion. The composition may comprise the polypeptides/polypeptide complexes in a sterile or isotonic medium. The compositions may be provided in fluid, including gel, form. Fluid formulations may be formulated for administration by injection or infusion (*e.g.* via cannula) to a blood vessel, or a selected region of the human or animal body, or to a tumor. The compositions may be provided in solid form, *e.g.* in lyophilized form.

**Sequence identity**

As used herein, 'sequence identity' refers to the percent of nucleotides/amino acid residues in a subject sequence that are identical to nucleotides/amino acid residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum percent sequence identity between the sequences. Pairwise and multiple sequence alignment for the purposes of determining percent sequence identity between two or more amino acid or nucleic acid sequences can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as ClustalOmega (Söding, J. Bioinformatics (2005) 21:951-960), T-coffee (Notredame *et al.* J Mol Biol (2000) 302:205-217), Kalign (Lassmann and Sonnhammer BMC Bioinformatics (2005) 6(298)) and

MAFFT (Katoh and Standley Molecular Biology and Evolution (2013) 30(4):772–780) software. When using such software, the default parameters, *e.g.* for gap penalty and extension penalty, are preferably used.

In preferred embodiments, for the purposes of evaluating sequence identity between amino acid sequences, the hydroxylation status of amino acid residues of the sequences is ignored. Thus, *e.g.* lysine and hydroxylysine residues are treated as identical amino acids for the purposes of sequence comparison, and similarly proline and hydroxyproline residues are treated as identical amino acids.

**Sequences**

| SEQ ID NO: | DESCRIPTION | SEQUENCE |
|---|---|---|
| 1 | Lysine hydroxylation site consensus | $X_1KX_2$<br><br>wherein $X_1$ = any amino acid, $X_2$ = G, S or A |
| 2 | Lysine hydroxylation site consensus – G at pos 3 | $X_1KG$<br><br>wherein $X_1$ = any amino acid |
| 3 | Lysine hydroxylation site consensus – A at pos 3 | $X_1KA$<br><br>wherein $X_1$ = any amino acid |
| 4 | Lysine hydroxylation site consensus – S at pos 3 | $X_1KS$<br><br>wherein $X_1$ = any amino acid |
| 5 | TCB mAb lysine hydroxylation site consensus | STKGP |
| 6 | Lysine hydroxylation peptide sequence 1 | SSASTK |
| 7 | Lysine hydroxylation peptide sequence 1 + G | SSASTKG |
| 8 | Lysine hydroxylation peptide sequence 2 | TLVTVSSASTK |
| 9 | Lysine hydroxylation peptide sequence 2 + G | TLVTVSSASTKG |
| 10 | Lysine hydroxylation peptide sequence 3 | LTVLSSASTK |
| 11 | Lysine hydroxylation peptide sequence 3 + G | LTVLSSASTKG |
| 12 | Lysine hydroxylation peptide sequence 4 | IKGGLFADIASHPWQAAIFAK |
| 13 | Lysine hydroxylation peptide sequence 5 | ILGNEGSFLTKGFSK |
| 14 | Lysine hydroxylation peptide sequence 6 | DCPTSCPTTCANGWECCKGYPCVNKACSGCTH |
| 15 | Lysine hydroxylation peptide sequence 7 | RSVDSTNNLPPRERKAGCKNFYWKGFTSC |
| 16 | Lysine hydroxylation peptide sequence 8 | $X_1X_2X_3X_4X_5X_6X_7X_8X_9$WGQGTLVTVSSASTK<br><br>wherein $X_1$ = any amino acid, $X_2$ = any amino acid, $X_3$ = any amino acid, $X_4$ = any amino acid, $X_5$ = any amino acid, $X_6$ = any amino acid, $X_7$ = any amino acid, $X_8$ = any amino acid, $X_9$ = any amino acid |
| 17 | Lysine hydroxylation peptide sequence 9 | $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$KGR<br><br>wherein $X_1$ = any amino acid, $X_2$ = any amino acid, $X_3$ = any amino acid, $X_4$ = any amino acid, $X_5$ = any amino acid, $X_6$ = any amino acid, $X_7$ = any amino acid, $X_8$ = any |

| | | |
|---|---|---|
| | | amino acid, $X_9$ = any amino acid, $X_{10}$ = any amino acid, $X_{11}$ = any amino acid, $X_{12}$ = any amino acid |
| 18 | Lysine hydroxylation peptide sequence 10 | $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}K$<br><br>wherein $X_1$ = any amino acid, $X_2$ = any amino acid, $X_3$ = any amino acid, $X_4$ = any amino acid, $X_5$ = any amino acid, $X_6$ = any amino acid, $X_7$ = any amino acid, $X_8$ = any amino acid, $X_9$ = any amino acid, $X_{10}$ = any amino acid, $X_{11}$ = any amino acid, $X_{12}$ = any amino acid |
| 19 | Lysine hydroxylation peptide sequence 11 | DSTYSLSSTLTLSK |
| 20 | Lysine hydroxylation peptide sequence 12 | LTVLSSASTKGPSVFPLAPSSK |
| 21 | Lysine hydroxylation peptide sequence 13 | $X_1X_2X_3X_4X_5X_6$WGQGVLVTVSSASTKGPSVFPLAPSSK<br><br>wherein $X_1$ = any amino acid, $X_2$ = any amino acid, $X_3$ = any amino acid, $X_4$ = any amino acid, $X_5$ = any amino acid, $X_6$ = any amino acid |
| 22 | Lysine hydroxylation peptide sequence 14 | LKSGTASVVCLLNNFYPR |
| 23 | Lysine hydroxylation peptide sequence 15 | DSTYSLSSTLTLSKADYEK |
| 24 | Lysine hydroxylation peptide sequence 16 | $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}$KGR<br><br>wherein $X_1$ = any amino acid, $X_2$ = any amino acid, $X_3$ = any amino acid, $X_4$ = any amino acid, $X_5$ = any amino acid, $X_6$ = any amino acid, $X_7$ = any amino acid, $X_8$ = any amino acid, $X_9$ = any amino acid, $X_{10}$ = any amino acid, $X_{11}$ = any amino acid |
| 25 | Lysine hydroxylation peptide sequence 17 | NQVSLSCAVK |
| 26 | Lysine hydroxylation peptide sequence 18 | $X_1X_2X_3X_4X_5X_6$WGQGVLVTVSSASTK<br><br>wherein $X_1$ = any amino acid, $X_2$ = any amino acid, $X_3$ = any amino acid, $X_4$ = any amino acid, $X_5$ = any amino acid, $X_6$ = any amino acid |
| 27 | Proline hydroxylation site consensus | $X_1$PG<br><br>wherein $X_1$ = any amino acid |
| 28 | V9 (scFab)-Fc(hole) P329G LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG<br>VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP<br>SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK<br>DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE<br>GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF<br>TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN<br>SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS<br>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS<br>SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPK<br>PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 29 | LC007 (VH-CH1)-Fc(knob) P329G LALA | EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDG<br>SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSA<br>STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL<br>QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP<br>APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP<br>REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD<br>SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 30 | LC007 (VL-CL) | DIVLTQSPSSLSASLGDRVTISCSASQGIRNYLNWYQQRPDGTVKLLIYYTSSLHSGV<br>PSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLPWTFGGGTKLEIKRTVAAPSV<br>FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS<br>TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 31 | LC007 (scFab)-Fc(hole) P329G LALA | DIVLTQSPSSLSASLGDRVTISCSASQGIRNYLNWYQQRPDGTVKLLIYYTSSLHSGV<br>PSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLPWTFGGGTKLEIKRTVAAPSV<br>FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS<br>TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSEGG<br>GSEGGGSEGGGSEGGGSGGGSGEVQLQESGPGLVKPSQSLSLTCSVTGYSITSG<br>YYWNWIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTE<br>DTATYYCADFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF<br>PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS<br>NTKVDKKVEPKSCDGGGGSGGGGSGAQDKTHTCPPCPAPEAAGGPSVFLFPPKP |

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| | | KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTK<br>NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 32 | V9 (VH-CH1) -<br>Fc(knob) LALA | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYK<br>GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD<br>VWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS<br>GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP<br>KSCDGGGGSGGGGSGAQDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPE<br>VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW<br>LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK<br>GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC<br>SVMHEALHNHYTQKSLSLSP |
| 33 | V9 (VL-CL) | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG<br>VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP<br>SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK<br>DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 34 | V9 (scFab)-LC007<br>(VH-CH1)-Fc(knob)<br>wt | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG<br>VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP<br>SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK<br>DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE<br>GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF<br>TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN<br>SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS<br>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS<br>SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLQESGPGLVKP<br>SQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISIT<br>RDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSASTKGPSVFPLAPSS<br>KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV<br>PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP<br>KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDEL<br>TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK<br>SRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 35 | LC007 (VH-CH1)-<br>Fc(hole) wt | EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDG<br>SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSA<br>STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL<br>QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP<br>APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP<br>REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD<br>SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 36 | V9 (scFab)-LC007<br>(VH-CH1)-Fc(knob)<br>LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG<br>VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP<br>SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK<br>DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE<br>GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF<br>TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN<br>SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS<br>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS<br>SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLQESGPGLVKP<br>SQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISIT<br>RDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSASTKGPSVFPLAPSS<br>KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV<br>PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP<br>PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDE<br>LTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD<br>KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 37 | LC007 (VH-CH1)-<br>Fc(hole) LALA | EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDG<br>SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSA<br>STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL<br>QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP<br>APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP<br>REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD<br>SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |

