Glycosylation of immunoglobulin single variable domains

FIELD OF THE INVENTION

The present technology relates to the field of glycosylation of immunoglobulin single variable domains (ISVDs). More specifically, the present technology provides specific positions within the amino acid sequence of an ISVD for use as glycosylation acceptor sites. The present technology also relates to ISVDs modified with specific glycans at the specified glycosylation acceptor sites and to conjugates thereof. The present technology also relates to polypeptides comprising at least one ISVD of the present technology, at least one ISVD with specific glycans or at least one conjugate thereof. The invention furthermore relates to a nucleotide sequence or nucleic acid encoding such ISVD and/or polypeptide, a method of making such ISVDs and/or polypeptides, and a composition comprising such ISVDs, conjugates and/or polypeptides.

BACKGROUND OF THE INVENTION

Over the past few years, antibody therapy has been rapidly gaining traction in the medical world. The ability to selectively target only the tissues affected by disease, while leaving healthy tissue intact, is very desirable for patients' quality of life.

Although antibody therapy is target specific, there are often issues encountered by working with such complex biologic molecules. For instance, issues in stability or solubility. Consequently, there is a constant drive to improve upon existing antibody therapies.

Glycosylation is a commonly applied post-translational modification in antibody therapeutics, specifically for monoclonal antibodies. Glycosylation is a process that may be required to obtain the desired therapeutic efficacy. Additionally, glycosylation can facilitate in the conjugation of one antibody to another and/or to other moieties that may provide additional functions to the antibody.

Immunoglobulin single variable domains (ISVDs) present interesting therapeutic potential within the field of antibody therapeutics due to their small size. Furthermore, production is much simpler, quicker and cheaper than the production of monoclonal antibodies.

Glycosylation of ISVDs at specified glycosylation acceptor sites has been described in WO 2016/150845, WO 2018/206734, and WO 2021/116252.

However, glycosylation of ISVDs has proven to be challenging. Depending on the host cell/organism used, glycosylation will not occur at all, or it occurs in limited ways. Additionally, introducing glycosylation

acceptor sites frequently resulted in interference in the folding of the ISVD proteins, with loss of affinity and/or stability and thus a loss of functionality as a consequence.
However, since glycosylation facilitates in conjugation of the ISVD to other moieties and can have an impact on therapeutic efficacy, a need remains for effective and stable glycosylated ISVDs.

SUMMARY OF THE INVENTION

The present technology aims to resolve issues related to the glycosylation of ISVDs. Therefore, in a first aspect, the present technology relates to a polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

In another aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

In another aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108, and 110, according to Kabat numbering.

In a further aspect, the present technology relates to a polypeptide comprising or (essentially) consisting of two ISVDs, wherein at least one of said two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 105, 108, and 110, according to Kabat numbering.

In yet a further aspect, the present technology relates to a polypeptide comprising or (essentially) consisting of two ISVDs, wherein at least one of said two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 55, 105 and 108, according to Kabat numbering.

In another aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of two ISVDs, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 53, 55, 68, 73, 75, 105, 108 and 110, according to Kabat numbering.

In yet another aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of two ISVDs, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

In a further aspect, the present technology concerns a polypeptide comprising at least three ISVDs, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

In yet another aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of three ISVDs, wherein at least one of said three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

In another aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of three ISVDs, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 55, 73, 75, 76, 105, 108, and 110, according to Kabat numbering.

In a further aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of three ISVDs, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, and 105, according to Kabat numbering.

In yet a further aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of three ISVDs, wherein the ISVD that is neither at the C-terminal end nor at the N-terminal end comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

The present technology also relates to such polypeptides that are glycosylated at one or more of these glycosylation acceptor sites.

In another aspect, the present technology relates to a nucleotide sequence or nucleic acid that encodes a polypeptide according to the present technology.

In a further aspect, the present technology concerns a method for producing a polypeptide according to the present technology, comprising the step of:

- Expressing the nucleotide sequence or nucleic acid according to the present technology in a suitable host cell or host organism,

wherein the host cell or host organism is capable of glycosylating the expressed polypeptide.

In yet a further aspect, the present technology relates to a method for the conjugation of the polypeptide to a moiety.

The present technology also provides a conjugate comprising the glycosylated polypeptide and a conjugated moiety, wherein the moiety is conjugated to the glycan on the polypeptide.

In another aspect, the present technology relates to a composition comprising the polypeptide, the nucleic acid or the conjugate according to the present technology.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the glycan profile as measured by LC-QTOF of glycosylated ISVD T043800005.

Figure 2 shows the glycan profile as measured by LC-QTOF of glycosylated ISVD T043800002.

Figure 3 shows the MALDI-TOF measurement of conjugates of ISVD T043800005 with bis-Mannose-6-Phosphate as described in Example 12.

Figure 4 shows the MALDI-TOF measurement of conjugates of ISVD T043800002 with bis-Mannose-6-Phosphate as described in Example 12.

Figure 5 shows the flow cytometry measurement to assess the TNF internalization in Jurkat cells (**A**) or K562 cells (**B**) after treatment with TNF complex and anti-TNF ISVD or bisM6P-anti-TNF ISVD conjugate at various concentrations for 1 and 4 hrs.

Figure 6 shows the fold increase in mean fluorescence intensity (MFI) in Jurkat cells (**A**) or K562 cells (**B**) which indicates the TNF internalization by bisM6P-anti-TNF ISVD conjugate over the anti-TNF ISVD alone.

Figure 7 shows Western blotting of TNF-α after treatment with either anti-TNF ISVD or bisM6P-anti-TNF ISVD at various timepoints in **(A)** cell lysates, after internalization in K562 cells or Jurkat cells at 37 °C for 2 hrs, followed by a treatment with or without bafilomycin for 4 and 24 hrs, and **(B)** in supernatant from the same K562 cell culture.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Sambrook et al. (Molecular Cloning: A Laboratory Manual (2nd.Ed.) Vols. 1-3, Cold Spring Harbor Laboratory Press, 1989), F. Ausubel et al. (Current protocols in molecular biology, Green Publishing and Wiley Interscience, New York, 1987), Lewin (Genes II, John Wiley & Sons, New York, N.Y., 1985), Old et al. (Principles of Gene Manipulation: An Introduction to Genetic Engineering (2nd edition) University of California Press, Berkeley, CA, 1981); Roitt et al. (Immunology (6th. Ed.) Mosby/Elsevier, Edinburgh, 2001), Roitt et al. (Roitt's Essential Immunology (10th Ed.) Blackwell Publishing, UK, 2001), and Janeway et al. (Immunobiology (6th Ed.) Garland Science Publishing/Churchill Livingstone, New York, 2005), as well as to the general background art cited herein.

Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein; as well as to for example the following reviews Presta (Adv. Drug Deliv. Rev. 58 (5-6): 640-56, 2006), Levin and Weiss (Mol. Biosyst. 2(1): 49-57, 2006), Irving et al. (J. Immunol. Methods 248(1-2): 31-45, 2001), Schmitz et al. (Placenta 21 Suppl. A: S106-12, 2000), Gonzales et al. (Tumour Biol. 26(1): 31-43, 2005), which describe techniques for protein engineering, such as affinity maturation and other techniques for improving the specificity and other desired properties of proteins such as immunoglobulins.

The term "sequence" as used herein (for example in terms like "immunoglobulin sequence", "antibody sequence", "variable domain sequence", "VHH sequence" or "protein sequence"), should generally be understood to include both the relevant amino acid sequence as well as nucleic acids or nucleotide sequences encoding the same, unless the context requires a more limited interpretation.

A nucleic acid or amino acid is considered to be "(in) (essentially) isolated (form)" - for example, compared to the reaction medium or cultivation medium from which it has been obtained - when it has been separated from at least one other component with which it is usually associated in said source or medium, such as another nucleic acid, another protein/polypeptide, another biological component or macromolecule or at least one contaminant, impurity or minor component. In particular, a nucleic acid or amino acid is considered "(essentially) isolated" when it has been purified at least 2-fold, in particular at least 10-fold, more in particular at least 100-fold, and up to 1000-fold or more. A nucleic acid or amino acid that is "in (essentially) isolated form" is preferably essentially homogeneous, as determined using a suitable technique, such as a suitable chromatographical technique, such as polyacrylamide-gel electrophoresis.

When a nucleotide sequence or amino acid sequence is said to "comprise" another nucleotide sequence or amino acid sequence, respectively, or to "essentially consist of" another nucleotide sequence or amino acid sequence, this may mean that the latter nucleotide sequence or amino acid sequence has been incorporated into the first mentioned nucleotide sequence or amino acid sequence, respectively, but more usually this means that the first mentioned nucleotide sequence or amino acid sequence comprises within its sequence a stretch of nucleotides or amino acid residues, respectively, that has the same

nucleotide sequence or amino acid sequence, respectively, as the latter sequence, irrespective of how the first mentioned sequence has actually been generated or obtained (which may for example be by any suitable method described herein). By means of a non-limiting example, when a polypeptide is said to comprise an immunoglobulin single variable domain, this may mean that said immunoglobulin single variable domain sequence has been incorporated into the sequence of the polypeptide, but more usually this means that the polypeptide contains within its sequence the sequence of the immunoglobulin single variable domains irrespective of how said polypeptide has been generated or obtained. Also, when a nucleic acid or nucleotide sequence is said to comprise another nucleotide sequence, the first mentioned nucleic acid or nucleotide sequence is preferably such that, when it is expressed into an expression product (e.g. a polypeptide), the amino acid sequence encoded by the latter nucleotide sequence forms part of said expression product (in other words, that the latter nucleotide sequence is in the same reading frame as the first mentioned, larger nucleic acid or nucleotide sequence).
By "(essentially) consists of" is meant that the later nucleic acid sequence or amino acid sequence, either is exactly the same as the polypeptide (e.g. the CDR region; the ISVD) or corresponds to the polypeptide (e.g. the CDR region; the ISVD) which has a limited number of amino acid residues, such as 1-20 amino acid residues, for example 1-10 amino acid residues and preferably 1-6 amino acid residues, such as 1, 2, 3, 4, 5 or 6 amino acid residues, added at the amino terminal end, at the carboxy terminal end, or at both the amino terminal end and the carboxy terminal end of the immunoglobulin single variable domain.

Amino acid residues will be indicated according to the standard three-letter or one-letter amino acid code. Reference is made to Table A-2 on page 48 of WO 08/020079.

2. Polypeptides and ISVDs with glycosylation acceptor sites

ISVD glycosylation has posed challenging. The inventors have now surprisingly found that glycosylation acceptor sites can be introduced at previously unknown positions, and that these glycosylation acceptor sites provide ISVDs with good glycosylation properties while the glycosylation does not interfere with the binding and folding of the ISVD, which in turn provides good conjugation properties, e.g. in production of ISVD conjugates.

Glycosylation is the reaction in which a carbohydrate (or 'glycan'), i.e. a glycosyl donor, is attached to a hydroxyl or other functional group of another molecule (a glycosyl acceptor) in order to form a

glycoconjugate. In biology, glycosylation usually refers to an enzyme-catalysed reaction. Glycosylation is a form of co-translational and post-translational modification. The majority of proteins synthesized in the rough endoplasmic reticulum undergoes glycosylation. Glycosylation is also present in the cytoplasm and nucleus as the O-GlcNAc modification. Different classes of glycans are produced: N-linked glycans attached to a nitrogen of asparagine or arginine sidechains; O-linked glycans attached to the hydroxyl oxygen of serine, threonine, tyrosine, hydroxylysine, or hydroxyproline sidechains, or to oxygens on lipids such as ceramide; phosphoglycans linked through the phosphate of a phosphoserine; and C-linked glycans, a rare form of glycosylation where a sugar is added to a carbon on a tryptophan sidechain.

A "glycan" as used herein generally refers to glycosidically linked monosaccharides, oligosaccharides and polysaccharides. Hence, carbohydrate portions of a glycoconjugate, such as a glycoprotein, glycolipid, or a proteoglycan are referred to herein as a "glycan". Glycans can be homo- or heteropolymers of monosaccharide residues and can be linear or branched. N-linked glycans may be composed of Galactose, neuraminic acid, N-acetylglucosamine (GalNAc), Fucose, Mannose, and other monosaccharides, as also exemplified further herein. In eukaryotes, O-linked glycans are assembled one sugar at a time on a serine or threonine residue of a peptide chain in the Golgi apparatus. Unlike N-linked glycans, there are no known consensus sequences but the position of a proline residue at either -1 or +3 relative to the serine or threonine is favourable for O-linked glycosylation.

The term “immunoglobulin single variable domain” (ISVD), interchangeably used with “single variable domain”, defines immunoglobulin molecules wherein the antigen binding site is present on, and formed by, a single immunoglobulin domain. This sets immunoglobulin single variable domains apart from “conventional” immunoglobulins (e.g. monoclonal antibodies) or their fragments (such as Fab, Fab’, F(ab’)2, scFv, di-scFv), wherein two immunoglobulin domains, in particular two variable domains, interact to form an antigen binding site. Typically, in conventional immunoglobulins, a heavy chain variable domain (VH) and a light chain variable domain (VL) interact to form an antigen binding site. In this case, the complementarity determining regions (CDRs) of both VH and VL will contribute to the antigen binding site, i.e. a total of 6 CDRs will be involved in antigen binding site formation.

In view of the above definition, the antigen-binding domain of a conventional 4-chain antibody (such as an IgG, IgM, IgA, IgD or IgE molecule; known in the art) or of a Fab fragment, a F(ab')2 fragment, an Fv fragment such as a disulfide linked Fv or a scFv fragment, or a diabody (all known in the art) derived from

such a conventional 4-chain antibody, would normally not be regarded as an immunoglobulin single variable domain, as, in these cases, binding to the respective epitope of an antigen would normally not occur by one (single) immunoglobulin domain but by a pair of (associating) immunoglobulin domains such as light and heavy chain variable domains, i.e., by a VH-VL pair of immunoglobulin domains, which jointly bind to an epitope of the respective antigen.

In contrast, immunoglobulin single variable domains are capable of specifically binding to an epitope of the antigen without pairing with an additional immunoglobulin variable domain. The binding site of an immunoglobulin single variable domain is formed by a single VH, a single VHH or single VL domain.

As such, the single variable domain may be a light chain variable domain sequence (e.g., a VL-sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (e.g., a VH-sequence or VHH sequence) or a suitable fragment thereof; as long as it is capable of forming a single antigen binding unit (i.e., a functional antigen binding unit that essentially consists of the single variable domain, such that the single antigen binding domain does not need to interact with another variable domain to form a functional antigen binding unit).

An immunoglobulin single variable domain (ISVD) can for example be a heavy-chain ISVD, such as a VHH, including a humanized VHH, a VH, including a camelized VH and a human VH. In one embodiment, it is a VHH, a camelized VH or humanized VHH. Heavy chain ISVDs can be derived from a conventional four-chain antibody or from a heavy chain antibody.

For example, the immunoglobulin single variable domain may be a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), a "dAb" or dAb (or an amino acid sequence that is suitable for use as a dAb) or a NANOBODY® ISVD (as defined herein and including, but not limited to, a VHH); other single variable domains, or any suitable fragment of any one thereof. [Note: NANOBODY ® and NANBODIES® are registered trademarks of Ablynx N.V.]

In particular, the immunoglobulin single variable domain may be a NANOBODY® ISVD (such as a VHH, including a humanized VHH or camelized VH) or a suitable fragment thereof.
“VHH domains”, also known as VHHs or VHH antibody fragments, have originally been described as the antigen binding immunoglobulin variable domain of “heavy chain antibodies” (i.e., of “antibodies devoid

of light chains"; Hamers-Casterman et al. Nature 363: 446-448, 1993). The term "VHH domain" has been chosen in order to distinguish these variable domains from the heavy chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "VH domains") and from the light chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "VL domains"). For a further description of VHHs, reference is made to the review article by Muyldermans (Reviews in Molecular Biotechnology 74: 277-302, 2001).

The generation of immunoglobulin sequences, such as VHHs, has been described extensively in various publications, among which WO 94/04678, Hamers-Casterman et al. 1993 and Muyldermans et al. 2001 (Reviews in Molecular Biotechnology 74: 277-302, 2001). In these methods, camelids are immunized with the target antigen in order to induce an immune response against said target antigen. The repertoire of VHHs obtained from said immunization is further screened for VHHs that bind the target antigen. In these instances, the generation of antibodies requires purified antigen for immunization and/or screening. Antigens can be purified from natural sources, or in the course of recombinant production. Immunization and/or screening for immunoglobulin sequences can be performed using peptide fragments of such antigens.

Immunoglobulin sequences of different origin, comprising mouse, rat, rabbit, donkey, human and camelid immunoglobulin sequences can be used in the present technology. Also, fully human, humanized or chimeric sequences can be used. For example, camelid immunoglobulin sequences and humanized camelid immunoglobulin sequences, or camelized domain antibodies, e.g., camelized dAbs as described by Ward et al (see for example WO 94/04678 and Riechmann, Febs Lett., 339:285-290, 1994 and Prot. Eng., 9:531-537, 1996) can be used herein. The ISVDs can be fused forming a multivalent and/or multispecific construct (for multivalent and multispecific polypeptides containing one or more VHH domains and their preparation, reference is also made to Conrath et al. 2001 (J. Biol. Chem., Vol. 276, 10. 7346-7350), as well as for example WO 96/34103 and WO 99/23221).

However, it should be noted that the ISVD comprised in the present technology is not limited to the origin of the ISVD sequence (or to the nucleotide sequence used to express it), nor to the way that the ISVD sequence or nucleotide sequence is (or has been) generated or obtained. Thus, the ISVD sequences may be naturally occurring sequences (from any suitable species) or synthetic or semi-synthetic sequences. In a specific but non-limiting aspect, the ISVD sequence is a naturally occurring sequence (from any suitable

species) or a synthetic or semi-synthetic sequence, including but not limited to "humanized" (as defined herein) immunoglobulin sequences (such as partially or fully humanized camelid, mouse or rabbit immunoglobulin sequences, and in particular partially or fully humanized VHH sequences), "camelized" (as defined herein) immunoglobulin sequences (and in particular camelized VH sequences), as well as ISVDs that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing.

Similarly, nucleotide sequences may be naturally occurring nucleotide sequences or synthetic or semi-synthetic sequences, and may for example be sequences that are isolated by PCR from a suitable naturally occurring template (e.g. DNA or RNA isolated from a cell), nucleotide sequences that have been isolated from a library (and in particular, an expression library), nucleotide sequences that have been prepared by introducing mutations into a naturally occurring nucleotide sequence (using any suitable technique known per se, such as mismatch PCR), nucleotide sequences that have been prepared by PCR using overlapping primers, or nucleotide sequences that have been prepared using techniques for DNA synthesis known per se.

A "humanized VHH" comprises an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring VHH domain, but that has been "humanized", i.e. by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring VHH sequence (and in particular in the framework sequences) by one or more of the amino acid residues that occur at the corresponding position(s) in a VH domain from a conventional 4-chain antibody from a human being. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the prior art (e.g., WO 2008/020079). Again, it should be noted that such humanized VHHs can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VHH domain as a starting material.

A "camelized VH" comprises an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring VH domain, but that has been "camelized", i.e. by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring VH domain from a conventional 4-chain

antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a VHH domain of a (camelid) heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the description in the prior art (e.g., Davies and Riechman (1994 and 1996), supra). Such "camelizing" substitutions are inserted at amino acid positions that form and/or are present at the VH-VL interface, and/or at the so-called Camelidae hallmark residues, as defined herein (see for example WO 94/04678 and Davies and Riechmann (1994 and 1996), supra). In one embodiment, the VH sequence that is used as a starting material or starting point for generating or designing the camelized VH is a VH sequence from a mammal, such as the VH sequence of a human being, such as a VH3 sequence. However, it should be noted that such camelized VH can be obtained in any suitable manner known per se and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring VH domain as a starting material.

The structure of an immunoglobulin single variable domain sequence can be considered to be comprised of four framework regions ("FRs"), which are referred to in the art and herein as "Framework region 1" ("FR1"); "Framework region 2" ("FR2"); "Framework region 3" ("FR3"); and "Framework region 4" ("FR4"), respectively; which framework regions are interrupted by three complementary determining regions ("CDRs"), which are referred to in the art and herein as "Complementarity Determining Region 1" ("CDR1"); "Complementarity Determining Region 2" ("CDR2"); and "Complementarity Determining Region 3" ("CDR3"), respectively.

As further described in paragraph q) on pages 58 and 59 of WO 08/020079 , the amino acid residues of an ISVD can be numbered according to the general numbering for VH domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muyldermans, 2000 (J. Immunol. Methods 240 (1-2): 185-195; see for example Figure 2 of this publication). In the present application, unless specified otherwise, the sequence of the ISVDs is determined according to Kabat numbering.

It should be noted that, as is well known in the art for VH domains and for VHH domains, the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering. That is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino

acid residues than the number allowed for by the Kabat numbering. This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence. The total number of amino acid residues in a VH domain and a VHH domain will usually be in the range of from 110 to 120, often between 112 and 115. It should however be noted that smaller and longer sequences may also be suitable for the purposes described herein.

Determination of CDR regions may also be done according to different methods. In the CDR determination according to Kabat, FR1 of an ISVD comprises the amino acid residues at positions 1-30, CDR1 of an ISVD comprises the amino acid residues at positions 31-35, FR2 of an ISVD comprises the amino acids at positions 36-49, CDR2 of an ISVD comprises the amino acid residues at positions 50-65, FR3 of an ISVD comprises the amino acid residues at positions 66-94, CDR3 of an ISVD comprises the amino acid residues at positions 95-102, and FR4 of an ISVD comprises the amino acid residues at positions 103-113.

The framework sequences are (a suitable combination of) immunoglobulin framework sequences or framework sequences that have been derived from immunoglobulin framework sequences (for example, by humanization or camelization). For example, the framework sequences may be framework sequences derived from a light chain variable domain (e.g., a VL sequence) and/or from a heavy chain variable domain (e.g., a VH sequence or VHH sequence). In one particular aspect, the framework sequences are either framework sequences that have been derived from a VHH sequence (in which said framework sequences may optionally have been partially or fully humanized) or are conventional VH sequences that have been camelized (as defined herein).

In particular, the framework sequences present in the ISVD sequences used in the methods described herein may contain one or more of Hallmark residues (as defined herein below), such that the ISVD sequence is a NANOBODY® ISVD, such as e.g., a VHH, including a humanized VHH, or camelized VH. Non-limiting examples of (suitable combinations of) such framework sequences will become clear from the further disclosure herein.

Generally, NANOBODY® ISVDs (in particular VHH sequences, including (partially) humanized VHH sequences and camelized VH sequences) can be characterized by the presence of one or more "Hallmark residues" (as described herein) in one or more of the framework sequences (again as further described

herein). Thus, generally, a NANOBODY® ISVD can be defined as an immunoglobulin sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which one or more of the Hallmark residues are as further defined herein.

In particular, a NANOBODY® ISVD can be an immunoglobulin sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which the framework sequences are as further defined herein.

More in particular, a NANOBODY® ISVD can be an immunoglobulin sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table 1 below.

Table 1: Hallmark Residues in NANOBODY® ISVDs

Position	Human VH3	Hallmark Residues
11	L, V; predominantly L	L, S, V, M, W, F, T, Q, E, A, R, G, K, Y, N, P, I; preferably L
37	V, I, F; usually V	$F^{(1)}$, Y, V, L, A, H, S, I, W, C, N, G, D, T, P, preferably $F^{(1)}$ or Y
$44^{(8)}$	G	$E^{(3)}$, $Q^{(3)}$, $G^{(2)}$, D, A, K, R, L, P, S, V, H, T, N, W, M, I; preferably $G^{(2)}$, $E^{(3)}$ or $Q^{(3)}$;most preferably $G^{(2)}$ or $Q^{(3)}$.
$45^{(8)}$	L	$L^{(2)}$, $R^{(3)}$, P, H, F, G, Q, S, E, T, Y, C, I, D, V; preferably $L^{(2)}$ or $R^{(3)}$
$47^{(8)}$	W, Y	$F^{(1)}$, $L^{(1)}$ or $W^{(2)}$ G, I, S, A, V, M, R, Y, E, P, T, C, H, K, Q, N, D; preferably $W^{(2)}$, $L^{(1)}$ or $F^{(1)}$
83	R or K; usually R	R, $K^{(5)}$, T, $E^{(5)}$, Q, N, S, I, V, G, M, L, A, D, Y, H; preferably K or R; most preferably K
84	A, T, D; predominantly A	$P^{(5)}$, S, H, L, A, V, I, T, F, D, R, Y, N, Q, G, E; preferably P
103	W	$W^{(4)}$, $R^{(6)}$, G, S, K, A, M, Y, L, F, T, N, V, Q, $P^{(6)}$, E, C; preferably W
104	G	G, A, S, T, D, P, N, E, C, L; preferably G
108	L, M or T; predominantly L	Q, $L^{(7)}$, R, P, E, K, S, T, M, A, H; preferably Q or $L^{(7)}$
Notes:		

(1)	In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.
(2)	Usually as GLEW at positions 44-47.
(3)	Usually as KERE or KQRE at positions 43-46, e.g. as KEREL, KEREF, KQREL, KQREF, KEREG, KQREW or KQREG at positions 43-47. Alternatively, also sequences such as TERE (for example TEREL), TQRE (for example TQREL), KECE (for example KECEL or KECER), KQCE (for example KQCEL), RERE (for example REREG), RQRE (for example RQREL, RQREF or RQREW), QERE (for example QEREG), QQRE, (for example QQREW, QQREL or QQREF), KGRE (for example KGREG), KDRE (for example KDREV) are possible. Some other possible, but less preferred sequences include for example DECKL and NVCEL.
(4)	With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.
(5)	Often as KP or EP at positions 83-84 of naturally occurring VHH domains.
(6)	In particular, but not exclusively, in combination with GLEW at positions 44-47.
(7)	With the proviso that when positions 44-47 are GLEW, position 108 is always Q in (non-humanized) V_{HH} sequences that also contain a W at 103.
(8)	The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEW, DLEW, GIEW, ELEW, GPEW, EWLP, GPER, GLER and ELEW.

In a first aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

The term "Glycosylation acceptor site" refers to a position within the ISVD, which can be N- or O-glycosylated. N-linked glycans are typically attached to asparagine (Asn), while O-linked glycans are commonly linked to the hydroxyl oxygen of serine, threonine, tyrosine, hydroxylysine, or hydroxyproline sidechains. In an embodiment, the glycosylation acceptor site is an N-glycosylation site. In an embodiment, the glycosylation acceptor site for N-glycosylation is asparagine (Asn). In an embodiment, the glycosylation acceptor site for O-glycosylation is serine, threonine, tyrosine, hydroxylysine, or hydroxyproline.

Surprisingly, the inventors found that besides known glycosylation acceptor sites, new glycosylation acceptor sites can be created in the ISVD amino acid sequence at position previously unknown to be suitable for glycosylation.

For efficient transfer of the glycan, in an embodiment, asparagine is located in a specific consensus sequence in the primary structure of the polypeptide (NXS, NXT or NXC). As such, a glycosylation acceptor site for N-linked glycosylation will usually be contained in an NXT motif or NXS motif (in which X can be any amino acid residue) which motif is glycosylated on the asparagine (N) residue. Thus, in the present technology, when it is stated that a position can be glycosylated, and it is intended that this position is N-glycosylated, then the primary sequence of the ISVD according the present technology will generally

contain an NXT or NXS motif (suitably introduced by mutating and/or substituting the relevant amino acid position(s)) such that the asparagine residue of said motif is present at the position to be glycosylated, i.e., at the glycosylation acceptor site.

Thus, in a further embodiment, the glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

In another embodiment, the glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 19, 26, and 105, according to Kabat numbering.

ISVDs with one of these three glycosylation acceptor positions 19, 26 and 105 were found to have significantly high degrees of glycosylation in any format, monovalent or multivalent, regardless of the ISVD position within a construct when used in such a construct.