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| 38 | V9 (scFab)-LC007 (VH-CH1)-Fc(knob) P329G LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLQESGPGLVKP SQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISIT RDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 39 | LC007 (VH-CH1)-Fc(hole) P329G LALA | EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDG SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 40 | V9 (scFab)-LC007 (VH-CH1)-Fc(knob) P329G LALA N297D | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLQESGPGLVKP SQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISIT RDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYDSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 41 | LC007 (VH-CH1)-Fc(hole) P329G LALA N297D | EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDG SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYDSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 42 | V9 (VL-CH1)-LC007 (VH-CH1)-Fc(hole) P329G LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSEV QLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDGSN NYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE PQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 43 | Fc(knob) wt | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS P |

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| 44 | V9 (VH-CL) | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYK<br>GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD<br>VWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD<br>NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK<br>SFNRGEC |
| 45 | FN18 (VL-CH1)-<br>LC007 (VH-CH1)-<br>Fc(hole) P329G LALA | DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLINW<br>ASTRESGVPDRFTGSGSRTDFTLTISSVKAEDLAVYFCQQFYSYPPTFGGGTKLEIK<br>SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP<br>AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGG<br>GGSEVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYIT<br>YDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTV<br>SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP<br>AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP<br>CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV<br>HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKG<br>QPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV<br>LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 46 | FN18 (VH-CL) | QVQLQQSEAELARPGASVKMSCKASGYTFTDYTIHWLKQRPGQGLDWIGYFNPSS<br>ESTEYNRKFKDRTILTADRSSTTAYMQLSSLTSEDSAVYYCSRKGEKLLGNRYWYF<br>DVWGAGTSVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV<br>DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT<br>KSFNRGEC |
| 47 | V9 (scFab)-Fc(knob)<br>P329G LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG<br>VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP<br>SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK<br>DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE<br>GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF<br>TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN<br>SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS<br>TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS<br>SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPK<br>PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV<br>VSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 48 | GA201 (VH-CH1)-<br>Fc(hole) P329G LALA | QVQLVQSGAEVKKPGSSVKVSCKASGFTFTDYKIHWVRQAPGQGLEWMGYFNPN<br>SGYSTYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSPGGYYVMDAW<br>GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL<br>TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP<br>IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE<br>NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS<br>P |
| 49 | GA201 (VL-CL) | DIQMTQSPSSLSASVGDRVTITCRASQGINNYLNWYQQKPGKAPKRLIYNTNNLQTG<br>VPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSFPTFGQGTKLEIKRTVAAPSVF<br>IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST<br>YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 50 | GA201 (scFab)-<br>Fc(knob) P329G<br>LALA | DIQMTQSPSSLSASVGDRVTITCRASQGINNYLNWYQQKPGKAPKRLIYNTNNLQTG<br>VPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSFPTFGQGTKLEIKRTVAAPSVF<br>IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST<br>YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSEGGG<br>SEGGGSEGGGSEGGGSGGGSGQVQLVQSGAEVKKPGSSVKVSCKASGFTFTDYK<br>IHWVRQAPGQGLEWMGYFNPNSGYSTYAQKFQGRVTITADKSTSTAYMELSSLRS<br>EDTAVYYCARLSPGGYYVMDAWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTA<br>ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ<br>TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI<br>SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL<br>HQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSL<br>WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG<br>NVFSCSVMHEALHNHYTQKSLSLSP |
| 51 | V9 (VH-CH1)-<br>Fc(hole) P329G LALA | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYK<br>GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD<br>VWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS<br>GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP<br>KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV<br>KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL<br>GAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG |

| | | |
|---|---|---|
| | | QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSP |
| 52 | V9 (scFab)-GA201 (VH-CH1)-Fc(knob) P329G LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSQVQLVQSGAEVKKP GSSVKVSCKASGFTFTDYKIHWVRQAPGQGLEWMGYFNPNSGYSTYAQKFQGRV TITADKSTSTAYMELSSLRSEDTAVYYCARLSPGGYYVMDAWGQGTTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREP QVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 53 | 3F2 (scFab)-Fc(knob) P329G LALA | EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSGGEVQLLESGGGLVQPGGSLRLSCAASGFTFSSY AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKGWFGGFNYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSP |
| 54 | V9 (scFab)-3F2 (VH-CH1)-Fc(knob) P329G LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSE GGGSEGGGSEGGGSEGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGYSF TGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMN SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLLESGGGLVQP GGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV YTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 55 | 3F2 (VH-CH1)-Fc(hole) P329G LALA | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSG GSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKT ISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 56 | 3F2 (VL-CL) | EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 57 | CH1A1A (VH-CH1)-V9 (VL-CH1)-Fc(knob) P329G LALA | QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTK TGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKA PKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQ GTKVEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS |

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| | | GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 58 | CH1A1A (VH-CH1)-Fc(hole) P329G LALA | QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTK TGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA PIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SP |
| 59 | CH1A1A (VL-CL) | DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 60 | V9(VH-CL)-LC007(VL-CL) | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYK GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD VWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGECDGGGGSGGGGSDIVLTQSPSSLSASLGDRVTISCSASQGIRNYLNWYQ QRPDGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLP WTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC |
| 61 | Fc(knob) P329G LALA | DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP IEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS P |
| 62 | V9(VL-CH1) | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC |
| 63 | V9(VH-CL)-Fc(knob) P329G LALA | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYK GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD VWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSP |
| 64 | LC007(VH-CH1)-V9(VH-CL)-Fc(knob) P329G LALA | EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDG SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGG GSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINP YKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWY FDVWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSP |
| 65 | M4-3 ML2(VL-CL) | DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLNWYQQKPGKAPKLLIYYTSSLHSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSKLPWTFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 66 | V9(VL-CH1)-M4-3 ML2(VH-CH1)-Fc(knob) P329G LALA | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCGGGGSGGGGSQV QLQESGPGLVKPSQTLSLTCTVSGGSITSGYYWNWIRQHPGKGLEWIGYITYDGSN NYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCADFDYWGQGTLVTVSSAST |

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|---|---|---|
| | | KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS<br>SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE<br>AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT<br>KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE<br>PQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD<br>GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 67 | M4-3 ML2(VH-CH1)-Fc(hole) P329G LALA | QVQLQESGPGLVKPSQTLSLTCTVSGGSITSGYYWNWIRQHPGKGLEWIGYITYDG<br>SNNYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCADFDYWGQGTLVTVSS<br>ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV<br>LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP<br>APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP<br>REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD<br>SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 68 | V9(VH-CL)-M4-3 ML2(VH-CH1)-Fc(knob) P329G LALA | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYK<br>GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD<br>VWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD<br>NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK<br>SFNRGECGGGGSGGGGSQVQLQESGPGLVKPSQTLSLTCTVSGGSITSGYYWNW<br>IRQHPGKGLEWIGYITYDGSNNYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYY<br>CADFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV<br>SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD<br>KKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE<br>DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS<br>NKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY<br>TQKSLSLSP |
| 69 | CH1A1A(VH-CH1)-V9(VH-CL)-Fc(knob) P329G LALA | QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTK<br>TGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDY<br>WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG<br>ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK<br>SCGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAP<br>GKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCA<br>RSGYYGDSDWYFDVWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN<br>FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC<br>EVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT<br>PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ<br>DWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC<br>LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF<br>SCSVMHEALHNHYTQKSLSLSP |
| 70 | H2C(VL-CH1) | QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWVQQKPGQAPRGLIGGTKFL<br>APGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNRWVFGGGTKLTVLSS<br>ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV<br>LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC |
| 71 | H2C(VH-CL)-M4-3 ML2(VH-CH1)-Fc(knob) P329G LALA | EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKY<br>NNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISY<br>WAYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK<br>VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV<br>TKSFNRGECGGGGSGGGGSQVQLQESGPGLVKPSQTLSLTCTVSGGSITSGYYW<br>NWIRQHPGKGLEWIGYITYDGSNNYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTA<br>VYYCADFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP<br>VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK<br>VDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS<br>HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK<br>VSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAV<br>EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN<br>HYTQKSLSLSP |
| 72 | 431/26(VL-CL) | DIQMTQSPSSLSASVGDRVTITCSTSSSVSYMHWYQQKPGKAPKLLIYSTSNLASGV<br>PSRFSGSGSGTDFTFTISSLQPEDIATYYCHQWSSYPTFGQGTKVEIKRTVAAPSVFI<br>FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST<br>YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 73 | 431/26(VH-CH1)-V9(VH-CL)-Fc(knob) P329G LALA | QVQLQESGPGLVRPSQTLSLTCTVSGFTISSGYSWHWVRQPPGRGLEWIGYIQYS<br>GITNYNPSLKSRVTMLVDTSKNQFSLRLSSVTAADTAVYYCAREDYDYHWYFDVWG<br>QGSLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT<br>SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCG<br>GGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKG<br>LEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGY<br>YGDSDWYFDVWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR |

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| | | EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSP |
| 74 | 431/26(VH-CH1)-Fc(hole) P329G LALA | QVQLQESGPGLVRPSQTLSLTCTVSGFTISSGYSWHWVRQPPGRGLEWIGYIQYS GITNYNPSLKSRVTMLVDTSKNQFSLRLSSVTAADTAVYYCAREDYDYHWYFDVWG QGSLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIE KTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 75 | CH1A1A(VL-CL)-V9 (VH-CL) | DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSG GGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKG LEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGY YGDSDWYFDVWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC |
| 76 | CH1A1A(VH-CH1)-Fc(knob) P329G LALA | QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTK TGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SP |
| 77 | Fc(hole) P329G LALA | DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS P |
| 78 | CH2527(VL-CH1) | QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKR APGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC |
| 79 | CH2527(VH-CL)-LC007(VH-CH1)-Fc(knob) P329G LALA | EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKY NNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSW FAYWGQGTLVTVSAASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGECGGGGSGGGGSEVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWN WIRQFPGNKLEWMGYITYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTAT YYCADFDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSP |
| 80 | LC007(VH-CH1)-CH2527(VH-CL)-Fc(knob) P329G LALA | EVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITYDG SNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCADFDYWGQGTTLTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGG GSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRS KYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYV SWFAYWGQGTLVTVSAASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSP |

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|---|---|---|
| 81 | TYRP1 VH-CH1 (EE) – $CD3_{orig}$/$CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYFLHWVRQAPGQGLEWMGWINPDNGNT VYAQKFQGRVTMTADTSTSTVYMELSSLRSEDTAVYYCTRRDYTYEKAALDYVVGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDGGGGSGGGGSQ AVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPGTP ARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSP |
| 82 | TYRP1 VH-CH1 (EE) – Fc (hole, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYFLHWVRQAPGQGLEWMGWINPDNG NTVYAQKFQGRVTMTADTSTSTVYMELSSLRSEDTAVYYCTRRDYTYEKAALDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK GQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 83 | TYRP1 VL-CL (RK) | DIQMTQSPSSLSASVGDRVTITCRASGNIYNYLAWYQQKPGKVPKLLIYDAKTLADGVP SRFSGSGSGTDFTLTISSLQPEDVATYYCQHFWSLPFTFGQGTKLEIKRTVAAPSVFIFP PSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 84 | $CD3_{orig}$ VH-CL | EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIRSKYNNYA TYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQG TLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 85 | $CD3_{opt}$ (P033.078) VH-CL | EVQLLESGGGLVQPGGSLRLSCAASGFTPESYAMNVVVRQAPGKGLEWVSRIRSKY NEYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRASNFPSSFVSYF GYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC |
| 86 | $CD3_{opt}$ (P035.093) VH-CL | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSRIRSKY NNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRASNFPASYVSY FAYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC |
| 87 | $CD3_{opt}$ (P035.064) VH-CL | EVQLLESGGGLVQPGGSLRLSCAASGFDFDNYAMNWVRQAPGKGLEWVSRIRSKH NGYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRASNFPSSYVSYF GYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC |
| 88 | $CD3_{opt}$ (P021.045) VH-CL | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSRIRSKY NNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRASNFPSSYVSY FAYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC |
| 89 | $CD3_{opt}$ (P004.042) VH-CL | EVQLLESGGGLVQPGGSLRLSCAASGFQFDNYAMNWVRQAPGKGLEWVSRIRTKYN EYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRASNFPQSYVSYFGY WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC |
| 90 | CEACAM5 VH-CH1 (EE) – $CD3_{orig}$/$CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDTYMHWVRQAPGQGLEWMGRIDPANGN SKYVPKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCAPFGYYVSDYAMAYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDGGGGSGGG GSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEICPGQAFRGLIGGTNKR APGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDEL TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 91 | CEACAM5 VH-CH1 (EE) – Fc (hole, PGLALA) | QVQLVQSGAEVKKPGSSVKVSCKASGFNIKDTYMHWVRQAPGQGLEWMGRIDPAN GNSKYVPKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYCAPFGYYVSDYAMAYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALT |

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|---|---|---|
| | | SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCD KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 92 | CEACAM5 VL-CL (RK) | EIVLTQSPATLSLSPGERATLSCRAGESVDIFGVGFLHWYQQKPGQAPRLLIYRASN RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQTNEDPYTFGQGTKLEIKRTVA APSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 93 | GPRC5D VH-CH1 (EE) – $CD3_{orig}/CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWVASISTGGVN TYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATHTGDYFDYWGQGTMVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDGGGGSGGG GSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNK RAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLP PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 94 | GPRC5D VH-CH1 (EE) – Fc (hole, PGLALA) | EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMAWVRQAPGKGLEWVASISTGG VNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATHTGDYFDYWGQGT MVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS KAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 95 | GPRC5D VL-CL (RK) | EIVLTQSPGTLSLSPGERATLSCRASQSVSISGINLMNWYQQKPGQQPKLLIYHASILAS GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTRESPLTFGQGTRLEIKRTVAAPSV FIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 96 | CD19(2B11) VH-CH1 (EE) – $CD3_{orig}/CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQAPGQGLEWMGYINPYN DGSKYTEKFQGRVTMTSDTSISTAYMELSRLRSDDTAVYYCARGTYYYGPQLFDYW GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC DGGGGSGGGGGQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPG QAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWV FGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SP |
| 97 | CD19(2B11) VH-CH1 (EE) – $CD3_{orig}/CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQAPGQGLEWMGYINPYN DGSKYTEKFQGRVTMTSDTSISTAYMELSRLRSDDTAVYYCARGTYYYGPQLFDYW GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC DGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPG QAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWV FGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SP |
| 98 | CD19(2B11) VH-CH1 (EE) – Fc (hole, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQAPGQGLEWMGYINPYN DGSKYTEKFQGRVTMTSDTSISTAYMELSRLRSDDTAVYYCARGTYYYGPQLFDYW GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS P |

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|---|---|---|
| 99 | CD19 VL-CL (RK) | DIVMTQTPLSLSVTPGQPASISCKSSQSLETSTGTTYLNWYLQKPGQSPQLLIYRVS<br>KRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQLLEDPYTFGQGTKLEIKRT<br>VAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE<br>QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 100 | CD19(018) VH-CH1 (EE) – $CD3_{orig}/CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQAPGQGLEWMGYINPYN<br>DGSKYTEKFQGRVTMTSDTSISTAYMELSRLRSDDTAVYYCARGTYYYGSALFDYW<br>GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL<br>TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC<br>DGGGGSGGGGGQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPG<br>QAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWV<br>FGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG<br>ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK<br>SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF<br>NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA<br>PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP<br>ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>SP |
| 101 | CD19(018) VH-CH1 (EE) – $CD3_{orig}/CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQAPGQGLEWMGYINPYN<br>DGSKYTEKFQGRVTMTSDTSISTAYMELSRLRSDDTAVYYCARGTYYYGSALFDYW<br>GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL<br>TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC<br>DGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPG<br>QAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWV<br>FGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG<br>ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK<br>SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF<br>NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA<br>PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP<br>ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>SP |
| 102 | CD19(018) VH-CH1 (EE) – Fc (hole, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQAPGQGLEWMGYINPYN<br>DGSKYTEKFQGRVTMTSDTSISTAYMELSRLRSDDTAVYYCARGTYYYGSALFDYW<br>GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL<br>TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC<br>DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP<br>IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE<br>NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS<br>P |
| 103 | CD19(018) VL-CL (RK) | DIVMTQTPLSLSVTPGQPASISCKSSQSLENPNGNTYLNWYLQKPGQSPQLLIYRVS<br>KRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQLTHVPYTFGQGTKLEIKRT<br>VAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE<br>QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 104 | $CD3_{orig}/CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA | QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNK<br>RAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSS<br>ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV<br>LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP<br>APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP<br>REPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD<br>SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 105 | Fc (hole, PGLALA) | DKTHTCPPCPAPEAAGGPSVFLEPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP<br>IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE<br>NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS<br>P |
| 106 | LCFR4-CH1_1 | FGQGTKVEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG<br>ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK<br>SC |
| 107 | LCFR4-CH1_2 | FGGGTKLEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG<br>ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK<br>SC |
| 108 | LCFR4-CH1_2 | FGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG<br>ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK<br>SC |
| 109 | LC MCSP ML2 (G3) | DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLNWYQQKPGKAPKLLIYYTSSLHSG<br>VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSALPWTFGQGTKVEIKRTVAAPS |

| | | |
|---|---|---|
| | | VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 110 | LC CD3 CH2527 (Crossfab, VL-CH1) | QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNK RAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC |
| 111 | MCSP M4-3 (C1) (VH-CH1) - CD3 CH2527 (Crossfab VH-Ck) - Fc(knob) P329GLALA | QVQLQESGPGLVKPSQTLSLTCTVSGGSITSGYYWNWIRQHPGKGLEWIGYITFDG SNNYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCADFDYWGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGG GSEVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIRS KYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYV SWFAYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSP |
| 112 | MCSP M4-3 (C1) (VH-CH1) - Fc(hole) P329GLALA | QVQLQESGPGLVKPSQTLSLTCTVSGGSITSGYYWNWIRQHPGKGLEWIGYITFDG SNNYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCADFDYWGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQP REPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 113 | LC CEA 2F1 | DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIKRTVAAPS VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 114 | CEA CH1A1A 98/99 - CD3 CH2527 (Crossfab VH-Ck) - Fc(knob) P329GLALA | QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTK TGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMDY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAP GKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYY CVRHGNFGNSYVSWFAYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSP |
| 115 | TYRP1 VH-CH1(EE) - CD3orig/CD3opt VL-CH1 - Fc (knob, PGLALA) | QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYFLHWVRQAPGQGLEWMGWINPD NGNTVYAQKFQGRVTMTADTSTSTVYMELSSLRSEDTAVYYCTRRDYTYEKAALDY WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPK SCDGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEK PGQAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNL WVFGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSP |
| 116 | CD3opt VH-CL | EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQAPGKGLEWVSRIRSKY NNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHTTFPSSYVSY YGYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC |
| 117 | EGFRvIII VH-CH1(EE) - CD3orig/CD3opt VL-CH1 - Fc (knob, PGLALA) | EVQLVQSGAEVKKPGESLKISCKGSGYSFDSYWIAWVRQMPGKGLEWMGVIHPYD SDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARVSRSSYAFDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCD GGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQ AFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVF |

| | | |
|---|---|---|
| | | GGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SP |
| 118 | EGFRvIII VH-CH1(EE) -Fc (hole, PGLALA) | EVQLVQSGAEVKKPGESLKISCKGSGYSFDSYWIAWVRQMPGKGLEWMGVIHPYD SDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARVSRSSYAFDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSCDK THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP |
| 119 | EGFRvIII VL-CL(RK) | DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQQRDGPPVTFGQGTKV EIKRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 120 | CH2527 VH | EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIRSKY NNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVS WFAYWGQGTLVTVSS |
| 124 | CH2527 HC-CDR1 | GFTFSTYA |
| 125 | CH2527, Clone 22, P035.093 HC-CDR2 | IRSKYNNYAT |
| 126 | CH2527 HC-CDR3 | VRHGNFGNSYVSWFAY |
| 127 | CH2527, Clone 22, P035.093 VL | QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNK RAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL |
| 128 | CH2527, Clone 22, P035.093 LC-CDR1 | TGAVTTSNY |
| 129 | CH2527, Clone 22, P035.093 LC-CDR2 | GTN |
| 130 | CH2527, Clone 22, P035.093 LC-CDR3 | ALWYSNLWV |
| 131 | Clone 22 VH | EVQLLESGGGLVQPGGSLRLSCAASGFQFSSYAMNWVRQAPGKGLEWVSRIRSKY NNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHTTFPSSYVSY YGYWGQGTLVTVSS |
| 132 | Clone 22 HC-CDR1 | GFQFSSYA |
| 133 | Clone 22 HC-CDR3 | VRHTTFPSSYVSYYGY |
| 134 | P035.093 VH | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSRIRSKY NNYATYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRASNFPASYVSY FAYWGQGTLVTVSS |
| 135 | P035.093 HC-CDR1 | GFTFSSYA |
| 136 | P035.093 HC-CDR3 | VRASNFPASYVSYFAY |
| 137 | 40G5C VH | EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYIHWVRQAPGQGLEWIGWIYPGD GNTKYNEKFKGRATLTADTSTSTAYLELSSLRSEDTAVYYCARDSYSNYYFDYWGQ GTLVTVSS |
| 138 | 40G5C HC-CDR1 | GYTFTNYY |
| 139 | 40G5C HC-CDR2 | IYPGDGNT |
| 140 | 40G5C HC-CDR3 | ARDSYSNYYFDY |
| 141 | 40G5C VL | DIVMTQSPDSLAVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKPGQPPKLLIYWA STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCTQSFILRTFGQGTKVEIK |
| 142 | 40G5C LC-CDR1 | QSLLNSRTRKNY |
| 143 | 40G5C LC-CDR2 | WAS |
| 144 | 40G5C LC-CDR3 | TQSFILRT |
| 145 | V9 VH | EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYK GVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFD VWGQGTLVTVSS |
| 146 | V9 HC-CDR1 | GYSFTGYT |
| 147 | V9 HC-CDR2 | INPYKGVS |
| 148 | V9 HC-CDR3 | ARSGYYGDSDWYFDV |
| 149 | V9 VL | DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIK |
| 150 | V9 LC-CDR1 | QDIRNY |
| 151 | V9 LC-CDR2 | YTS |
| 152 | V9 LC-CDR3 | QQGNTLPWT |

| | | |
|---|---|---|
| 153 | HLA-G VH-CH1 (EE) – $CD3_{orig}$/$CD3_{opt}$ VL-CH1 – Fc (knob, PGLALA) | QVQLQQSGPGLLKPSQTLSLTCAISGDSVSSNRAAWNWIRQSPSRGLEWLGRTYY RSKWYNDYAVSVQGRITLIPDTSKNQFSLRLNSVTPEDTAVYYCASVRAVAPFDYW GQGVLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC DGGGGSGGGGGQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPG QAFRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWV FGGGTKLTVLSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGA PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SP |
| 154 | HLA-G VL-CL(RK) | DIVMTQSPDSLAVSLGERATINCKSSQSVLNPSNNKNNLAWYQQQPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQYYRTPWTFGQGTKVEI KRTVAAPSVFIFPPSDRKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |
| 155 | HLA-G VH-CH1(EE) - Fc (hole, PGLALA) | QVQLQQSGPGLLKPSQTLSLTCAISGDSVSSNRAAWNWIRQSPSRGLEWLGRTYY RSKWYNDYAVSVQGRITLIPDTSKNQFSLRLNSVTPEDTAVYYCASVRAVAPFDYW GQGVLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDEKVEPKSC DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAP IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS P |
| 156 | HLA-G VH | QVQLQQSGPGLLKPSQTLSLTCAISGDSVSSNRAAWNWIRQSPSRGLEWLGRTYY RSKWYNDYAVSVQGRITLIPDTSKNQFSLRLNSVTPEDTAVYYCASVRAVAPFDYW GQGVLVTVSS |
| 157 | HLA-G HC-CDR1 | SNRAAWN |
| 158 | HLA-G HC-CDR2 | RTYYRSKWYNDYAVSVQG |
| 159 | HLA-G HC-CDR3 | VRAVAPFDY |
| 160 | HLA-G VL | DIVMTQSPDSLAVSLGERATINCKSSQSVLNPSNNKNNLAWYQQQPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQYYRTPWTFGQGTKVEI K |
| 161 | HLA-G LC-CDR1 | KSSQSVLNPSNNKNNLA |
| 162 | HLA-G LC-CDR2 | WASTRES |
| 163 | HLA-G LC-CDR3 | QQYYRTPWT |
| 164 | P035.093 HC-CDR1 (Kabat) | SYAMN |
| 165 | P035.093 HC-CDR2 (Kabat) | RIRSKYNNYATYYADSVKG |
| 166 | P035.093 HC-CDR3 (Kabat) | ASNFPASYVSYFAY |
| 167 | P035.093 LC-CDR1 (Kabat) | GSSTGAVTTSNYAN |
| 168 | P035.093 LC-CDR2 (Kabat) | GTNKRAP |
| 169 | Lysine hydroxylation peptide sequence 19 | YNNYATYYADSVK |
| 170 | Lysine hydroxylation peptide sequence 19 + G | YNNYATYYADSVKG |
| 171 | Lysine hydroxylation peptide sequence 20 | GTYYYGPQLFDYWGQGTTVTVSSASTK |
| 172 | Lysine hydroxylation peptide sequence 20 + G | GTYYYGPQLFDYWGQGTTVTVSSASTKG |
| 173 | Lysine hydroxylation peptide sequence 21 | AEDTAVYYCATHTGDYFDYWGQGTMVTVSSASTK |
| 174 | Lysine hydroxylation peptide sequence 21 + G | AEDTAVYYCATHTGDYFDYWGQGTMVTVSSASTKG |

***

The present disclosure includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organisational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present disclosure will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word 'comprise,' and variations such as 'comprises' and 'comprising,' will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

As used herein, an amino acid sequence, or a region of a polypeptide which 'corresponds' to a specified reference amino acid sequence or region of a polypeptide has at least 60%, *e.g.* one of at least ≥65%, ≥70%, ≥75%, ≥80%, ≥85%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99% or 100% sequence identity to the amino acid sequence of the amino acid sequence/polypeptide/region. An amino acid sequence/region/position of a polypeptide/amino acid sequence which 'corresponds' to a specified reference amino acid sequence/region/position of a polypeptide/amino acid sequence can be identified by sequence alignment of the subject sequence to the reference sequence, *e.g.* using sequence alignment software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960).

Where an amino acid (other than glycine) is referred to herein, both L and D enantiomers of the relevant amino acid are expressly contemplated. In some embodiments, except where specifically indicated otherwise, reference to an amino acid herein refers particularly to the L enantiomer, this being the form of the amino acid as it occurs in nature.

It must be noted that, as used in the specification and the appended claims, the singular forms 'a', 'an', and 'the' include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from 'about' one particular value, and/or to 'about' another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent 'about', it will be understood that the particular value forms another embodiment.

Where a nucleic acid sequence is disclosed herein, the reverse complement thereof is also expressly contemplated.

Methods described herein may preferably be performed *in vitro*. The term '*in vitro*' is intended to encompass procedures performed with cells in culture whereas the term '*in vivo*' is intended to encompass procedures with/on intact multi-cellular organisms.

Values may be expressed herein as 'about' a particular value. Similarly, ranges may be expressed herein as from 'about' a particular value, and/or to 'about' another particular value. The term 'about' in relation to a numerical value is optional, and means for example +/- 10%. By way of illustration, reference *e.g.* to 'about 10%' is to be construed as 9% to 11%. In instances herein where 'about' is recited, the value it precedes is also specifically contemplated. By way of illustration, reference *e.g.* to 'about 10%' also specifically contemplates 10%.

**Brief Description of the Figures**

Embodiments and experiments illustrating the principles of the present disclosure will now be discussed with reference to the accompanying figures.

**Figures 1A to 1G. Conformation of hydroxylysine modification and relevance for tryptic digestion.** **1A**) Schematic model of a 2+1 T-cell bsmAb (TCB) (dotted lines: disulfide bridge). **1B**) Example XIC of modified (upper panel) and unmodified (bottom panel) tryptic peptide HCAA-350 of GPRC5D TCB (TCB mAb 3) with zero missed cleavage LTVLSSASTK (SEQ ID NO:10) and the tryptic peptide with one missed cleavage LTVLSSASTKGPSVFPLAPSSK (SEQ ID NO:20). The bold dots indicate the time points when the MS/MS fragmentations of the precursors has been triggered, that are representing the main species of the respective XIC signals. Note the difference in the intensity scale of both unmodified peptides compared to the respective modified peptide. **1C**) Overview of HCD fragment spectra of the modified (upper panel) and unmodified peptide (bottom panel) HCAA-350 of GPRC5D TCB. **1D**) Zoomed view of modified (upper panel) and unmodified peptide (lower panel). The shift of 15.9950 Da of the y1-ion confirms the modification on lysine. **1E**) Example overview HCD fragment spectra of the modified (upper panel) and unmodified peptide (bottom panel) with one missed cleavage. **1F**) Zoomed view of the same MS/MS scans with focus on the fragment ions y12 and y13 that belongs to the KG motif. The distance between both fragments is equivalent to the mass of a HyL-residue with -5.6 ppm deviation (theoretical mass of HyL = 144.0899). **1G**) Identified sites and levels of cleaved and missed cleaved tryptic peptides of three different TCB molecules (arrowheads indicate HyL modification site in respective TCB molecule).