In an embodiment, the polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD. In this embodiment it may be that said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

In a further embodiment, the polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD. In this embodiment it may be that said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108 and 110, according to Kabat numbering.

The process of designing/selecting and/or preparing a polypeptide, starting from an immunoglobulin single variable domain such as a VHH, humanized VHH, camelized VH, domain antibody or dAb, is also referred to herein as “formatting” said immunoglobulin single variable domain; and an immunoglobulin single variable domain that is made part of a polypeptide is said to be “formatted” or to be “in the format of” said polypeptide. Examples of ways in which an immunoglobulin single variable domain can be

formatted, and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted immunoglobulin single variable domain form a further aspect of the present technology.

For example, and without limitation, one or more immunoglobulin single variable domains may be used as a "binding unit", "binding domain" or "building block" (these terms are used interchangeable) for the preparation of a polypeptide, which may optionally contain one or more further immunoglobulin single variable domains that can serve as a binding unit.

The present technology also provides a polypeptide or construct that comprises or essentially consists of one or more immunoglobulin single variable domain(s). Monovalent polypeptides comprise or essentially consist of only one binding unit (such as e.g., immunoglobulin single variable domains). Polypeptides that comprise two or more binding units (such as e.g., immunoglobulin single variable domains) will also be referred to herein as "multivalent" polypeptides, and the binding units/immunoglobulin single variable domains present in such polypeptides will also be referred to herein as being in a "multivalent format". For example a "bivalent" polypeptide may comprise two immunoglobulin single variable domains, optionally linked via a linker sequence, whereas a "trivalent" polypeptide may comprise three immunoglobulin single variable domains, optionally linked via two linker sequences; whereas a "tetravalent" polypeptide may comprise four immunoglobulin single variable domains, optionally linked via three linker sequences; whereas a "pentavalent" polypeptide may comprise five immunoglobulin single variable domains, optionally linked via four linker sequences; whereas a "hexavalent" polypeptide may comprise six immunoglobulin single variable domains, optionally linked via five linker sequences, etc.

In another aspect, the present technology concerns a polypeptide comprising or (essentially) consisting of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

In an embodiment, the present technology concerns a polypeptide comprising or (essentially) consisting of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108 and 110, according to Kabat numbering.

In another embodiment, the present technology concerns a monovalent polypeptide that comprises or (essentially) consists of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

In a further embodiment, the present technology concerns a monovalent polypeptide that comprises or (essentially) consists of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108 and 110, according to Kabat numbering.

The inventors surprisingly found that positions 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108 and 110 are glycosylation acceptor sites in an ISVD that can be glycosylated to have functional, and sufficiently glycosylated ISVDs, e.g., for use as monovalent polypeptide and/or in conjugation with further moieties.

In an aspect of the present technology, said polypeptide according to the present technology comprises at least two ISVDs. In this embodiment it may be that at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

In an embodiment, at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

The inventors surprisingly found that glycosylation acceptors sites present at positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110 could provide a good degree of glycosylation in a multivalent polypeptide as well, such as e.g., in a polypeptide comprising at least two ISVDs. Interestingly, another position for use as a glycosylation acceptor site was found, namely position 3, which gave an insufficient degree of glycosylation in a monovalent format, but very high degrees of glycosylation in a multivalent polypeptide. In particular, positions 3, 19, 26, 55, 105, and 108 gave high degrees of glycosylation regardless of the position of the ISVD within the multivalent polypeptide, such as e.g., a polypeptide comprising at least two ISVDs.

In a further embodiment, said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs. In another embodiment, at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 105, 108 and 110, according to Kabat numbering.

In another embodiment, said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs. In another embodiment, at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

Accordingly, in another aspect, the present technology relates to a polypeptide comprising or (essentially) consisting of two ISVDs, wherein at least one of said two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

In an embodiment, at least one of said two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

The inventors surprisingly found that, for use of some positions as glycosylation acceptor site, the location of the ISVD in the polypeptide had an effect on the degree of glycosylation obtained. Specifically, in some embodiments, the degree of glycosylation depended on whether the ISVD with the glycosylation acceptor site was the C-terminal ISVD or the N-terminal ISVD. For use of positions 3, 19, 26, 55, 105 and 108 as glycosylation acceptor site, the location of the ISVD with the glycosylation acceptor site in the polypeptide had no influence on the degree of glycosylation obtained. However, for use of positions 53, 68, 75, and 110 as glycosylation acceptor site, the inventors surprisingly found that poor glycosylation was obtained when the ISVD comprising the glycosylation acceptor site was the C-terminal ISVD. Additionally, for position 1 poor glycosylation was obtained when the ISVD comprising the glycosylation site was the N-terminal ISVD, while good glycosylation was obtained when the ISVD comprising the glycosylation site was the C-terminal ISVD. Position 15 was found to have decent, but not high, degrees of glycosylation in the

bivalent format regardless of the location of the ISVD. Furthermore, position 102 was found to have poor glycosylation when in bivalent format, but good glycosylation in trivalent format.

Consequently, in another embodiment, the N-terminal ISVD of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 53, 55, 68, 73, 75, 105, 108 and 110, according to Kabat numbering.

In a further embodiment, the C-terminal ISVD of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 19, 26, 55, 73,105, and 108, according to Kabat numbering.

As such, in a further aspect, the present technology relates to a polypeptide comprising or (essentially) consisting of two ISVDs, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 53, 55, 68, 73, 75, 105, 108 and 110, according to Kabat numbering.

In a further embodiment, the C-terminal ISVD of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 19, 26, 55, 73, 105 and 108, according to Kabat numbering.

As mentioned before, the inventors surprisingly found that position 3 as a glycosylation acceptor site only provided good degrees of glycosylation in a multivalent format, including a bivalent polypeptide. Additionally, they found that position 102 provided good degrees of glycosylation in monovalent and multivalent format, except for the bivalent format. The other disclosed glycosylation acceptor sites provided good degrees of glycosylation in both monovalent and multivalent formats, including a bivalent polypeptide.

In another aspect, said polypeptide according to the present technology comprises at least three ISVDs. In an embodiment, at least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

The inventors were surprised to find that a glycosylation acceptor site present in the ISVD at position 15, resulted in a high degree of glycosylation of the ISVD, only when the ISVD was in (at least) trivalent format. Insufficient degrees of glycosylation were observed with a glycosylation acceptor site present in an ISVD at position 15 when the ISVD was in a monovalent format, and only a just acceptable degree of glycosylation was observed in bivalent format. However, when such an ISVD was formatted in the trivalent polypeptide, surprisingly high degrees of glycosylation were observed. Additionally, glycosylation acceptor sites present in the ISVD at already previously discussed positions 1, 3, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110 also provided high degrees of glycosylation when the ISVD was in (at least) trivalent format.

In an embodiment, at least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, and 105, according to Kabat numbering.

The inventors surprisingly found that when the glycosylation acceptor site was at amino acid position 3, 15, 19, 26 or 105 in an at least trivalent polypeptide, the location of the ISVD within the polypeptide had no influence on the degree of glycosylation.

In another embodiment, said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs. As mentioned above, the presence in the ISVD of a glycosylation acceptor site at position 15 surprisingly provided high degrees of glycosylation in a trivalent polypeptide. Additionally, glycosylation acceptor sites present in the ISVD at positions 1, 3, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110 provided high degrees of glycosylation in a multivalent format, including in a trivalent polypeptide.

The inventors surprisingly found that, for use of some positions as glycosylation acceptor sites, the location of the ISVD in the formatted polypeptide had an effect on the degree of glycosylation obtained, also in trivalent format. Specifically, in some cases, the degree of glycosylation depended on whether the ISVD with the glycosylation acceptor site was the C-terminal ISVD, the N-terminal ISVD or an ISVD that was neither at the C-terminal end nor at the N-terminal end (i.e., an ISVD between the C-terminal ISVD and the N-terminal ISVD, such as the middle ISVD). For use of positions 3, 15, 19, 26, 55, 73, 105 and 108

as glycosylation acceptor site, the location of the ISVD with the glycosylation acceptor site in the polypeptide had no influence on the degree of glycosylation obtained. However, for use of positions 53, 68, 75, 102 and 110 as glycosylation acceptor site, the inventors surprisingly found that poor glycosylation was obtained when the ISVD comprising the glycosylation acceptor site was the C-terminal ISVD. Additionally, they inventors found that a glycosylation acceptor site at position 1 could only be present in the C-terminal ISVD to obtain high degrees of glycosylation. For use of positions 68, 75, and 110 as glycosylation acceptor site, any other location, besides the C-terminal, of the ISVD within the polypeptide would result in a high degree of glycosylation.

Thus, in an embodiment, the N-terminal ISVD of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 55, 73, 75, 76, 105, 108, and 110 according to Kabat numbering.

In another embodiment, the C-terminal ISVD of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, and 105, according to Kabat numbering.

In yet another embodiment, at least one of the at least three ISVDs that is neither at the C-terminal end nor at the N-terminal end comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

Here below specific items for every specific glycosylation acceptor site are provided. The invention should not be considered to be limited to these items.

Position 1

Item 1.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering.

Item 1.2. The polypeptide according to item 1.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 1.3. The polypeptide according to item 1.1 or 1.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site.

Item 1.4. The polypeptide according to any one of items 1.1-1.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 1.5. The polypeptide according to any one of items 1.1-1.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 1.6. The polypeptide according to item 1.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 1.

Item 1.7. The polypeptide according to item 1.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 1.

Item 1.8. The polypeptide according to item 1.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering.

Item 1.9. The polypeptide according to any one of items 1.5-1.8, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 1.10. The polypeptide according to any one of items 1.1-1.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 1.11. The polypeptide according to item 1.10, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 1.

Item 1.12. The polypeptide according to item 1.10, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 1.

Item 1.13. The polypeptide according to item 1.10, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 1.

Item 1.14. The polypeptide according to item 1.10, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 1, according to Kabat numbering.

Item 1.15. The polypeptide according to items 1.10-1.14, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 3

Item 3.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering.

Item 3.2. The polypeptide according to item 3.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 3.3. The polypeptide according to item 3.1 or 3.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 3, according to Kabat numbering.

Item 3.4. The polypeptide according to any one of items 3.1-3.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 3.5. The polypeptide according to item 3.4, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.6. The polypeptide according to item 3.5, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.7. The polypeptide according to item 3.5, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.8. The polypeptide according to item 3.4, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering.

Item 3.9. The polypeptide according to items 3.4-3.8, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 3.10. The polypeptide according to items 3.1-3.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 3.11. The polypeptide according to item 3.10, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.12. The polypeptide according to item 3.11, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.13. The polypeptide according to item 3.11, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.14. The polypeptide according to item 3.11, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.15. The polypeptide according to item 3.10, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 3.

Item 3.16. The polypeptide according to item 3.10, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 3, according to Kabat numbering.

Item 3.17. The polypeptide according to any one of items 3.10-3.16, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 15

Item 15.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 15, according to Kabat numbering.

Item 15.2. The polypeptide according to item 15.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 15.3. The polypeptide according to item 15.1 or 15.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue

of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 15, according to Kabat numbering

Item 15.4. The polypeptide according to any one of items 15.1-15.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 15.5. The polypeptide according to item 15.4, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 15, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 15.

Item 15.6. The polypeptide according to item 15.5, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 15, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 15.

Item 15.7. The polypeptide according to item 15.5, wherein only an ISVD that is neither at the C-terminal end nor the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 15, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 15.

Item 15.8. The polypeptide according to item 15.5, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 15, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 15.

Item 15.9. The polypeptide according to item 15.4, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 15, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 15.

Item 15.10. The polypeptide according to item 15.4, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 15, according to Kabat numbering.

Item 15.11. The polypeptide according to any one of items 15.4-15.10, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 19

Item 19.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering.

Item 19.2. The polypeptide according to item 19.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 19.3. The polypeptide according to item 19.1 or 19.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 19, according to Kabat numbering.

Item 19.4. The polypeptide according to any one of items 19.1-19.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 19.5. The polypeptide according to any one of items 19.1-19.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 19.6. The polypeptide according to item 19.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.7. The polypeptide according to item 19.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat

numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.8. The polypeptide according to item 19.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.9. The polypeptide according to item 19.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering.

Item 19.10. The polypeptide according to any one of items 19.5-19.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 19.11. The polypeptide according to any one of items 19.1-19.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 19.12. The polypeptide according to item 19.11, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.13. The polypeptide according to item 19.12, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.14. The polypeptide according to item 19.12, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.15. The polypeptide according to item 19.12, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.16. The polypeptide according to item 19.11, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 19.

Item 19.17. The polypeptide according to item 19.11, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 19, according to Kabat numbering.

Item 19.18. The polypeptide according to any one of items 19.11-19.17, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 26

Item 26.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering.

Item 26.2. The polypeptide according to item 26.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 26.3. The polypeptide according to item 26.1 or 26.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 26, according to Kabat numbering.

Item 26.4. The polypeptide according to any one of items 26.1-26.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 26.5. The polypeptide according to any one of items 26.1-26.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 26.6. The polypeptide according to item 26.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.7. The polypeptide according to item 26.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.8. The polypeptide according to item 26.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.9. The polypeptide according to item 26.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering.

Item 26.10. The polypeptide according to any one of items 26.5-26.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 26.11. The polypeptide according to any one of items 26.1-26.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 26.12. The polypeptide according to item 26.11, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.13. The polypeptide according to item 26.12, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.14. The polypeptide according to item 26.12, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.15. The polypeptide according to item 26.12, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.16. The polypeptide according to item 26.11, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 26.

Item 26.17. The polypeptide according to item 26.11, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 26, according to Kabat numbering.

Item 26.18. The polypeptide according to items 26.11-26.17, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 53

Item 53.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering.

Item 53.2. The polypeptide according to item 53.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 53.3. The polypeptide according to item 53.1 or 53.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 53, according to Kabat numbering.

Item 53.4. The polypeptide according to any one of items 53.1-53.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 53.5. The polypeptide according to any one of items 53.1-53.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 53.6. The polypeptide according to item 53.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 53.

Item 53.7. The polypeptide according to item 53.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 53.

Item 53.8. The polypeptide according to item 53.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering.

Item 53.9. The polypeptide according to any one of items 53.5-53.8, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 53.10. The polypeptide according to any one of items 53.1-53.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 53.11. The polypeptide according to item 53.10, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 53.

Item 53.12. The polypeptide according to item 53.11, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 53.

Item 53.13. The polypeptide according to item 53.11, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 53.

Item 53.14. The polypeptide according to item 53.10, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 53.

Item 53.15. The polypeptide according to item 53.10, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 53, according to Kabat numbering.

Item 53.16. The polypeptide according to any one of items 53.10-53.15, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 55

Item 55.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering.

Item 55.2. The polypeptide according to item 55.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 55.3. The polypeptide according to item 55.1 or 55.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 55, according to Kabat numbering.

Item 55.4. The polypeptide according to any one of items 55.1-55.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 55.5. The polypeptide according to any one of items 55.1-55.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 55.6. The polypeptide according to item 55.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.7. The polypeptide according to item 55.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.8. The polypeptide according to item 55.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.9. The polypeptide according to item 55.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering.

Item 55.10. The polypeptide according to any one of items 55.5-55.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 55.11. The polypeptide according to any one of items 55.1-55.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 55.12. The polypeptide according to item 55.11, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.13. The polypeptide according to item 55.12, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.14. The polypeptide according to item 55.12, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.15. The polypeptide according to item 55.12, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.16. The polypeptide according to item 55.11, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 55.

Item 55.17. The polypeptide according to item 55.11, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 55, according to Kabat numbering.

Item 55.18. The polypeptide according to items 55.11-55.17, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 68

Item 68.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering.

Item 68.2. The polypeptide according to item 68.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 68.3. The polypeptide according to item 68.1 or 68.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 68, according to Kabat numbering.

Item 68.4. The polypeptide according to any one of items 68.1-68.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 68.5. The polypeptide according to any one of items 68.1-68.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 68.6. The polypeptide according to item 68.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 68.

Item 68.7. The polypeptide according to item 68.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 68, according to Kabat

numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 68.

Item 68.8. The polypeptide according to item 68.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering.

Item 68.9. The polypeptide according to any one of items 68.5-68.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 68.10. The polypeptide according to any one of items 68.1-68.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 68.11. The polypeptide according to item 68.10, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 68.

Item 68.12. The polypeptide according to item 68.11, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 68.

Item 68.13. The polypeptide according to item 68.11, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 68.

Item 68.14. The polypeptide according to item 68.10, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 68.

Item 68.15. The polypeptide according to item 68.10, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 68, according to Kabat numbering.

Item 68.15. The polypeptide according to items 68.10-68.15, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 73

Item 73.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering.

Item 73.2. The polypeptide according to item 73.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 73.3. The polypeptide according to items 73.1 or 73.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 73, according to Kabat numbering.

Item 73.4. The polypeptide according to any one of items 73.1-73.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 73.5. The polypeptide according to any one of items 73.1-73.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 73.6. The polypeptide according to item 73.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.7. The polypeptide according to item 73.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat

numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.8. The polypeptide according to item 73.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.9. The polypeptide according to item 73.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering.

Item 73.10. The polypeptide according to items 73.5-73.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 73.11. The polypeptide according to any one of items 73.1-73.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 73.12. The polypeptide according to item 73.11, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.13. The polypeptide according to item 73.12, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.14. The polypeptide according to item 73.12, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.15. The polypeptide according to item 73.12, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.16. The polypeptide according to item 73.11, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 73.

Item 73.17. The polypeptide according to item 73.11, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 73, according to Kabat numbering.

Item 73.18. The polypeptide according to items 73.11-73.17, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 75

Item 75.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering.

Item 75.2. The polypeptide according to item 75.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 75.3. The polypeptide according to item 75.1 or 75.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 75, according to Kabat numbering.

Item 75.4. The polypeptide according to any one of items 75.1-75.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 75.5. The polypeptide according to any one of items 75.1-75.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 75.6. The polypeptide according to item 75.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 75.

Item 75.7. The polypeptide according to item 75.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 75.

Item 75.8. The polypeptide according to item 75.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering.

Item 75.9. The polypeptide according to items 75.5-75.8, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 75.10. The polypeptide according to any one of items 75.1-75.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 75.11. The polypeptide according to item 75.10, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 75.

Item 75.12. The polypeptide according to item 75.11, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 75.

Item 75.13. The polypeptide according to item 75.11, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 75.

Item 75.14. The polypeptide according to item 75.10, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 75.

Item 75.15. The polypeptide according to item 75.10, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 75, according to Kabat numbering.

Item 75.16. The polypeptide according to any one of items 75.10-75.15, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 76

Item 76.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering.

Item 76.2. The polypeptide according to item 76.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 76.3. The polypeptide according to item 76.1 or 76.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 76, according to Kabat numbering.

Item 76.4. The polypeptide according to any one of items 76.1-76.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 76.5. The polypeptide according to any one of items 76.1-76.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 76.6. The polypeptide according to item 76.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.7. The polypeptide according to item 76.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.8. The polypeptide according to item 76.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.9. The polypeptide according to item 76.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering.

Item 76.10. The polypeptide according to any one of items 76.5-76.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 76.11. The polypeptide according to any one of items 76.1-76.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 76.12. The polypeptide according to item 76.11, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.13. The polypeptide according to item 76.12, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.14. The polypeptide according to item 76.12, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.15. The polypeptide according to item 76.12, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.16. The polypeptide according to item 76.11, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 76.

Item 76.17. The polypeptide according to item 76.11, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 76, according to Kabat numbering.

Item 76.18. The polypeptide according to any one of items 76.11-76.17, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 102

Item 102.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 102, according to Kabat numbering.

Item 102.2. The polypeptide according to item 1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 102.3. The polypeptide according to item 102.1 or 102.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 102, according to Kabat numbering.

Item 102.4. The polypeptide according to any one of items 102.1-102.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 102.5 The polypeptide according to any one of items 102.1-102.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 102.6. The polypeptide according to item 102.5, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 102, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 102.

Item 102.7. The polypeptide according to item 102.6, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 102, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 102.

Item 102.8. The polypeptide according to item 102.6, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 102, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 102.

Item 102.9. The polypeptide according to item 102.5, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 102, according to Kabat

numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 102.

Item 102.10. The polypeptide according to item 102.5, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 102, according to Kabat numbering.

Item 102.11. The polypeptide according to any one of items 102.5-102.10, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 105

Item 105.1. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering.

Item 105.2. The polypeptide according to item 105.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 105.3. The polypeptide according to item 105.1 or 105.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 105, according to Kabat numbering.

Item 105.4. The polypeptide according to any one of items 105.1-105.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 105.5. The polypeptide according to any one of items 105.1-105.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 105.6. The polypeptide according to item 105.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat

numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.7. The polypeptide according to item 105.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.8. The polypeptide according to item 105.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.9. The polypeptide according to item 105.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering.

Item 105.10. The polypeptide according to any one of items 105.5-105.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 105.11. The polypeptide according to any one of items 105.1-105.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 105.12. The polypeptide according to item 105.11, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.13. The polypeptide according to item 105.12, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.14. The polypeptide according to item 105.12, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.15. The polypeptide according to item 105.12, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.16. The polypeptide according to item 105.11, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 105.

Item 105.17. The polypeptide according to item 105.11, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 105, according to Kabat numbering.

Item 105.18. The polypeptide according to any one of items 105.11-105.17, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 108

Item 108.1. The polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering.

Item 108.2. The polypeptide according to item 108.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 108.3. The polypeptide according to item 108.1 or 108.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue

of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 108, according to Kabat numbering.

Item 108.4. The polypeptide according to any one of items 108.1-108.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 108.5. The polypeptide according to any one of items 108.1-108.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 108.6. The polypeptide according to item 108.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.7. The polypeptide according to item 108.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.8. The polypeptide according to item 108.6, wherein only the C-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining N-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.9. The polypeptide according to item 108.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering.

Item 108.10. The polypeptide according to any one of items 108.5-108.9, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 108.11. The polypeptide according to any one of items 108.1-108.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 108.12. The polypeptide according to item 108.11, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.13. The polypeptide according to item 108.12, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.14. The polypeptide according to item 108.12, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.15. The polypeptide according to item 108.12, wherein only the C-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining N-terminal and middle ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.16. The polypeptide according to item 108.11, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 108.

Item 108.17. The polypeptide according to item 108.11, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 108, according to Kabat numbering.

Item 108.18. The polypeptide according to any one of items 108.11-108.17, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Position 110

Item 110.1. The polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering.

Item 110.2. The polypeptide according to item 110.1, wherein said glycosylation acceptor site is an N-glycosylation site.

Item 110.3. The polypeptide according to item 110.1 or 110.2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site and is present at the amino acid position 110, according to Kabat numbering.

Item 110.4. The polypeptide according to any one of items 110.1-110.3, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Item 110.5. The polypeptide according to any one of items 110.1-110.3, wherein said polypeptide comprises or (essentially) consists of at least two ISVDs.

Item 110.6. The polypeptide according to item 110.5, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 110.

Item 110.7. The polypeptide according to item 110.6, wherein only the N-terminal ISVD of the at least two ISVDs comprises a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering, and the remaining C-terminal ISVD does not comprise a glycosylation acceptor site present at the amino acid position 110.

Item 110.8. The polypeptide according to item 110.5, wherein both of said at least two ISVDs comprise a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering.

Item 110.9. The polypeptide according to any one of items 110.5-110.8, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Item 110.10. The polypeptide according to any one of items 110.1-110.3, wherein said polypeptide comprises or (essentially) consists of at least three ISVDs.

Item 110.11. The polypeptide according to item 110.10, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering, and the remaining two ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 110.

Item 110.12. The polypeptide according to item 110.11, wherein only the N-terminal ISVD of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering, and the remaining middle and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 110.

Item 110.13. The polypeptide according to item 110.11, wherein only an ISVD that is neither at the C-terminal end nor at the N-terminal end of the at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering, and the remaining N-terminal and C-terminal ISVDs do not comprise a glycosylation acceptor site present at the amino acid position 110.

Item 110.14. The polypeptide according to item 110.10, wherein at least two of said at least three ISVDs comprises a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering, and the remaining ISVD does not comprise a glycosylation acceptor site present at the amino acid position 110.

Item 110.15. The polypeptide according to item 110.10, wherein all three of the at least three ISVDs comprise a glycosylation acceptor site present at the amino acid position 110, according to Kabat numbering.

Item 110.16. The polypeptide according to any one of items 110.10-110.15, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Combinations of glycosylation acceptor sites

Item A. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 1, in combination with at least one further glycosylation acceptor site present at any of positions 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item B. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 3, in combination with at least one further glycosylation acceptor site present at any of positions 1, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item C. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 15, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item D. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 19, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item E. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 26, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 53, 55, 68, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item F. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino

acid position 53, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 55, 68, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item G. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 55, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 68, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item H. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 68, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 73, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item I. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 73, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 68, 75, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item J. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 75, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 76, 102, 105, 108 and/or 110, according to Kabat numbering.

Item K. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 76, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108 and/or 110, according to Kabat numbering.

Item L. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 102, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 105, 108 and/or 110, according to Kabat numbering.

Item M.A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 105, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 108 and/or 110, according to Kabat numbering.

Item N. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 108, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105 and/or 110, according to Kabat numbering.

Item O. A polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at the amino acid position 110, in combination with at least one further glycosylation acceptor site present at any of positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, and/or 108, according to Kabat numbering.

3. ISVD glycoproteins

The glycosylation acceptor site present in the polypeptides can be modified (but not necessarily) with an N- or an O-linked glycan. The present technology also relates to the polypeptides of the present technology that are glycosylated at one or more of the specified glycosylation acceptor sites. Upon expression of a polypeptide with said glycosylation acceptor site in a host or host cell capable of glycosylating polypeptides (as further defined herein), the produced polypeptide will be directly modified in the host or host cell with one or more glycans. The obtained glycosylated polypeptide, also referred to herein as an ISVD glycoprotein, comprises one or more glycans. In one embodiment the polypeptide may comprise one or more glycans with a terminal N-acetyl glucosamine (GlcNAc), a (terminal) mannose, a (terminal) sialic acid, a (terminal) galactose or a combination thereof. As such, the present technology provides a polypeptide comprising an ISVD as described herein, wherein the polypeptide is glycosylated with one or more glycans selected from N-acetyl glucosamine (GlcNAc), mannose, galactose, fucose and sialic acid.

In some embodiments, the ISVD glycoproteins of the present technology show a high affinity. In some embodiments, the ISVD glycoproteins of the present technology have the same affinity compared to the polypeptide without the glycosylation at the specified glycosylation acceptor site.

The affinity is a measure for the binding strength between a moiety and a binding site on the target molecule: the lower the value of the dissociation constant (K_D), the stronger the binding strength between a target molecule and a targeting moiety. Typically, binding units used in the present technology, such as ISVDs, will bind to their targets with a K_D of 10^{-5} to 10^{-12} moles/liter or less, 10^{-7} to 10^{-12} moles/liter or less, or 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/moles or more, 10^7 to 10^{12} liter/moles or more, or 10^8 to 10^{12} liter/moles). Any K_D value greater than 10^{-4} mol/liter (or any K_A value lower than 10^4 liters/mol) is generally considered to indicate non-specific binding. The K_D for biological interactions, such as the binding of immunoglobulin sequences to an antigen, which are considered specific are typically in the range of 10^{-5} moles/liter (10000 nM or 10 μM) to 10^{-12} moles/liter (0.001 nM or 1 pM) or less.

The dissociation constant may be the actual or apparent dissociation constant, as will be clear to the skilled person. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned below. In this respect, it will also be clear that it may not be possible to measure dissociation constants of more than 10^{-4} moles/liter or 10^{-3} moles/liter (e.g., of 10^{-2} moles/liter). Optionally, as will also be clear to the skilled person, the (actual or apparent) dissociation constant may be calculated on the basis of the (actual or apparent) association constant, by means of the relationship [$K_D = 1/K_A$].