**Figures 2A to 2G. Localization of HyL modification hotspot and structural consequence in TCB mAbs.** **2A**) Several exposed lysine residues were detected to by hydroxylated in TCB mAbs (arrowheads). The hotspot (dark arrowhead) with the highest HyL modification levels is similar to previous observations by Xie *et al.*, MAbs. (2016) 8(2): 371-378 (dark arrowhead). **2B**) Probability analysis of amino acids in position -2 to +2 identified in hydroxylated peptides identified by LC-MS. **2C**) The percentage distribution of detected HyL modified peptides differ for the three analyzed TCB mAbs. HLA-G TCB (TCB mAb 2) is the only molecule where the identical hotspot motif LTVLSSASTK (SEQ ID NO:10) and modified K125 is located in the N-terminal Fab binder. **2D**) Structure of the crossed Fab as

represented in ribbons with HyL350 (ball & stick) and the respective domain annotations. The identified hotspot HyL350 TCB mAbs is located immediately C-terminal to the interface of VL to CH1 in the HC knob polypeptide chain. **2E**) Predicted influence of hydroxylysine modification on hydrophobicity, positive and negative charge patches. The prediction is based on in-silico protonation, energy minimization, and protein patch calculation in MOE 2022. **2F**) Structure of Trastuzumab Fab domain (PDB code: 1n8z), as an exemplary example for a generic IgG Fab, is represented in darker and lighter grey for HC and LC, respectively. **2G**) Overlap of a crossed Fab and a generic Fab using the two constant regions CH1 and κ CL. The overlap of the structures reveals a difference in the elbow segment and the orientation of the neighbored loop.

**Figures 3A to 3J. Variability of HyL modification during cell culture is dependent on iron availability. 3A**) Time course of HyL350 levels of CD19 TCB (TCB mAb 1) during a representative fed-batch fermentation process show a drop in late process phase. The horizontal bar represents the period of time where the HyL modification level shifted (*i.e.* the 'shift phase'). **3B**) Kinetic of the per day delta product increase of the fermentation process peaks in the middle of the process. **3C**) Simulated kinetic of %HyL350 levels new produced to describe the observed kinetic of product HyL30 modification in 3A. **3D**) Mechanistic description of the enzymatic reactions of PLODs. In proteins, a XKG (SEQ ID NO:2) consensus sequence is recognized by the PLOD enzymes and hydroxylated by using 2-oxoglutarate and oxygen and the cofactor $Fe^{2+}$ to a 5R-hydrosy-L-lysyl residue, succinate and carbon dioxide. **3E**) Free iron ($Fe^{2+}$) concentrations measured in the cell-free cell culture supernatant during the fed batch cultivation process. The iron cell density concentrations at a specific process time point were used to calculate the iron availability per cell (**3F**) and the cell-specific iron uptake rate (**3G**). Both indicate a stagnation in trends in the late process phase and suggest a switch in iron metabolism, which is not seen in global iron concentration measurements. Analysis of Iron concentration effect on CD19 TCB hydroxylation for K350 hot spot motif (**3H**) and the lower abundant K67 motif (**3I**). **3J**) Iron response curve for HyL modification stimulation was created by fitting the normalized K350 and K67 hydroxylation levels for the tested iron concentrations.

**Figures 4A to 4C. Variability of HyL modification during cell culture is dependent on iron availability.** Analysis of the effect of iron concentration on hydroxylation of lysines of hotspot motifs. Graphs show data points and fitted iron concentration:response curves for normalized levels of HyL modification at the indicated lysine residues, for CD19 TCB (TCB mAb 1) (**4A**), HLA-G TCB (TCB mAb 2) (**4B**), and GPRC5D TCB (TCB mAb 3) (**4C**).

**Figures 5A to 5C. Variability of HyL modification during cell culture is dependent on iron availability.** Analysis of Iron concentration effect on CD19 TCB hydroxylation for the K67 motif (**5A**), the K125 motif (**5B**) and the K350 motif (**5C**).

**Figures 6A to 6C. Variability of HyL modification during cell culture is dependent on iron availability.** Analysis of Iron concentration effect on GPRC5D TCB hydroxylation for the K67 motif (**6A**), the K121 motif (**6B**) and the K346 motif (**6C**).

## Examples

### Example 1: Materials and Methods

#### 1.1 Cell culture

CHO cells were cultured in a proprietary DMEM/F12-based medium in 125-500 mL shake flask vessels at 150 rpm, 37°C, 80% rH, and 5% $CO_2$. Cells were passaged at a seeding density of 3-6 x $10^5$ cells/mL every 3-4 days. Pools of cells stably expressing bispecific antibody molecules were generated as described in Carver *et al.*, Biotechnol Prog. (2020) 36(4):e2967. Briefly, expression plasmids were transfected into CHO cells by MaxCyte STX electroporation (MaxCyte Inc., Rockville, Maryland, USA). Transfected cells were then selected and antibody expression was confirmed by analysis by flow cytometry using a BD FACS Canto II flow cytometer (BD, Eysins, Switzerland) of cells stained with antibody specific for human IgG. Antibody-expressing CHO cell clones were selected after single cell-cloning by limited dilution, titer and binder validation by ELISA, and evaluation of cell growth and productivity performance in fed batch production assays in ambr250 bioreactors (Sartorius AG, Göttingen, Germany).

#### 1.2 Fed-batch cultures

Fed-batch production cultures were performed in 24-deep well plates, shake flasks, or ambr250 bioreactors (Sartorius AG, Göttingen, Germany) with proprietary, chemically-defined production media. Cells may also be cultured in commercially-available cell culture media, such as CD-CHO, ProCHO, or DMEM/F12 media. Cells were seeded at between 2-15 x $10^6$ cells/ml on day 0 of the production stage after adaptation to production media during 2 passages. Cultures received daily proprietary feed medium after day 3, and additional bolus feeds on days 3 and 7, with optional bolus feed at day 10. Cells were cultivated for 14 days. Production in the ambr250 system were operated at set points of 35°C, DO 30%, pH 7.0, and an agitation rate of 1300 rpm with shift to 1600 rpm on day 3.5.

#### 1.3 Batch cultures

Batch production cultures were performed in 24-deep well plates, with proprietary chemically-defined production media lacking iron. Cells were seeded at 5 x $10^6$ cells/ml on day 0 of the production stage. Cultures received daily medium feeds after day 3, and an additional bolus feed on days 3 and 7. Cells were cultivated for 5 days. Iron was added manually to the cultures (to achieve the indicated concentrations shown in Figures 4A to 4C) at day 0.

#### 1.4 Off-line sample analysis

Process parameters were analyzed with a Cedex Bio HT Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) for the measurement of antibody production, and for selected metabolite concentrations, including iron. Total cell count, viable cell concentration, and average cell diameter was measured by Cedex HiRes Analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Integrated viable cell density (IVCD) and specific productivity rates for each condition were calculated as followed:

$$IVCD_n = IVCD_{n-1} + \frac{VCD_n + VCD_{n-1}}{2} * (t_n - t_{n-1}) \quad \text{(Equ 1)}$$

The cell-specific productivity qP and the cell-specific iron consumption rate $qS_{Iron}$ were calculated as follows:

$$\frac{dS/P}{dt} = qS/P * X \quad \text{(Equ 2)}$$

$$qP = \frac{1}{X} * \frac{P_i - P_{i-1}}{t_i - t_{i-1}} \quad \text{(Equ 3)}$$

$$qS_{Iron} = \frac{1}{X} * \frac{S_i - S_{i-1}}{t_i - t_{i-1}} \quad \text{(Equ 4)}$$

A negative and positive value for qS and qP represent consumption and production of a compound, respectively.

1.5 Structural Modeling Analysis

A homology model of the CrossFab moiety was constructed utilizing MoFvAb/IgNORANT, with the incorporation of hydroxylysine achieved through MOE 2022. This process involved the protonation of K350 and subsequent energy minimization. The Trastuzumab Fab domain (PDB code 1n8z) served as a representative example of a generic IgG Fab moiety.

For the evaluation of chemico-physical parameters, such as hydrophobicity and the presence of positive or negative charge patches, both the lysine and hydroxylysine forms of the SSASTK360 motif (SEQ ID NO:6) of VL-CH1 were analyzed using MOE 2022.

1.6 TCB mAb aggregate and fragment analytics

Cell culture supernatants were clarified (by centrifugation at 1000 x *g* for 30 min at 4°C, and 1.2 µm filtration, using AcroPrep 96 Filter Plates, Pall Cooperation). Analytical protein A chromatography was performed by UHPLC with UV detection (Dionex Ultimate 3000 UHPLC fitted with POROS™ A 20 µm Column, Thermo Fisher Scientific Inc.).

Antibody integrity was analyzed after protein A affinity chromatography (PreDictor RoboColumn MabSelect SuRe, Cytiva) and normalization with protein quantitation using UV measurement (Nanoquant Infinite M200, Tecan). Percentage of correctly assembled antibodies (Main-Peak) was assessed by CE-SDS (HT Antibody Analysis 200 assay on the LabChip GXII system, PerkinElmer) under non-reducing conditions by relative quantification of the expected protein size to total protein content.

Size exclusion chromatography (SEC) for the determination of the aggregation and oligomeric state of recombinant immunoglobulins was performed by HPLC chromatography. Briefly, protein A-purified product was applied to a TSKgel QC-PAK GFC 300 column (Tosoh Bioscience) or to a Tosoh TSKgel UP-SW3000 column in 250 mM KCl, 200 mM K2HPO4/KH2PO4 buffer (pH 6.2) on a Dionex Ultimate® HPLC system (Thermo Fischer Scientific, Waltham, Massachusetts, USA). The eluted antibody was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard #151–1901 served as a gel filtration calibration standard.