The affinity of a molecular interaction between two molecules can be measured via different techniques known per se, such as the well-known surface plasmon resonance (SPR) biosensor technique (see for example Ober et al. 2001, Intern. Immunology 13: 1551-1559). The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, where one molecule is immobilized on the biosensor chip and the other molecule is passed over the immobilized molecule under flow conditions yielding k_{on}, k_{off} measurements and hence K_D (or K_A) values. This can for example be performed using the well-known BIAcore® system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jonsson et al. 1993 (Ann.

Biol. Clin. 51: 19-26), Jonsson et al. 1991 (Biotechniques 11: 620-627), Johnsson et al. 1995 (J. Mol. Recognit. 8: 125-131), and Johnnson et al. 1991 (Anal. Biochem. 198: 268-277).

Another well-known biosensor technique to determine affinities of biomolecular interactions is bio-layer interferometry (BLI) (see for example Abdiche et al. 2008, Anal. Biochem. 377: 209-217). The term "bio-layer Interferometry" or "BLI", as used herein, refers to a label-free optical technique that analyzes the interference pattern of light reflected from two surfaces: an internal reference layer (reference beam) and a layer of immobilized protein on the biosensor tip (signal beam). A change in the number of molecules bound to the tip of the biosensor causes a shift in the interference pattern, reported as a wavelength shift (nm), the magnitude of which is a direct measure of the number of molecules bound to the biosensor tip surface. Since the interactions can be measured in real-time, association and dissociation rates and affinities can be determined. BLI can for example be performed using the well-known Octet® Systems (ForteBio, a division of Pall Life Sciences, Menlo Park, USA).

Alternatively, affinities can be measured in Kinetic Exclusion Assay (KinExA) (see for example Drake et al. 2004, Anal. Biochem., 328: 35-43), using the KinExA® platform (Sapidyne Instruments Inc, Boise, USA). The term "KinExA", as used herein, refers to a solution-based method to measure true equilibrium binding affinity and kinetics of unmodified molecules. Equilibrated solutions of an antibody/antigen complex are passed over a column with beads precoated with antigen (or antibody), allowing the free antibody (or antigen) to bind to the coated molecule. Detection of the antibody (or antigen) thus captured is accomplished with a fluorescently labeled protein binding the antibody (or antigen).

The GYROLAB® immunoassay system provides a platform for automated bioanalysis and rapid sample turnaround (Fraley et al. 2013, Bioanalysis 5: 1765-74).

In some embodiments, glycosylated polypeptides of the present technology specifically bind to their target with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, 10^{-7} to 10^{-12} moles/liter or less, or 10^{-8} to 10^{-12} moles/liter, as determined by Surface Plasmon Resonance. In some embodiments, glycosylated polypeptides of the present technology specifically bind to their target with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, 10^{-7} to 10^{-12} moles/liter or less, or 10^{-8} to 10^{-12} moles/liter, as determined by Meso Scale Discovery.

In some embodiments, the ISVD glycoproteins of the present technology have (essentially) the same or a higher melting temperature (Tm) compared to the polypeptide without the glycosylation at the specified glycosylation acceptor site. The denaturation midpoint of a protein is defined as the temperature (Tm) or concentration of denaturant at which both the folded and unfolded states are equally populated at equilibrium (assuming two-state protein folding). Tm is often determined using a thermal shift assay, such as the thermal shift assay described in the Examples section of the present application. Melting temperature is defined as the temperature of a protein at which 50% of said protein is unfolded.

The components, e.g., the ISVDs, present in the polypeptide may be linked to each other by one or more suitable linkers, such as peptidic linkers. The use of linkers to connect two or more (poly)peptides is well known in the art. One often used class of peptidic linker are known as the "Gly-Ser" or "GS" linkers. These are linkers that essentially consist of glycine (G) and serine (S) residues, and usually comprise one or more repeats of a peptide motif such as the GGGGS (SEQ ID NO: 150) motif (for example, have the formula (Gly-Gly-Gly-Gly-Ser)n in which n may be 1, 2, 3, 4, 5, 6, 7 or more). Some often-used examples of such GS linkers are 9GS linkers (GGGGSGGGS, SEQ ID NO: 151), 15GS linkers (n=3; SEQ ID NO: 152) and 35GS linkers (n=7; SEQ ID NO: 153). Reference is for example made to Chen et al. 2013 (Adv. Drug Deliv. Rev. 65(10): 1357–1369) and Klein et al. 2014 (Protein Eng. Des. Sel. 27 (10): 325-330). In one embodiment of the polypeptide of the present technology, 9GS linkers to link the components of the polypeptide to each other, are used. In one embodiment of the polypeptide of the present technology, 35GS linkers to link the components of the polypeptide to each other, are used.

The present technology also provides sequence optimized ISVDs and polypeptides that show increased stability upon storage during stability studies. The sequence optimized ISVDs and polypeptides show reduced pyroglutamate post-translational modification of the N-terminus and hence have increased product stability. Pyroglutamate modification leads to heterogeneity of the final product and needs to be avoided. The possibility of pGlu post-translational modification of the N-terminus was eliminated by changing the N-terminal Glutamic acid (E) into an Aspartic acid (D) which led to increased product stability. Accordingly, the present invention also relates to ISVDs and polypeptides as described above wherein the Glutamic acid at position 1 (said position determined according to Kabat numbering) is changed into an Aspartic acid (E1D).

The present technology also provides sequence optimized ISVDs and polypeptides that are "humanized", i.e. in which one or more amino acid residues in the amino acid sequence of said naturally occurring VHH sequence (and in particular in the framework sequences) are replaced by one or more of the amino acid residues that occur at the corresponding position(s) in a VH domain from a conventional 4-chain antibody from a human being (e.g. indicated above). Accordingly, the present invention also relates to ISVDs and polypeptides as described above that are humanized.

The present technology also provides sequence optimized ISVDs and polypeptides that exhibit reduced binding by pre-existing antibodies present in human serum. To this end, in one embodiment, the polypeptide comprises a valine (V) at amino acid position 11 and a leucine (L) at amino acid position 89 (according to Kabat numbering) in at least one ISVD. In one embodiment, the polypeptide comprises a valine (V) at amino acid position 11 and a leucine (L) at amino acid position 89 (according to Kabat numbering) in each ISVD. Accordingly, the present technology also relates to ISVDs and polypeptides as described above that have been sequence optimized with a valine (V) at amino acid position 11 and a leucine (L) at amino acid position 89 (according to Kabat numbering) in at least one ISVD, such as in all ISVDs.

In one embodiment, the ISVD or polypeptide has a C-terminal end of the sequence VTVSS(X)n (SEQ ID NO: 154), in which n is 1 to 10, preferably 1 to 5, such as 1, 2, 3, 4 or 5, and in which each X is an amino acid residue that is independently chosen. In one embodiment, the polypeptide comprises such an ISVD at its C-terminal end. In one embodiment, n is 1 or 2, such as 1. In one embodiment, X is a naturally occurring amino acid. In one embodiment, X is chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L) or isoleucine (I).

In another embodiment, the polypeptide comprises a lysine (K) or glutamine (Q) at position 110 (according to Kabat numbering) in at least one ISVD. In another embodiment, the ISVD comprises a lysine (K) or glutamine (Q) at position 112 (according to Kabat numbering) in at least one ISVD. In these embodiments, the C-terminus of the ISVD is VKVSS (SEQ ID NO: 155), VQVSS (SEQ ID NO: 156), VTVKS (SEQ ID NO: 157), VTVQS (SEQ ID NO: 158), VKVKS (SEQ ID NO: 159), VKVQS (SEQ ID NO: 160), VQVKS (SEQ ID NO: 161), or VQVQS (SEQ ID NO: 162) such that after addition of a single alanine the C-terminus of the polypeptide for example comprises the sequence VTVSSA (SEQ ID NO: 163), VKVSSA (SEQ ID NO: 164), VQVSSA (SEQ ID NO: 165), VTVKSA (SEQ ID NO: 166), VTVQSA (SEQ ID NO: 167), VKVKSA (SEQ ID NO: 168), VKVQSA (SEQ

ID NO: 169), VQVKSA (SEQ ID NO: 170), or VQVQSA (SEQ ID NO: 171). In one embodiment, the polypeptide comprises a valine (V) at amino acid position 11 and a leucine (L) at amino acid position 89 (according to Kabat numbering) in each ISVD, optionally a lysine (K) or glutamine (Q) at position 110 (according to Kabat numbering) in at least one ISVD, and comprises an extension of 1 to 5 (naturally occurring) amino acids (as defined above), such as a single alanine (A) extension, at the C-terminus of the C-terminal ISVD, such that the C-terminus of the polypeptide for example comprises the sequence VTVSSA (SEQ ID NO: 163), VKVSSA (SEQ ID NO: 164) or VQVSSA (SEQ ID NO: 165). See e.g., WO2012/175741 and WO2015/173325 for further information in this regard.

Provided are some embodiments for ISVD glycoproteins that had particularly high degrees of glycosylation when the glycosylation acceptor site in the ISVD is as described herein.

In an aspect of the present technology, the ISVD glycoprotein is a monovalent polypeptide, wherein the sequence of the polypeptide is selected from SEQ ID NO: 11, SEQ ID NO: 14, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NOs: 73-78, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NOs: 89-91, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 96, and SEQ ID NOs: 177-179.

In another aspect of the present technology, the ISVD glycoprotein is a bivalent polypeptide, wherein the sequence of the polypeptide is selected from SEQ ID NOs: 99-102, SEQ ID NOs: 105-108, SEQ ID NO: 110, SEQ ID NOs: 116-118, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NOs: 123-125, SEQ ID NOs: 128-133, SEQ ID NOs: 141-143, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 182, SEQ ID NO: 183, and SEQ ID NO: 187.

In a further aspect of the present technology, the ISVD glycoprotein is a trivalent polypeptide, wherein the sequence of the polypeptide is selected from SEQ ID NO: 20, SEQ ID NOs: 22-24, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NOs: 44-52, SEQ ID NOs: 55-57, SEQ ID NOs: 59-61, SEQ ID NOs: 63-65, SEQ ID NO: 186, SEQ ID NO: 190, and SEQ ID NO: 191.

4. Conjugates

The present technology also relates to a conjugate comprising a polypeptide according to the present technology and a conjugated moiety, which is conjugated to the glycan. The polypeptides modified with

glycans at the specified glycosylation acceptor sites are an ideal starting point for glycan-based conjugation due to their high degree of glycosylation.

Conjugation can be performed either chemically (e.g., using periodate oxidation of the glycan component and subsequent conjugation via methods known in the art such as oxime ligation, hydrazone ligation, or via reductive amination) or enzymatically (e.g., using Galactose Oxidase to oxidize Galactose and subsequent conjugation via oxime ligation, hydrazone ligation, or via reductive amination). Alternatively, tagged glycan residues may be incorporated to allow subsequent conjugation reactions (e.g., incorporation of GalNAz in the glycan chain using a mutant galactosyltransferase, and subsequent conjugation reaction via click chemistry).

In certain embodiments the conjugate comprises a linker between the glycan and the conjugated moiety. The use of a specific linker will depend on the application and will be clear to the skilled person. For example, oximes and hydrazones, in particular derived from aliphatic aldehydes, show less stability over time in water or at lower pH. Aromatically stabilized structures can be more useful to stably link a glycan to a conjugated moiety. Such stabilized linkers are also within the scope of the present application, as they can limit adverse effects due to premature release of the conjugated moiety, particularly when the conjugated moiety is a toxic substance, e.g., intended for killing of a tumor cell. Of particular interest are BICYCLO[6.1.0]NON-4-YNE REAGENTS as well as aromatically stabilized triazole linkers and sulfamide linkers. It is within common technical knowledge that increased stability of a conjugate can also result from reduced aggregation tendency of any of the moieties comprised within said conjugate. For the production of ISVD conjugates with increased stability the reader is non-exclusively referred to WO2013036748, WO2014065661, WO2015057064 and WO2016053107 as well as to other patent applications filed by Synaffix B.V.

Various linkers known in the art can be used to link the glycosylated polypeptide and the conjugated moiety. As should be clear, cleavable and non-cleavable linkers can be employed to achieve the desired release profile. In general, the optimal combination of linker and conjugation chemistry must be uniquely tailored to correlate each unique facet: the glycosylated polypeptide, the conjugated moiety, and the profile of the disease to be treated. For reviews on antibody-drug conjugates and linkers used herein see for example McCombs and Owen 2015 (AAPS J. 17(2), 2015) and Lu et al. 2016 (Int. J. Mol. Sci. 17(4: 561);

doi: 10.3390/ijms17040561) and Pillow et al. 2017 (Pharm Pat Anal. 6(1)) describing a novel quaternary ammonium salt linker useful in conjugates for the treatment of cancer and infectious diseases.

Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in polypeptides for pharmaceutical use. For instance, poly(ethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026. It is encompassed within the scope of the invention that the length, the degree of flexibility and/or other properties of the linker may have some influence on the properties of the final conjugate, including but not limited to the affinity, specificity or avidity for a specific target. Based on the disclosure herein, the skilled person will be able to determine the optimal linker for use in a specific conjugate, optionally after some limited routine experiments.

In some embodiments, the conjugate comprising a polypeptide according to the present technology and a conjugated moiety has at least one additional function or property as compared to the unconjugated polypeptide. For example, a conjugate comprising a polypeptide of the present technology and a cytotoxic drug being the conjugated moiety results in the formation of a binding polypeptide with drug cytotoxicity as second function (i.e., in addition to antigen binding conferred by the ISVD comprised in the polypeptide). As another example, the conjugation of a second binding polypeptide to the polypeptide of the present technology may confer additional binding properties. As a further example, the conjugated moiety may be another sugar moiety such as glucose, providing glucose transport function (Ancey et al. 2018, FEBS J. 285: 2926) or bis-mannose-6-phosphate providing degrader function. As yet another example, the conjugated moiety may be a PEG molecule, providing half-life extension and/or stabilizing/solubilizing function. As another example, the conjugated moiety may be a proteolysis targeting chimera (PROTAC), providing degrader function (Sakamoto et al. 2001, PNAS 98: 8554–9). As a further example, the conjugated moiety may be a therapeutic moiety (e.g., anti-fungal, anti-bacterial, anti-viral, anti-parasitic, cytotoxic, radionucleotide), providing a therapeutic function. Other moieties that can be conjugated to the glycans present at the glycosylation acceptor site include half-life extending moieties (PEG or PEG mimetics, large polysaccharides), detection units (chromophore units, fluorescent units, phosphorescent units, luminescent units, light absorbing units, radioactive units), targeting moieties (e.g., small molecules, antibodies).