1.7 LC-MS Peptide map procedure for hydroxylysine analysis

Expressed and protein-A purified antibodies were denatured and reduced with 6 M guanidine and 16 mM DTT at pH 7, 37° C for 1 hour. The denatured reduced protein was then carboxymethylated using 73 mM IAA-C12 (Fluka) and then buffer exchanged on a NAP-5 (GE Healthcare Life Sciences) into 50 mM TRIS, 2 mM CaCl2 at pH7.5 and digested by Trypsin (Promega) at 37° C for 1 hour.

The digested samples were then analyzed by liquid chromatography with tandem mass spectrometry (LC-MS-MS). Liquid chromatography was performed on a Waters Acquity UPLC (Waters) with a reversed-phase column Acquity CSH C18, 1.7µm, 130A, 2.1 x 150mm (Waters). The aqueous mobile phase (mobile phase A) contained 0.1% (v/v) formic acid (FA) in HPLC grade water. The organic mobile phase (mobile phase B) contained 0.1% (v/v) FA in acetonitrile. The gradient that was utilized in this experiment used a 2-step linear gradient of 1% to 30% mobile phase B from 2 min to 33 min and to 60% until 42 min followed by an increase to 90% between 42.5 and 44.5 min, a decrease back to 50% between 44.6 min and 50 min followed by a re-equilibration at 1% eluent B from 50 min to 56 min. The column temperature was set to 65°C.

The UPLC was coupled to an Orbitrap FusionTM mass spectrometer (Thermo Scientific). MS1 spectra were acquired with the Orbitrap mass analyzer with a resolution of 120000 while MS/MS data were acquired in the Orbitrap with a resolution of 50000. The MS/MS event on the Orbitrap was repeated for the topN precursor ions with dynamic exclusion of 4.5 sec enabled. The resulting MS data were processed by ByosTM & ByonicTM (Protein Metrics Inc). Manual data interpretation was performed using ByologicTM (Protein Metrics Inc). The MS/MS ByosTM search settings include a precursor mass tolerance of 5 ppm and a fragment mass tolerance of 20 ppm. The enzyme specificity was set to fully specific with one allowed missed cleavage. The quantification of relative hydroxylysine (HyL; "Kox", lysine oxidized)-modified tryptic peptides considering missed cleavage products (0 mc and 1 mc) compared to unmodified peptides ("K") was calculated as followed:

$$rel.area\ Hyl\ modified = \frac{[area\ Kox\ (0\ mc) + area\ Kox\ (1\ mc)]}{[area\ K\ (0\ mc) + area\ K\ (1\ mc)] + [area\ Kox\ (0\ mc) + area\ Kox\ (1\ mc)]} \quad \text{(Equ 5)}$$

**Example 2: Results**

2.1 Identification of Hydroxylysine Modification in TCB mAbs

Using a spectrometric tryptic peptide mapping approach, the inventors identified several +15.9950 Da mass shifts in three different, recombinantly-produced 2+1 IgG format T-cell bispecific (TCB) antibodies: CD19 TCB (also referred to herein as "TCB mAb 1"), HLA-G TCB (also referred to herein as "TCB mAb 2") and GPRC5D TCB (forimtamig; also referred to herein as "TCB mAb 3"), expressed by three different recombinant CHO cell lines (Figure 1B).

| TCB | Constituent polypeptides |
|---|---|
| CD19 TCB (TCB mAb 1) | SEQ ID NO:97 + SEQ ID NO:98 + SEQ ID NO:99 + SEQ ID NO:116 |
| HLA-G TCB (TCB mAb 2) | SEQ ID NO:86 + SEQ ID NO:153 + SEQ ID NO:154 + SEQ ID NO:155 |

| | |
|---|---|
| GPRC5D TCB (TCB mAb 3) | SEQ ID NO:93 + SEQ ID NO:94 + SEQ ID NO:95 + SEQ ID NO:116 |

The levels of the peptide modifications varied based on the positions in the TCB molecules (see Table 1).

| **CD19 TCB** | | | | | | |
|---|---|---|---|---|---|---|
| **CD19 TCB domain** | **Cleaved** | **Sequence** | **Start AA** | **End AA** | **Var. Pos.** | **CD19 TCB [%]** |
| LC-B | nd | XXXXXXXXXXXX**K**GR (SEQ ID NO:17) | 55 | 69 | 67 | <0.1 |
| LC-B | nd | XXXXXXXXXXXX**K** (SEQ ID NO:18) | 53 | 67 | 54 | <0.1 |
| LC-A & B | nd | DSTYSLSSTLTLS**K** (SEQ ID NO:19) | 175/188 | 188/201 | 188/201 | <0.1 |
| HC-K | s | LTVLSSAST**K** (SEQ ID NO:10) | 341 | 350 | 350 | 4.9 |
| | l | LTVLSSAST**K**GPSVFPLAPSSK (SEQ ID NO:20) | 341 | 362 | 350 | 7.4 |
| | | | | | sum | 12.3 |
| **HLA-G TCB** | | | | | | |
| **HLA-G TCB domain** | **Cleaved** | **Sequence** | **Start AA** | **End AA** | **Var. Pos.** | **HLA-G TCB [%]** |
| LC-A | nd | XXXXXXXXXXXX**K**GR (SEQ ID NO:17) | 55 | 69 | 67 | <0.1 |
| HC-K | s | XXXXXXWGQGVLVTVSSAST**K** (SEQ ID NO:26) | 104 | 125 | 125 | 0.5 |
| | l | XXXXXXWGQGVLVTVSSAST**K**GPSVFPLAPSSK (SEQ ID NO:21) | 104 | 137 | 125 | <0.1 |
| | | | | | sum | 0.6 |
| LC-B | nd | L**K**SGTASVVCLLNNFYPR (SEQ ID NO:22) | 131 | 148 | 132 | <0.1 |
| LC-A & B | s | DSTYSLSSTLTLS**K** (SEQ ID NO:19) | 188/176 | 201/189 | 201/189 | <0.1 |
| | l | DSTYSLSSTLTLS**K**ADYEK (SEQ ID NO:23) | 188/176 | 206/198 | 201/198 | <0.1 |
| | | | | | sum | <0.1 |
| HC-K | s | LTVLSSAST**K** (SEQ ID NO:10) | 341 | 350 | 350 | 1.1 |
| | l | LTVLSSAST**K**GPSVFPLAPSSK (SEQ ID NO:20) | 341 | 362 | 350 | 1.2 |
| | | | | | sum | 2.2574 |
| **GPRC5D TCB** | | | | | | |
| **GPRC5D TCB domain** | **Cleaved** | **Sequence** | **Start AA** | **End AA** | **Var. Pos.** | **GPRC5D TCB [%]** |
| LC-A | s | XXXXXXXXXXXX**K** (SEQ ID NO:18) | 55 | 67 | 67 | 0.1 |
| | l | XXXXXXXXXXXX**K**GR (SEQ ID NO:17) | 55 | 69 | 67 | <0.1 |
| | | | | | sum | 0.1 |
| HC-H | nd | NQVSLSCAV**K** (SEQ ID NO:25) | 361 | 370 | 370 | 0.1 |
| HC-K | s | LTVLSSAST**K** (SEQ ID NO:10) | 337 | 346 | 346 | 3.6 |
| | l | LTVLSSAST**K**GPSVFPLAPSSK (SEQ ID NO:20) | 337 | 358 | 346 | 3.2 |
| | | | | | sum | 6.8 |

**Table 1: Hydroxylysine (HyL) levels of TCBs produced by recombinant expression.** The modified lysine residue is highlighted in bold. In case different cleavage versions are quantified together, the single quantities are summed to build a total for the respective locus. The cleaved peptide is marked with an "s" (short) and the non-cleaved version with a "l" (long). Note, not for every locus a peptide with missed

cleavage was detected and indicated in the table by "nd" (not detected); "<0.1" means confirmed detection of modification, but no valid concentrations can be generated; *semi quantitative

In the variable regions of the TCBs, HyL was observed at low levels (<1%), and at high levels in the CH1 regions of heavy chain knob (HC-K) polypeptide (up to 12.3%) C-terminal to the VL region of crossFab moieties (Table 1, Figure 1G). The detected motif with the +15.9950 Da modification in the prominent CH1 element is similar to the motif identified by Xie *et al.*, MAbs. (2016) 8(2): 371-378, but differs in that in the TCB molecules of the present disclosure, the CH1 region is provided in VL-CH1 form (Figure 1A).

Xi *et al.* attributed the +16 Da modification to hydroxylation of the lysine residue at the terminal lysine of SEQ ID NO:16. Based on this previous observation, the inventors investigated the possibility of HyL modification being the reason for the mass shifts observed in the three analyzed TCB mAbs. For this, the exact amino acid positions of the modifications were determined by analysis of the HCD (higher energy collisional dissociation) fragmentation data (Table 1). The location of the +15.9950 Da modification in the prominent LTVLSSASTK peptide (amino acids 341-350; SEQ ID NO:10) of HC-K was attributed to oxidation and confirmed at lysine K350 in the analyzed peptide, by using extracted ion chromatograms of the modified and the unmodified peptide LTVLSSASTK350 (SEQ ID NO:10) for both with and without missed cleavage (Figure 1C). The hydroxylation of lysine results in decreased hydrophobicity of the tryptic peptide with an expected earlier retention time compared to the unmodified peptide. This is confirmed by the XIC comparison of both and is in line with data as reported by Xie *et al.* (Figure 1B).

The influence of HyL residue on the selectivity of trypsin has been reported previously (Molony *et al.*, Tech Prot Chem. (1995) 6:91-98, Molony *et al.*, Anal Biochem. (1998) 258(1):136-137). To investigate possible loss of trypsin selectivity due to hydroxylation of lysine to HyL, the tryptic peptide with one missed cleavage LTVLSSASTKGPSVFPLAPSSK350 (SEQ ID NO:20) was also evaluated for the presence of HyL. Comparison of the XIC intensity scales and calculation of the ratios separately for the different cleavage species revealed that HyL was present in approximately 99% of the peptides with missed cleavage, while HyL was only present in approximately 5% of the correctly-cleaved peptides (Figure 1B). These data suggest that lysine hydroxylation influences the efficiency of trypsin cleavage.

For some samples, the unmodified peptide with missed cleavage (SEQ ID NO:20) could not be detected based on MS2, because the precursor intensity was below the MS2 trigger limit. The tryptic peptide with one missed cleavage was only found in its modified form. A comparison of modified and unmodified peptide based on XIC and retention time was not possible. However, the MS/MS fragment spectrum of the modified peptide revealed nearly 100% coverage, and confirmed the modification position for the same lysine K350 located in the HC-K CH1 domain (Figures 1C to 1F).

2.2 Hotspot modification of Lysine350 in crossed CH1-VL domain of the Knob Heavy Chain in TCB mAbs

The TCB mAbs comprise the lysine hydroxylation hot spot peptide LTVLSSASTK (SEQ ID NO:10) in each of the three Fab moieties. Xie *et al.*, MAbs. (2016) 8(2): 371-378 previously reported hydroxylation

modification of CH1 lysine in an analyzed IgG, and attributed this to the presence of an XKG (SEQ ID NO:2) consensus sequence, which is a site of modification in several endogenous hydroxylated polypeptides. Remarkably, only the lysine in the XKG motif located in $C_H1$ heavy chain was identified by Xie *et al.* as a site for hydroxylation, despite the fact that several further sequences conforming to the XKG motif are present in the IgG.

Interestingly, the C-terminal lysine of the LTVLSSASTK350 (SEQ ID NO:10) peptide located in the crossed HC CH1 Fab domain (*i.e.* of the VL-CH1 region of the crossFab moiety) appears to be particularly susceptible to hydroxylation. HyL was detected in the CH1 region of the crossFab moiety in all three TCB mAb molecules investigated.

Although the hotspot motif is present in the CH1 domains of all three Fab moieties (*i.e.* the two traditional Fab moieties, and the crossFab moiety) in the different TCB mAb molecules investigated in the present studies, HyL modification was only detected in a Fab moiety other than the crossFab moiety in HLA-G TCB (particularly, at LTVLSSASTK125 in the CH1 domain of the HC-K chain of the Fab moiety; see Figure 1G and Figure 2C). HyL modification in the LTVLSSASTK125 peptide was 5 times lower compared to the LTVLSSASTK350 peptide located in VL-CH1 region of the crossFab moiety, indicating additional structural requirements for HyL modification efficiency than solely the amino acid sequence. No HyL modification of LTVLSSASTK125 was detected in CD19 TCB and GPRC5D TCB, despite their comprising the identical consensus sequence STKGP (SEQ ID NO:5) motif.

Minor yet measurable lysine hydroxylation was also detected in the three different TCB mAbs within alternative, similar XKG (SEQ ID NO:2) containing regions of the molecules, but also at sequence motifs representing XKA (SEQ ID NO:3) and XKS (SEQ ID NO:4) – see Figure 2A. However, the STKGP (SEQ ID NO:5) sequence motif dominated all hydroxylated peptide sequences detected (Figure 2B).

The prominent hotspot at the K350 in the crossed VL-CH1 region of the knob heavy chain is close to the transition to the chimeric VL domain, downstream of the intramolecular CDR (Figure 2D). The K350 is located within the unstructured CL-CH1 interface, close to an exposed loop in the CH1 domain. The inventors investigated whether the introduction of a hydroxyl group at this position altered the close spatial structure of the CH1 domain and chemico-physical parameters. Using a molecular prediction approach using artificial lysine protonation, energy minimization, and subsequent protein patch calculation, no substantial differences were observed for surface hydrophobicity, as well as for positive and negative charge patches, indicating that hydroxylation at K350 has either no or only minor effects on overall molecular properties (Figure 2E).

The inventors investigated potential structural differences between the LTVLSSASTK350 and LTVLSSASTK125 motifs using a structural model comparison approach, in an attempt to explain the observed preferential hydroxylation at K350. The Trastuzumab Fab domain, as an exemplary generic IgG Fab structure, was overlapped with a crossFab domain, using the two constant regions CH1 and κ CL for orientation alignment. Structural analysis of the crossFab domain by comparison with the structural

organization of the Fab domain of Trastuzumab revealed significant differences in the upstream elbow segment and for a neighboring exposed loop (Figure 2G). The elbow segment appears to be more exposed in the crossFab, as compared to the canonical Fab structure, which might be a consequence of the crossFab comprising an additional amino acid in this region. In addition, a K350 neighbored loop in the CH1 of the crossFab structure adopts a different conformation, indicating some structural flexibility. This might provide greater steric accessibility of the K350 region to lysyl hydroxylases.

2.3 Lysine hydroxylation is dynamic and dependent on iron availability in cell cultures

Upon examining the HyL level of CD19 TCB over the process duration, the inventors observed a trend of decreasing HyL level in the produced molecule, particularly at the prominent K350 residue located in the intersecting VL-CH1 domain during later process phases, beginning from day 6 on until day of harvest at day 10 (Figure 3A). The measured values are also shown in Table 2.

| Data point | Process time [d] | Hyl350 [%] |
|---|---|---|
| d2 | 1.80 | 16.855 |
| d6 | 5.80 | 15.504 |
| d8 | 7.80 | 11.974 |
| d9 | 8.80 | 10.872 |
| d10 | 9.80 | 12.249 |

**Table 2:** During a representative fed-batch fermentation process, Hyl350 levels of TCB1 were measured to generate the time course shown in Figure 3A.

The process productivity at various time points was determined by calculating the daily formation of new product, which contributed to the cumulative final product concentration at the day of harvest. A consistent augmentation in the levels of newly formed product was observed until the initiation of the shift phase on day 6, followed by a subsequent decline (Figure 3B). Intriguingly, through the simulation of the time-resolved levels of the newly modified HyL350 peptide between the sampling days using a sigmoid interpolation function, the inventors discerned a specific temporal decrease in the levels at the day with the onset of the global HyL350 shift at day 6, followed by a subsequent growth again (Figure 3B).

The inventors have previously determined that HyL modification of the TCB mAbs arises as a consequence of enzymatic PLOD activity (see *e.g.* WO 2023/232961 A1). Given that PLODs require $Fe^{2+}$ ions as cofactor for catalytic activity (Figure 3D), the inventors sought to analyze the available cell culture iron level during the process duration. A clear decrease in cell culture iron level was observed over time, which became more pronounced when examining the iron availability per cell, where iron levels stagnated at a low level from day 6 onwards (Figures 3E, 3F). Low iron levels were also accompanied by a very low steady-state level of cell-specific iron consumption rate $qS_{Iron}$ from day 6 on until the end of the process at day 10 (Figure 3G). Both the reduced iron availability and the lower cellular iron uptake occurs

contemporaneously with the shift phase to lower levels of HyL350 in the antibodies produced from the cells.

In further experiments, the inventors investigated whether the initial levels of iron in the cell culture media influence the HyL modification of TCB mAb motifs. The CD19 TCB expressing CHO cell line was cultured with ferrous ion levels ranging from 0 to 200 µM, and samples of the produced product were subsequently analyzed for HyL350 abundance. Analysis of the HyL modification levels of the hotspot motif K350 and the less abundant K67 location (located near to the CDR region of the N-terminal Fab domain), revealed a dose-dependent increase in HyL modification, reaching saturation with 80 µM iron in the culture (Figures 3H, 3I). The low level of HyL modification at 0 µM iron supplementation is likely induced by the residual iron transferred from the precursor culture used to inoculate the experiment.

These data suggest that at Fe ion concentrations below 40 µM, the availability of iron is a rate-limiting factor for HyL modification of polypeptides expressed from cells in culture. The fitting models suggested that cell culture medium Fe ion concentrations of less than 10 µM are adequate to elicit half-maximal activation of the hydroxylation reactions (Figure 3J).

2.4 Lysine hydroxylation is iron concentration-dependent

In further experiments, the inventors performed batch cultures (see Example 1.3) in which cells expressing CD19 TCB, HLA-G TCB or GPRC5D TCB were cultured in cell culture media comprising a range of concentrations of iron. The expressed TCB mAbs were subsequently purified and analyzed as described in Examples 1.6 and 1.7, to determine the extent of HyL modification at lysine residues of the TCB mAbs susceptible to hydroxylation. Concentration-response curves were fitted to the data, and iron concentrations achieving half-maximal level of HyL modification (*i.e.* EC50 values) for the given lysine residues of the different TCB mAbs were determined.

The results are shown in Figures 4A to 4C. For all positions analyzed, and in all of the TCB mAbs characterized, the proportion of species comprising HyL modification at the relevant position was found to be iron concentration-dependent. Iron concentrations achieving half-maximal HyL modification were remarkably similar for the different lysine residues and between the different TCB mAbs, and ranged from 3.58 µM to 7.43 µM.

**Example 3: Discussion**

HyL modifications of the expressed TCB mAbs were found to be predominantly located at the C-terminal K350 of the LTVLSSASTK peptide that is located within the unstructured, VL-CH1 interface of the crossFab moiety within the heavy knob chain, and to a much lesser content at K125 in a similar peptide LTVLSSASTK, located in N-terminal Fab moiety. The hotspot position is similar, yet not identical in respect to the upstream sequence and structural neighborhood as motif previously identified by Xie *et al.*, MAbs. (2016) 8(2): 371-378 (SEQ ID NO:16). The crossing of the chains in the cross-Fab moiety appears to result in the formation of a more flexible, exposed hotspot motif LTVLSSASTK350, compared to the

canonical VH-CH1 interface in Fab moieties. This might account for the increased HyL modification at this position in the crossFab moiety compared to the equivalent position of the Fab moiety.