5. Nucleic acid molecules

Another aspect of the present technology relates to a nucleotide sequence or nucleic acid encoding a polypeptide according to the present technology.

A "*nucleic acid molecule*" (used interchangeably with "*nucleic acid*") is a chain of nucleotide monomers linked to each other via a phosphate backbone to form a nucleotide sequence. A nucleic acid may be used to transform/transfect a host cell or host organism, e.g., for expression and/or production of a polypeptide. Suitable hosts or host cells for production purposes will be clear to the skilled person, is preferably a host organism that is capable of glycosylating the polypeptide, and may for example be any suitable fungal, prokaryotic or eukaryotic cell or cell line or any suitable fungal, prokaryotic or eukaryotic organism. A host or host cell comprising a nucleic acid encoding the polypeptide of the present technology is also encompassed by the present technology.

A nucleic acid may be for example DNA, RNA, or a hybrid thereof, and may also comprise (e.g., chemically) modified nucleotides, like PNA. It can be single- or double-stranded. In one embodiment, it is in the form of double-stranded DNA. For example, the nucleotide sequences of the present technology may be genomic DNA or cDNA.

The nucleic acids of the present technology can be prepared or obtained in a manner known per se, and/or can be isolated from a suitable natural source. Nucleotide sequences encoding naturally occurring (poly)peptides can for example be subjected to site-directed mutagenesis, so as to provide a nucleic acid molecule encoding the polypeptide with sequence variation. Also, as will be clear to the skilled person, to prepare a nucleic acid, or several nucleotide sequences, such as at least one nucleotide sequence encoding a targeting moiety and for example nucleic acids encoding one or more linkers, can be linked together in a suitable manner.

Techniques for generating nucleic acids will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers.

In an embodiment, said nucleotide sequence or nucleic acid is optimized for expression in a host cell or host organism that is capable of glycosylating the polypeptide encoded by the nucleotide sequence or nucleic acid.

In a further embodiment, said nucleotide sequence or nucleic is in the form of a construct or (expression) vector that can be expressed in a host cell or host organism that is capable of glycosylating the polypeptide encoded by the nucleotide sequence or nucleic acid. A vector as used herein is a vehicle suitable for carrying genetic material into a cell. A vector includes naked nucleic acids, such as plasmids or mRNAs, or nucleic acids embedded into a bigger structure, such as liposomes or viral vectors.

In some embodiments, vectors comprise at least one nucleic acid that is optionally linked to one or more regulatory elements, such as for example one or more suitable promoter(s), enhancer(s), terminator(s), etc.). In one embodiment, the vector is an expression vector, i.e., a vector suitable for expressing an encoded polypeptide or construct under suitable conditions, e.g., when the vector is introduced into a (e.g., human) cell. DNA-based vectors include the presence of elements for transcription (e.g., a promoter and a polyA signal) and translation (e.g., Kozak sequence).

In one embodiment, in the vector, said at least one nucleic acid and said regulatory elements are "operably linked" to each other, by which is generally meant that they are in a functional relationship with each other. For instance, a promoter is considered "operably linked" to a coding sequence if said promoter is able to initiate or otherwise control/regulate the transcription and/or the expression of a coding sequence (in which said coding sequence should be understood as being "under the control of" said promotor). Generally, when two nucleotide sequences are operably linked, they will be in the same orientation and usually also in the same reading frame. They will usually also be essentially contiguous, although this may also not be required.

In one embodiment, any regulatory elements of the vector are such that they are capable of providing their intended biological function in the intended host cell or host organism. For instance, a promoter, enhancer or terminator should be "operable" in the intended host cell or host organism, by which is meant that for example said promoter should be capable of initiating or otherwise controlling/regulating the

transcription and/or the expression of a nucleotide sequence - e.g., a coding sequence - to which it is operably linked.

In a further aspect, the present technology concerns a method for producing a polypeptide and/or ISVD glycoprotein according to the present technology, wherein the method comprises the step of:

- Expressing the nucleotide sequence or nucleic acid according to the present technology in a suitable (non-human) host cell or (non-human) host organism, wherein the host cell or host organism is capable of glycosylating the expressed polypeptide; optionally followed by
- isolating and/or purifying the obtained polypeptide and/or ISVD glycoprotein.

The host cell or host organism capable of glycosylating the expressed polypeptide will usually be a eukaryotic cell or organism. In one embodiment the host cell is a higher eukaryotic cell. A "higher eukaryotic cell" as used herein refers to eukaryotic cells that are not cells from unicellular organisms. In other words, a higher eukaryotic cell is a cell from (or derived from, in case of cell cultures) a multicellular eukaryote such as a human cell line or another mammalian cell line (e.g., a CHO cell line). Typically, the higher eukaryotic cells will not be fungal cells. Particularly, the term generally refers to mammalian cells, human cell lines and insect cell lines. More particularly, the term refers to vertebrate cells, even more particularly to mammalian cells or human cells. The higher eukaryotic cells as described herein will typically be part of a cell culture (e.g., a cell line, such as a HEK or CHO cell line), although this is not always strictly required (e.g., in case of plant cells, the plant itself can be used to produce a recombinant protein).

In one embodiment the host cell is a lower eukaryotic cell. By "lower eukaryotic cell" a filamentous fungus cell or a yeast cell is meant. Yeast cells can be from the species *Saccharomyces* (e g. *Saccharomyces cerevisiae*), *Hansenula* (e.g *Hansenula polymorpha*), *Arxula* (e.g. Arxula *adeninivorans*), *Yarrowia* (e.g. *Yarrowia lipolytica*), *Kluyveromyces* (e.g. *Kluyveromyces lactis*), *Aspergillus niger* or *Komagataella phaffii* (Kurtzman, C.P. (2009) J Ind Microbiol Biotechnol. 36(11) which was previously named and is better known under the old nomenclature as *Pichia pastoris*, and which naming will also be further used herein. According to a specific embodiment, the lower eukaryotic cells are *Pichia* cells, and in a most particular embodiment *Pichia pastoris* cells.

Some non-limiting examples of suitable mammalian cell lines or yeast strains include Chinese Hamster Ovary (CHO) cells, Human embryonic kidney (HEK) cells, Sp2/0 or Ns0 mouse myeloma cells and baby

hamster kidney (BHK) cells as well as other mammalian cell lines and yeast strains that can be used for the expression/production/ manufacture of polypeptides and proteins that are intended for administration to human subjects and/or for therapeutic use.

The present technology also pertains to such (non-human) host cells or (non-human) host organisms comprising the polypeptide or ISVD glycoprotein of the present technology, the nucleic acid encoding the polypeptide or ISVD glycoprotein of the present technology, and/or the vector comprising said nucleic acid molecule.

6. Composition

In yet a further aspect, the present technology relates to a composition comprising a polypeptide according to the present technology; a polypeptide or ISVD glycoprotein produced using the method of the present technology; a conjugate according to the present technology; a nucleic acid encoding the polypeptide of the present technology or a vector comprising such a nucleic acid molecule. The composition may be a pharmaceutical composition. The composition may further comprise at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally comprise one or more further pharmaceutically active polypeptides and/or compounds.

7. Uses

The polypeptide, ISVD glycoprotein or conjugate of the present technology, the nucleic acid molecule or vector as described herein, or the composition comprising the polypeptide, ISVD glycoprotein, conjugate, nucleic acid molecule or vector of the present technology are useful as a medicament. Accordingly, the present technology provides the polypeptide, ISVD glycoprotein or conjugate of the present technology, the nucleic acid molecule or vector as described herein, or the composition comprising the polypeptide, ISVD glycoprotein, conjugate, nucleic acid molecule or vector of the present technology for use as a medicament.

As such, further provided is a method for the diagnosis, prevention and/or treatment of at least one disease and/or disorder, comprising the administration, to a subject in need thereof, of a pharmaceutically active amount of at least one polypeptide, ISVD glycoprotein or conjugate of the present technology, the

nucleic acid molecule or vector as described herein, or a composition comprising the polypeptide, ISVD glycoprotein, conjugate, nucleic acid molecule or vector of the present technology.

8. Embodiments

The invention is further exemplified by, but not limited to, the following embodiments.

Embodiment 1. Polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

Embodiment 2. Polypeptide according to embodiment 1, wherein said glycosylation acceptor site is an N-glycosylation site.

Embodiment 3. Polypeptide according to embodiment 1 or 2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site.

Embodiment 4. Polypeptide according to any one of embodiments 1-3, wherein said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 105,108, and 110, according to Kabat numbering.

Embodiment 5. Polypeptide according to any one of embodiments 1-3, wherein said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 19, 26, 55, 73, 105, and 108, according to Kabat numbering.

Embodiment 6. Polypeptide according to any one of embodiments 1-3, wherein said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108, and 110, according to Kabat numbering.

Embodiment 7. Polypeptide according to embodiment 6, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

Embodiment 8. Polypeptide comprising or (essentially) consisting of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

Embodiment 9. Polypeptide according to embodiment 8, wherein the glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108, and 110, according to Kabat numbering.

Embodiment 10. Polypeptide according to any one of embodiments 1-3, wherein said polypeptide comprises at least two ISVDs.

Embodiment 11. Polypeptide according to embodiment 10, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

Embodiment 12. Polypeptide according to embodiment 10, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 19, 26, 53, 55, 68, 73, 75, 105, 108 and 110, according to Kabat numbering.

Embodiment 13. Polypeptide according to embodiment 10, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

Embodiment 14. Polypeptide according to embodiment 10, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

Embodiment 15. Polypeptide according to embodiment 14, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 53, 55, 68, 73, 75, 105, 108, and 110, according to Kabat numbering.

Embodiment 16. Polypeptide according to embodiment 14, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

Embodiment 17. Polypeptide according to embodiment 14, wherein at least one of the two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

Embodiment 18. Polypeptide comprising or (essentially) consisting of two ISVDs, wherein at least one of said two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 105, 108 and 110 according to Kabat numbering.

Embodiment 19. Polypeptide according to embodiment 18, wherein at least one of the two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

Embodiment 20. Polypeptide according to embodiment 18, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 53, 55, 68, 73, 75, 105, 108, and 110, according to Kabat numbering.

Embodiment 21. Polypeptide according to embodiment 18, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

Embodiment 22. Polypeptide comprising or (essentially) consisting of two ISVDs, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 19, 26, 53, 55, 68, 73, 75, 105, 108 and 110, according to Kabat numbering.

Embodiment 23. Polypeptide comprising or (essentially) consisting of two ISVDs, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 19, 26, 55, 105, and 108, according to Kabat numbering.

Embodiment 24. Polypeptide according to any one of embodiments 1-3, wherein said polypeptide comprises at least three ISVDs.

Embodiment 25. Polypeptide according to embodiment 24, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

Embodiment 26. Polypeptide according to embodiment 24, wherein least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, and 105, according to Kabat numbering.

Embodiment 27. Polypeptide according to any one of embodiment 24-26, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Embodiment 28. Polypeptide according to embodiment 24, 25 or 27, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 55, 73, 75, 76, 105, 108, and 110, according to Kabat numbering.

Embodiment 29. Polypeptide according to embodiment 24, 25 or 27, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, and 105, according to Kabat numbering.

Embodiment 30. Polypeptide according to embodiment 24, 25 or 27, wherein at least one of the at least three ISVDs that is neither at the C-terminal end nor at the N-terminal end comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

Embodiment 31. Polypeptide comprising or (essentially) consisting of at least three ISVDs, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110 according to Kabat numbering.

Embodiment 32. Polypeptide according to embodiment 31, wherein the N-terminal ISVD comprises a glycosylation acceptor site at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 55, 73, 75, 76, 105, 108 and 110, according to Kabat numbering.

Embodiment 33. Polypeptide according to embodiment 31, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 1, 3, 15, 19, 26, and 105, according to Kabat numbering.

Embodiment 34. Polypeptide according to embodiment 31, wherein at least one of the at least three ISVDs that is neither at the C-terminal end nor at the N-terminal end comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 3, 15, 19, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

Embodiment 35. Polypeptide according to any one of embodiments 31-34, wherein the polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

Embodiment 36. Polypeptide according to any one of embodiments 1-35 that is glycosylated at the glycosylation acceptor site with one or more glycans.

Embodiment 37. Polypeptide according to embodiment 36 wherein the glycan is selected from a terminal N-acetyl glucosamine (GlcNAc), a (terminal) mannose, a (terminal) sialic acid, a (terminal) galactose or a combination thereof.

Embodiment 38. The polypeptide according to embodiment 1, wherein the ISVD glycoprotein is a monovalent polypeptide, wherein the sequence of the polypeptide is selected from SEQ ID NO: 11, SEQ ID NO: 14, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NOs: 73-78, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO:

85, SEQ ID NO: 87, SEQ ID NOs: 89-91, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 96, and SEQ ID NOs: 177-179.

Embodiment 39. The polypeptide according to embodiment 1, wherein the ISVD glycoprotein is a bivalent polypeptide, wherein the sequence of the polypeptide is selected from SEQ ID NOs: 99-102, SEQ ID NOs: 105-108, SEQ ID NO: 110, SEQ ID NOs: 116-118, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NOs: 123-125, SEQ ID NOs: 128-133, SEQ ID NOs: 141-143, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 182, SEQ ID NO: 183, and SEQ ID NO: 187.

Embodiment 40. The polypeptide according to embodiment 1, wherein the ISVD glycoprotein is a trivalent polypeptide, wherein the sequence of the polypeptide is selected from SEQ ID NO: 20, SEQ ID NOs: 22-24, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NOs: 44-52, SEQ ID NOs: 55-57, SEQ ID NOs: 59-61, SEQ ID NOs: 63-65, SEQ ID NO: 186, SEQ ID NO: 190, and SEQ ID NO: 191.