The reason for high HyL modification at K350 of the crossFab moieties relative to the equivalent position in the canonical Fab moieties in the TCB mAb molecules might be that VL-CH1 provides a structure facilitating access of the lysyl hydroxylase PLOD enzymes to K350. The data suggest that higher-order structural features are important for enzyme-target-recognition rather than simplified, short consensus sequence definitions.

In addition to hydroxylation at the previously-identified XKG motif (SEQ ID NO:2), the present study also detected HyL at XKA (SEQ ID NO:3) and XKS (SEQ ID NO:4) motifs, suggesting a certain level of flexibility in consensus sequence substrates for PLOD enzymes.

The presence of a HyL in peptides is able to abolish the tryptic digestion (Markolovic et al., (2018) Nat Chem Biol. 14(7):688-695). In contrast to Xie *et al.*, in the present studies peptide mapping analysis of TCB mAbs showed a clear effect of HyL on digestion efficiency (Table 1). Approximately half of the prominent, highly-abundant peptides with HyL modification showed a tryptic miscleavage event.

Most therapeutic proteins, such as TCB mAbs and generic IgGs, use dynamic cell cultivation processes and high performance CHO production cell lines (see review by Kim *et al.* Appl Microbiol Biotechnol. (2012) 93(3):917-930). Though, these CHO production cell lines originate from the same CHO ancestor cell line and related CHO host cell sub lineages, each final production clone demonstrate a more or less unique metabolic profile (Popp *et al.*, Biotechnol Bioeng. (2016) 113(9):2005-2019). As consequence, the availability of specific substrates and cofactors needed for biocatalytical processes are tightly linked to cell-specific consumption rates of small molecules present in cell culture. In the present studies, the inventors demonstrate that iron, acting as a cofactor for enzymatic hydroxylation activity in PLODs, modulates the level of hydroxylation of lysine residues within consensus motifs in recombinantly-expressed TCB mAbs. Dynamic alteration in the per-cell iron availability and consumption rate was observed, with a reduction during the later stages of the cell cultivation process.

**Example 4: Further analysis of the influence of iron concentration in the cell culture medium on the level of lysine hydroxylation**

In further experiments, the influence of iron concentration in the cell culture medium on the level of hydroxylation of lysines of CD19 TCB or GPRC5D TCB expressed from CHO cells was further evaluated.

CHO cells transfected with vectors encoding TCB mAb1 or TCB mAb3 were cultivated using a regular, 3 to 4 day split method in seed train medium (a proprietary DMEM/F12-based medium, including iron) supplemented with puromycin, to obtain clones stably expressing CD19 TCB or GPRC5D TCB.

Prior to expression experiments, CHO clones stably expressing either TCB mAb1 or TCB mAb3 were cultivated in seed train medium for four days in 125 mL shake flasks containing a working volume of 25 mL, and subsequently split into 500 mL shake flasks with a working volume of up to 150 mL.

Briefly, cultivation was performed in 125 mL shake flasks with a working volume of 25 mL. The target seeding cell density was set at 2.5 x $10^6$ cells/mL. Medium exchange was performed via centrifugation at 220 x *g* for 5 min, with a maximum of 5% carryover of residual seed train medium. Cells were resuspended in cell culture media for evaluation of the effect of iron concentration on the level of lysine hydroxylation in molecules expressed from the cells.

Two different chemically-defined base cell culture media were used: (i) a proprietary DMEM/F12-based medium lacking iron, and (i) commercially-available alpha-MEM media (ThermoFisher Scientific, Cat No. 12000014), which also lacks iron. Cell culture medium lacking iron was used to enable control over the level of iron in the culture, by supplementation. The alpha-MEM media was supplemented with 10 g/L glucose and 4 mM L-glutamine prior to culture cultivation of cells for the expression of CD19 TCB or GPRC5D TCB.

CD19 TCB or GPRC5D TCB molecules were expressed from cells cultured in cell culture medium without iron supplementation (0 µM), or in culture medium supplemented from a 5 mM stock solution of iron to a final concentration in culture of 0 µM, 15 µM, 30 µM or 45 µM. Iron concentrations in cell culture medium were evaluated by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis.

Cell culture supernatants were clarified and analytical protein A chromatography was performed as described in Example 1.6. Lysine hydroxylation in expressed CD19 TCB and GPRC5D TCB molecules was analysed by LC-MS Peptide mapping as described in Example 1.7.

The results are shown in the tables below and in Figures 5A to 5C and 6A to 6C. A Fe concentration-dependent increase in lysine hydroxylation was observed in CD19 TCB and GPRC5D TCB molecules produced by expression in both the proprietary culture medium and commercially-available alpha-MEM medium. Increasing levels of lysine hydroxylation were observed with increasing Fe concentration in the cell culture medium across all sites comprising a lysine residue susceptible to hydroxylation, but the correlation was most particularly striking for the most abundant peptide LTVLSSASTK (SEQ ID NO:10; C-terminal K = position 350 for CD19 TCB, and position 346 for GPRC5D TCB). Levels of HyL increased across the full range of iron concentrations tested up to 45 µM, suggesting that even higher iron concentrations might be necessary to observe saturation or stagnation of HyL formation. The HyL observed at "0 µM" iron are likely due to the carryover of residual iron from the seedtrain cultivation (into the TCB mAb expression culture).

**CD19 TCB:**

| Medium | Fe conc. (µM) | YNNYATYYADSVK67 (SEQ ID NO:169) | GTYYYGPQLFDYWGQGTTVTVSSASTK125 (SEQ ID NO:171) | LTVLSSASTK350 (SEQ ID NO:10) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| alpha_MEM_0µM | < LOD (1.369) | 0.06% | 0.33% | 8.46% |
| alpha_MEM_15µM | 17.272 | 0.1% | 0.55% | 15.33% |
| alpha_MEM_30µM | 33.439 | 0.09% | 0.44% | 17.76% |
| alpha_MEM_45µM | 49.111 | 0.1% | 0.70% | 18.36% |
| Prop_0µM | <LOD (0.936) | 0.06% | 0.22% | 9.5% |
| Prop_15µM | 16.58 | 0.11% | 0.52% | 17.04% |
| Prop_30µM | 32.537 | 0.14% | 0.58% | 19.53% |
| Prop_45µM | 43.07 | 0.16% | 0.57% | 21.39% |

**GPRC5D TCB:**

| **Medium** | **Fe conc. (µM)** | **YNNYATYYADSVK67 (SEQ ID NO:169)** | **AEDTAVYYCATHTGDYFDYWGQGTMVTVSSASTK121 (SEQ ID NO:173)** | **LTVLSSASTK346 (SEQ ID NO:10)** |
|---|---|---|---|---|
| alpha_MEM_0µM | 3.148 | 0.08% | 0.13% | 7.76% |
| alpha_MEM_15µM | 15.765 | 0.11% | 0.21% | 12.86% |
| alpha_MEM_30µM | 21.562 | 0.13% | 0.26% | 15.02% |
| alpha_MEM_45µM | 49.433 | 0.14% | 0.29% | 13.32% |
| Prop_0µM | 2.467 | 0.08% | 0.13% | 6.27% |
| Prop_15µM | 17.068 | 0.14% | 0.23% | 13.31% |
| Prop_30µM | 32.963 | 0.19% | 0.24% | 16.63% |
| Prop_45µM | 49.113 | 0.20% | 0.20% | 17.04% |

These data indicate that expressing polypeptides comprising lysine residues susceptible to hydroxylation in cell culture medium having an iron concentration of <50 µM (*e.g.* <45 µM) reduces the proportion of molecules expressed molecules comprising HyL at the relevant position. These data also demonstrate that this technical effect is observed following expression from cells cultured different types of culture media.

**Claims**

1. A method for producing a polypeptide comprising an amino acid sequence including a lysine residue susceptible to hydroxylation, and/or including a proline residue susceptible to hydroxylation, wherein the method comprises culturing cells comprising nucleic acid for expressing the polypeptide for the majority of the period of culture in cell culture medium having an Fe ion concentration of less than 60 μM.

2. The method according to claim 1, wherein the method comprises seeding the cells in cell culture medium comprising an Fe ion concentration of less than 60 μM.

3. The method according to claim 1 or claim 2, wherein the method comprises culturing cells comprising nucleic acid for expressing the polypeptide in cell culture medium comprising an Fe ion concentration of less than 10 μM, for the majority of the period of culture.

4. The method according to any one of claims 1 to 3, wherein the method comprises seeding the cells in cell culture medium comprising an Fe ion concentration of less than 10 μM.

5. The method according to any one of claims 1 to 4, wherein the polypeptide comprises an amino acid sequence including a lysine residue susceptible to hydroxylation, and wherein the amino acid sequence including a lysine residue susceptible to hydroxylation comprises the amino acid sequence of SEQ ID NO:1.

6. The method according to any one of claims 1 to 5, wherein the polypeptide comprises an amino acid sequence including a lysine residue susceptible to hydroxylation, and wherein the polypeptide comprises an amino acid sequence selected from SEQ ID NOs:5, 7, 9 and 11.

7. The method according to any one of claims 1 to 6, wherein the polypeptide comprises the VL-CH1 region of a crossFab moiety.

8. The method according to any one of claims 1 to 7, wherein the polypeptide comprises an amino acid sequence including a lysine residue susceptible to hydroxylation, and wherein the polypeptide comprises an amino acid sequence selected from SEQ ID NOs:106, 107 and 108.

9. The method according to any one of claims 1 to 8, wherein the polypeptide is a constituent polypeptide of a multispecific antigen-binding molecule.