Embodiment 41. Nucleotide sequence or nucleic acid encoding a polypeptide according to any one of embodiments 1-40.

Embodiment 42. Nucleotide sequence or nucleic acid according to embodiment 41, that is optimized for expression in a host cell or host organism that is capable of glycosylating the polypeptide encoded by the nucleotide sequence or nucleic acid.

Embodiment 43. Nucleotide sequence or nucleic acid according to embodiment 41 or 42, that is in the form of a construct or (expression) vector that can be expressed in a host cell or host organism that is capable of glycosylating the polypeptide encoded by the nucleotide sequence or nucleic acid.

Embodiment 44. Method for producing a polypeptide according to any one of embodiments 1-40, wherein the method comprises the step of:

- Expressing the nucleotide sequence or nucleic acid according to any one of embodiments 41-43 in a suitable host cell or host organism,

wherein the host cell or host organism is capable of glycosylating the expressed polypeptide.

Embodiment 45. Method for the conjugation of a moiety to the polypeptide of any one of embodiments 1-40, comprising the step of:

- Oxidation of one or more of the glycans present on the polypeptide, optionally using periodate oxidation; and
- Conjugation of the oxidized glycan to the moiety.

Embodiment 46. Method according to embodiment 45, wherein the moiety is selected from (bis-)mannose-6-phosphate, a PROTAC and a PEG moiety.

Embodiment 47. Conjugate comprising the polypeptide according to any one of embodiments 1-40 and a conjugated moiety, wherein the moiety is conjugated to the glycan.

Embodiment 48. Conjugate according to embodiment 47, wherein the moiety is selected from (bis-)mannose-6-phosphate, a PROTAC and a PEG moiety.

Embodiment 49. Composition comprising a polypeptide according to any one of embodiments 1-40, a polypeptide produced using the method of embodiment 45 or 46, or a conjugate according to embodiment 47 or 48.

Embodiment 50. The polypeptide according to any one of embodiments 1-40, the nucleotide sequence or nucleic acid according to any one of embodiments 41-43, the conjugate according to embodiment 47 or 48, or the composition according to embodiment 49 for use as a medicament.

Embodiment 51. Use of the polypeptide according to any one of embodiments 1-40, the nucleotide sequence or nucleic acid according to any one of embodiments 41-43, the conjugate according to embodiment 47 or 48, or the composition according to embodiment 49 for the manufacture of a medicament.

Below in Table 2A and Table 2B are lists of preferred glycosylation acceptor sites present in ISVDs according to the present technology and preferred format(s) for such the ISVD with the glycosylation acceptor site.

Table 2A: Preferred positions in the ISVD for glycosylation acceptor sites (Kabat numbering)

Position	Valency
1	Monovalent, multivalent
3	Multivalent
15	Multivalent
19	Monovalent, multivalent
26	Monovalent, multivalent
53	Monovalent, multivalent
55	Monovalent, multivalent
68	Monovalent, multivalent
73	Monovalent, multivalent
75	Monovalent, multivalent
76	Multivalent
102	Monovalent, multivalent
105	Monovalent, multivalent
108	Monovalent, multivalent
110	Monovalent, multivalent

Table 2B: Further preferred positions in the ISVD for glycosylation acceptor sites (Kabat numbering)

Position	Valency
1	Monovalent; multivalent, preferably multivalent in C-terminal ISVD
3	Multivalent, preferably bivalent, more preferably trivalent
15	Multivalent, preferably bivalent, more preferably trivalent
19	Monovalent; multivalent

26	Monovalent; multivalent, preferably bivalent or trivalent
53	Monovalent; multivalent, preferably bivalent or trivalent in ISVD that is not C-terminal ISVD
55	Monovalent; multivalent, preferably trivalent, more preferably bivalent
68	Monovalent; multivalent, preferably bivalent or trivalent in ISVD that is not C-terminal ISVD
73	Monovalent; multivalent
75	Monovalent; multivalent, preferably in ISVD that is not C-terminal ISVD
76	Multivalent, preferably trivalent
102	Monovalent; multivalent, preferably trivalent in ISVD that is not C-terminal ISVD
105	Monovalent; multivalent, preferably bivalent or trivalent
108	Monovalent; multivalent, preferably trivalent, more preferably bivalent
110	Multivalent, preferably bivalent or trivalent in ISVD that is not C-terminal ISVD

The present technology and its embodiments will be further highlighted in the Examples section below.

EXAMPLES

Example 1: Generation of ISVD expression constructs

ISVD-encoding DNA fragments, obtained by PCR with specific combinations of forward FR1 and reverse FR4 primers each carrying a unique restriction site, were digested with the appropriate restriction enzymes, and ligated into the matching cloning cassettes of ISVD expression vectors. The ligation mixtures were then used to transform electrocompetent or chemically competent *Escherichia coli* TG1 (Lucigen, Cat. No. 60502 or custom-made, respectively) or TOP10 (ThermoFisher Scientific, Cat. No. C404052 or

C4081201, respectively) cells, which were grown under the appropriate antibiotic selection pressure (kanamycin or Zeocin). Resistant clones were verified by Sanger sequencing of plasmid DNA (LGC Genomics).

Preparation of constructs for expression of monovalent ISVDs in E. coli

Monovalent ISVDs were expressed in *E. coli* TG1 cells from a plasmid expression vector containing the lac promoter, a resistance gene for kanamycin, an *E. coli* replication origin and a ISVD cloning site preceded by the coding sequence for the OmpA signal peptide, which directs the expressed ISVDs to the periplasmic compartment of the bacterial host. In frame with the ISVD coding sequence, the vector codes for a C-terminal 3xFLAG and His6 tag.

Preparation of constructs for expression of ISVDs in CHO cells

The mammalian expression vectors used for expression of the ISVD proteins contained the RSV-LTR promoter, a resistance gene for Zeocin and the signal peptide of a mouse light chain. The DNA encoding the ISVD building blocks and GS linkers was cloned in the expression vector via Golden Gate cloning (Engler C, Marillonnet S. Golden Gate cloning. Methods Mol. Biol. 2014;1116:119-31). The expression vectors contained two BpiI restriction sites for the cloning of the PCR-amplified monovalent ISVDs DNA together with the GS linker DNA in one or multiple vectors. All these elements were flanked by BpiI sites. The use of unique nucleotide overhangs for each position of the cloning cassette allowed seamless ligation in a pre-defined order. After Sanger sequence confirmation, plasmid DNA derived from *E. coli* TOP10 was transfected into CHOEBNALT85 cells.

Example 2: Expression of ISVDs in *E. coli*

E. coli cells, containing the ISVD expression vector, were grown for 2 hours at 37°C followed by 29 hours at 30°C (250 rpm) in a baffled shaker flask containing "5052" auto-induction medium (50x stock: 25% glycerol, 2.5% glucose, 10% lactose). Cells were pelleted by centrifugation (20 minutes, 4500 rpm, 4°C), the supernatant was discarded, and pellets were frozen over night at -20°C. The frozen cell pellets were then dissolved in DPBS (Gibco, Cat. No. 14190-094) at 1/12.5th of the original culture volume and incubated at 4°C for 1 hour while gently rotating, to disrupt the outer membrane of the cells. The cells were pelleted again (20 minutes, 8500 rpm, 4°C) and the supernatant, containing the ISVDs, was collected and filtered to immediately proceed with purification.

ISVDs expressed in *E. coli* were considered controls as no glycosylation will occur in the expression through *E. coli*.

Example 3: Expression of ISVDs in mammalian cells

CHOEBNALT85-1E9 cells (QMCF Technology licensed from Icosagen) were seeded at a density of 1.5E06 cells/mL in 10 mL of CHO TF medium (Xell, Cat. No. 8860001) with GlutaMAX™ Supplement (Gibco, Cat. No. 35050-038) and transfected with a DNA/Transfection Reagent 007 complex. The complex was formed by mixing 10 µg of plasmid DNA in 300 µL of water and 50 µg of Transfection Reagent 007 (Icosagen, Cat. No. R007P001) in 200 µL of water and incubating for 5 minutes at room temperature. After incubation for 1.5 hours at 37°C, 10 mL of fresh CHO TF medium with GlutaMAX™ Supplement was added, and the cells were allowed to grow for 24 hours at 37°C. Subsequently, 10 mL of fresh CHO TF medium with GlutaMAX™ Supplement and Penicillin-Streptomycin (Gibco, Cat. No. 15140-122) was added, and the cells were incubated for another 72 hours at 37°C. Then, the cell density was determined and upon reaching 3.5E06 cells/mL with a viability of > 90%, 1.8 mL of Basic Feed (Xell, Cat. No. 1092-0001) was added to the cells and the incubation temperature was lowered to 30°C. Basic Feed was added again after 48 hours and once more after another 48 hours. After a final incubation of 72 hours, the cells were pelleted by centrifugation (10 minutes at 1000 rpm) after which the supernatant was transferred to another tube. The supernatant was centrifuged again to clear the remaining cell debris (30 minutes at 8500 rpm) and the medium containing the ISVDs was collected, filtered, and stored at -20°C until purification. All incubations were done in a humidified orbital shaker incubator at 200 rpm in the presence of 8% CO_2.

Example 4: Purification of ISVDs using Protein A affinity chromatography

ISVD constructs were purified on Protein A followed by a desalting step and if necessary, preparative SEC in D-PBS. Concentration was determined via OD280/OD340 measurement. Quality control was performed by SDS-PAGE and mass spectrometry.

The full list of generated ISVDs can be found in Table 3 shown here below in Example 5.

Example 5: Glycosylation analysis

The ISVD glycoproteins obtained from the mammalian cells were analysed by SDS page for their glycosylation at the different glycosylation acceptor sites. The N-linked oligosaccharides were removed by

PNGase to confirm that the shift in molecular weight (MW) in SDS-PAGE is in fact due to glycosylation. More specifically, if the shift in MW does not disappear upon PNGase treatment, in comparison to the wild-type, non-glycosylated control, it means that something other than an added sugar caused the increase in MW.

PNGase F step

N-linked oligosaccharides were removed from the ISVD glycoproteins by PNGase F (N-glycosidase F). The method was performed according to the manufacturer's instructions (NEB, Catalog # P0704S): 5-10 µg of glycosylated ISVD, 1 µL of 10x Glycoprotein Denaturing buffer and H_2O were added to a total volume of 10 µL. The mix was heated for 10 minutes at 100°C before chilling on ice and a short centrifugation at 10 seconds. Subsequently, 2 µL of 10x Glycobuffer, 2 µL 10 % NP-40 and 6 µL H_2O were added before the addition of 1 µL PNGase F. The reaction was gently mixed and incubated for 1 hour at 37°C. 2 µg of the mix was finally analysed using SDS-PAGE analysis, according to the protocol described here below.

Protocol SDS-PAGE analysis

2 µg sample was analysed on a precast 4-12% BT NuPAGE SDS PAGE gel (Invitrogen, #NP0321BOX or NP0323BOX), under both reducing (R), heated and non-reducing (NR), non-heated conditions. The loading buffer was NuPAGE, 4x LDS Sample Buffer (Invitrogen, #NR0008). Heating occurred at 98°C for 3 min. 5 µl of Marker (Sharp Pre-stained Protein Standard, Novex, #LC5800) was loaded before the gel was run in 1x MES running buffer, at a voltage of 180 V during 40min. Finally, the gel was stained with Instant Blue and destained with tap water for 8h.

Mass Spectrometry analysis

Intact mass LC-MS analyses were performed either on an Agilent 1290 series UHPLC coupled to an Agilent Q-TOF 6530 mass spectrometer (both from Agilent Technologies), or on a Vanquish Flex UHPLC coupled to a Q-Exactive Plus mass spectrometer (both from Thermo Fisher Scientific). After online desalting using a MassPREP Micro Desalting Column (Waters), the molecular mass of the main product(s) was (were) determined after charge state deconvolution of the raw MS data.

Results

Only ISVDs that had a glycosylation degree of over 50% were considered to be sufficiently glycosylated. The full list of the tested ISVDs can be found below in Table 3.

Table 3: Glycosylation analysis of generated ISVDs

ISVD ID	SEQ ID NO	Glycosylation acceptor site(s)	Valency/location mutation	Glycosylation degree
ALB00606	1	15N	Mono	N/A
ALB00607	2	19N	Mono	N/A
ALB00608	3	26N	Mono	N/A
ALB00609	4	55N	Mono	N/A
ALB00610	5	105N	Mono	N/A
ALB00611	6	108N	Mono	N/A
ALB00612	7	75T*	Mono	N/A
ALB00613	8	15N	Mono	20%
ALB00614	9	19N	Mono	50%
ALB00615	10	26N	Mono	60%
ALB00616	11	55N	Mono	100%
ALB00617	12	105N	Mono	40%
ALB00618	13	108N	Mono	20%
ALB00619	14	75T*	Mono	100%
ALB00620	15	3N, 5T**	Mono	5%
ALB00621	16	3N, 5T**	Mono	N/A
ALB00622	17	NONE	Mono	N/A
ALB00623	18	NONE	Mono	0%
T043800001	19	NONE	Tri	0%
T043800002	20	3N, 5T**	Tri / C-term	100%
T043800003	21	5N	Tri / C-term	10%
T043800004	22	15N	Tri / C-term	80%
T043800005	23	19N	Tri / C-term	95%
T043800006	24	26N	Tri / C-term	99%
T043800007	25	53N	Tri / C-term	10%
T043800008	26	55N	Tri / C-term	50%
T043800009	27	68N	Tri / C-term	0%
T043800010	28	A74N, T76**	Tri / C-term	10%
T043800011	29	102N	Tri / C-term	5%
T043800012	30	105N	Tri / C-term	70%
T043800013	31	108N	Tri / C-term	50%
T043800014	32	110N	Tri / C-term	0%
T043800015	33	75N	Tri / C-term	10%
T043800016	34	75T*	Tri / C-term	50%
T043800055	35	NONE	Tri	0%
T043800056	36	15N	Tri / N-term	60%

T043800057	37	19N	Tri / N-term	100%
T043800058	38	26N	Tri / N-term	100%
T043800059	39	53N	Tri / N-term	nd
T043800060	40	55N	Tri / N-term	100%
T043800061	41	68N	Tri / N-term	nd
T043800062	42	74N	Tri / N-term	60%
T043800063	43	102N	Tri / N-term	nd
T043800064	44	105N	Tri / N-term	100%
T043800065	45	75N	Tri / N-term	100%
T043800066	46	76N	Tri / N-term	100%
T043800067	47	15N	Tri / middle	90%
T043800068	48	19N	Tri / middle	100%
T043800069	49	26N	Tri / middle	100%
T043800070	50	53N	Tri / middle	90%
T043800071	51	55N	Tri / middle	100%
T043800072	52	68N	Tri / middle	100%
T043800073	53	74N	Tri / middle	50%
T043800074	54	102N	Tri / middle	90%
T043800075	55	105N	Tri / middle	100%
T043800076	56	75N	Tri / middle	100%
T043800089	57	3N, 5T**	Tri / N-term	90%
T043800090	58	5N	Tri / N-term	nd
T043800091	59	3N, 5T**	Tri / middle	100%
T043800092	60	108N	Tri / N-term	100%
T043800093	61	110N	Tri / N-term	100%
T043800094	62	5N	Tri / middle	0%
T043800095	63	108N	Tri / middle	100%
T043800096	64	110N	Tri / middle	100%
T043800097	65	76N	Tri / middle	100%
T043800098	66	NONE	Tri	0%
T043800118	67	3N, 5T**	Mono	20%
T043800119	68	110N	Mono	50%
T043800120	69	75N	Mono	100%
T043800121	70	75T*	Mono	100%
T043800122	71	5N	Mono	0%
T043800123	72	15N	Mono	10%
T043800124	73	19N	Mono	100%
T043800125	74	26N	Mono	90%
T043800126	75	55N	Mono	100%
T043800127	76	68N	Mono	90%

T043800128	77	105N	Mono	90%
T043800129	78	108N	Mono	90%
T043800130	79	NONE	Mono	0%
T043800131	80	5N	Mono	5%
T043800133	81	55N	Mono	90%
T043800134	82	68N	Mono	40%
T043800135	83	108N	Mono	90%
T043800136	84	110N	Mono	30%
T043800137	85	75T*	Mono	95%
T043800138	86	NONE	Mono	0%
T043800139	87	110N	Mono	80%
T043800143	88	15N	Mono	20%
T043800144	89	19N	Mono	80%
T043800145	90	26N	Mono	90%
T043800146	91	55N	Mono	80%
T043800147	92	68N	Mono	30%
T043800148	93	105N	Mono	90%
T043800149	94	108N	Mono	90%
T043800150	95	NONE	Mono	0%
T043800159	96	1N, 3S**	Mono	100%
T043800161	97	3N, 5T**	Mono	10%
T043800178	98	NONE	Bi/N-term	0%
T043800179	99	3N, 5T**	Bi/N-term	90%
T043800180	100	110N	Bi/N-term	99%
T043800181	101	75N	Bi/N-term	99%
T043800182	102	75T*	Bi/N-term	99%
T043800183	103	5N	Bi/N-term	0%
T043800184	104	15N	Bi/N-term	40%
T043800185	105	19N	Bi/N-term	100%
T043800186	106	26N	Bi/N-term	99%
T043800187	107	55N	Bi/N-term	99%
T043800188	108	108N	Bi/N-term	100%
T043800189	109	NONE	Bi/C-term	0%
T043800190	110	3N, 5T**	Bi/C-term	99%
T043800191	111	110N	Bi/C-term	0%
T043800192	112	75N	Bi/C-term	30%
T043800193	113	75T*	Bi/C-term	50%
T043800194	114	5N	Bi/C-term	0%
T043800195	115	15N	Bi/C-term	30%
T043800196	116	19N	Bi/C-term	100%

T043800197	117	26N	Bi/C-term	99%
T043800198	118	55N	Bi/C-term	90%
T043800199	119	68N	Bi/C-term	10%
T043800200	120	105N	Bi/C-term	90%
T043800201	121	108N	Bi/C-term	90%
T043800202	122	NONE	Bi/N-term	0%
T043800203	123	110N	Bi/N-term	99%
T043800204	124	75N	Bi/N-term	100%
T043800205	125	73N	Bi/N-term	100%
T043800206	126	5N	Bi/N-term	0%
T043800207	127	15N	Bi/N-term	50%
T043800208	128	19N	Bi/N-term	99%
T043800209	129	26N	Bi/N-term	100%
T043800210	130	55N	Bi/N-term	100%
T043800211	131	68N	Bi/N-term	100%
T043800212	132	105N	Bi/N-term	100%
T043800213	133	108N	Bi/N-term	100%
T043800214	134	3N, 5T**	Bi/N-term	60%
T043800215	135	NONE	Bi/C-term	0%
T043800216	136	110N	Bi/C-term	0%
T043800217	137	75N	Bi/C-term	40%
T043800218	138	73N	Bi/C-term	60%
T043800219	139	5N	Bi/C-term	0%
T043800220	140	15N	Bi/C-term	50%
T043800221	141	19N	Bi/C-term	90%
T043800222	142	26N	Bi/C-term	100%
T043800223	143	55N	Bi/C-term	80%
T043800224	144	68N	Bi/C-term	30%
T043800225	145	105N	Bi/C-term	90%
T043800226	146	108N	Bi/C-term	80%
T043800227	147	3N, 5T**	Bi/C-term	50%
T043800228	148	68N	Bi/N-term	95%
T043800229	149	105N	Bi/N-term	100%
T043800899	177	53N	Mono	100%
T043800901	178	53N	Mono	95%
T043800902	179	102N	Mono	100%
T043800969	180	1N	Bi/N-term	10%
T043800970	181	1N	Bi/N-term	10%
T043800971	182	1N	Bi/C-term	95%
T043800972	183	1N	Bi/C-term	90%

T043800973	184	1N	Tri/N-term	10%
T043800974	185	1N	Tri/middle	0%
T043800975	186	1N	Tri/C-term	100%
T043800976	187	53N	Bi/N-term	95%
T043800977	188	53N	Bi/C-term	5%
T043800978	189	53N	Tri/N-term	nd
T043800979	190	73N	Tri/N-term	90%
T043800980	191	73N	Tri/middle	100%
T043800981	192	102N	Bi/N-term	0%
T043800982	193	102N	Bi/C-term	0%
T043800983	194	102N	Tri/N-term	nd
T043800984	195	102N	Tri/middle	nd
T043800985	196	102N	Tri/C-term	0%

Notes:
N/A stands for "not applicable"
nd stands for "not determined"
*= actual glycosylation acceptor site is at position 73, but mutation was at position 75
**= the complete NXS- / NXT-motif was mutated; the actual glycosylation acceptor site is the Asn.

Surprisingly, the inventors found that some glycosylation acceptor sites were only glycosylated in some ISVD formats and/or in certain specific configurations of the ISVDs, while other glycosylation acceptor sites had different requirements to obtain high degrees of glycosylation.

Based on the glycosylation analysis performed it was concluded that the following positions in the ISVD for use as a glycosylation acceptor sites (Kabat numbering) had a good degree of glycosylation when the ISVD was in a monovalent as well as in a multivalent format: 1, 19, 26, 53, 55, 68, 73, 75, 102, 105, 108, and 110. Surprisingly, however, use of a glycosylation acceptor site present at one of positions 53, 68, 75, 102 and 110 in the C-terminal ISVD of a multivalent format resulted in a low degree of glycosylation while, use of glycosylation acceptor sites at these positions when not present in the C-terminal ISVD of a multivalent format, resulted in high degrees of glycosylation. For position 102, this was specifically in a trivalent, or higher, format.

In an opposite fashion, for position 1 it was observed that in a multivalent format, high degrees of glycosylation were observed only if the glycosylation acceptor site was present in the C-terminal ISVD.

For glycosylation acceptor sites at positions 3 and 15 in the ISVD, it was observed that high degrees of glycosylation could only be obtained when the ISVD was in a multivalent format. For glycosylation acceptor site at position 15 in the ISVD specifically, it was observed that high degrees of glycosylation could only be obtained when the ISVD was in the trivalent, or higher, format. Position 76 was only tested in a trivalent format and yielded high glycosylation degrees in this format.

Example 6: Thermal shift assay

In this assay it was determined if the glycosylation of ISVDs had an effect on melting temperature. The following ISVDs were used: ALB00606 (SEQ ID NO: 1), ALB00607 (SEQ ID NO: 2), ALB00608 (SEQ ID NO: 3), ALB00609 (SEQ ID NO: 4), ALB00610 (SEQ ID NO: 5), ALB00611 (SEQ ID NO: 6), ALB00612 (SEQ ID NO: 7), ALB00616 (SEQ ID NO: 11), ALB00621 (SEQ ID NO: 16), ALB00622 (SEQ ID NO: 17).

Of these ALB00606, ALB00607, ALB00608, ALB00609, ALB00610, ALB00611, ALB00612, ALB00621 and ALB00622 were produced in *E. coli* (in which no glycosylation occurs) and served as controls.

ALB00616 was produced in CHO cells, hence glycosylation would occur, and served as exemplary glycosylated ISVD. Since the sequence of ALB00616 is identical to the sequence of ALB00609, the only difference between these differently produced ISVDs is whether they are glycosylated or not.

The thermal shift assay (TSA) was performed in a 96-well plate on a qPCR machine (LightCycler 480II, Roche). Per row, one ISVD was analysed in the following pH range: 4, 5, 6, 7, 8 and 9. In some cases, six different formulation buffers were also included, namely 20 mM acetate (pH 5), 20 mM histidine (pH 6) or 20 mM phosphate (pH 7), either with or without 8% sucrose. Per well, 5 µL of ISVD sample (0.8 mg/mL in D-PBS) was added to 5 µL of Sypro Orange (40× in MilliQ water; Invitrogen, Cat. No. S6551) and 10 µL of buffer (100 mM phosphate, 100 mM borate, 100 mM citrate and 115 mM NaCl with a pH ranging from 4 to 9). A temperature gradient (37 to 99°C at a rate of 0.03°C/s) was applied, which induced unfolding of the ISVDs, and hence exposure of hydrophobic patches. Binding of Sypro Orange to those hydrophobic patches, caused increase in fluorescence intensity, which was measured (Ex/Em = 465/580 nm). The inflection point of the first derivative of the fluorescence intensity curve at pH 7 served as a measure of the melting temperature (Tm).

Results

Results of the thermal assay are shown below in Table 4.

Table 4: Measured melting temperatures of the ISVDs when produced in *E. coli* or CHO cells

ISVD ID	SEQ ID NO	Melting temperature (°C)
ALB00606	1	71
ALB00607	2	72
ALB00608	3	71
ALB00609	4	74
ALB00610	5	74
ALB00611	6	72
ALB00612	7	74
ALB00616	11	74
ALB00621	16	72
ALB00622	17	74

As can be seen from Table 4, the melting temperature was the same for the non-glycosylated and the glycosylated ISVDs. In particular, the ISVDs with an identical sequence ALB00609 (control ISVD produced in *E. coli*) and ALB00616 (exemplary ISVD produced in CHO cells) showed no difference in melting temperature whether it was glycosylated or not.

Consequently, it can be concluded that the melting temperatures of the glycosylated ISVDs are not affected by the glycosylation of said ISVDs.

Example 7: Affinity analysis of ISVD /Human Serum Albumin interaction using Surface Plasmon Resonance

The affinity of the ISVDs to human serum albumin (HSA) was determined by Surface Plasmon Resonance (SPR). T043800002 (SEQ ID NO: 20), T043800003 (SEQ ID NO: 21), T043800004 (SEQ ID NO: 22). T043800005 (SEQ ID NO: 23), T043800008 (SEQ ID NO: 26), and T043800012 (SEQ ID NO: 30) were tested. The wildtype ISVD T04380001 (SEQ ID NO: 19) was used as reference.

HSA (Sigma-Aldrich, Cat# A8763000) was directly immobilized on Flow Cell 2 (FC) of a C1 Biacore chip on a Biacore 8K(+) instrument: Chip activation was done by a 7 min injection of EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide, 200 mM, Sigma Aldrich Cat# 39391)/NHS (N-Hydroxysuccinimide, 50 mM, Sigma Aldrich Cat# 130672), HSA was diluted to 4 µg/ml in 10 mM Acetate buffer, pH 4.5 and flowed over channels 1 to 8 of FC1 for immobilization, prior to a deactivation step with ethanolamine HCl (1M, Cytiva) for 7min. Flow rate during activation, immobilization and deactivation was set on 10 µL/min. An affinity determination was set up with a 6-point dilution series ranging between 1000 and 1 nM of ISVD. The various concentrations were tested in a Multi Cycle Kinetic Mode with a flow rate of 30 µL/min, 120 s association time and 600 s dissociation time in 1x HBS-EP+ buffer. Regeneration conditions after each interaction analysis was done by flowing 10mM Glycine pH 1.5 buffer for 60 s at a flow rate of 30 µL/min over the chip surface. Data analysis was performed using the Biacore insight evaluation software.

Results

Results of the affinity measurements of the tested ISVDs can be found below in Table 5.

Table 5: affinity determination of ISVDs to HSA (SPR)

ISVD ID	**SEQ ID NO**	**Valency/ mutation in which ISVD**	**K_D (M)**
T04380001 (wildtype)	19	Tri / C-term	8,40E-09
T043800002	20	Tri / C-term	1,50E-08
T043800004	22	Tri / C-term	1,10E-08
T043800005	23	Tri / C-term	1,30E-08
T043800008	26	Tri / C-term	1,30E-08
T043800012	30	Tri / C-term	1,50E-08

As can be seen from Table 5, the affinity of the glycosylated ISVDs versus the wildtype reference ISVD is comparable. This shows that the glycosylated ISVDs maintain their high affinity for their target.

Example 8: Affinity analysis of ISVD/EGFR interaction using Surface Plasmon Resonance

The affinity of the ISVDs to EGFR was determined by Surface Plasmon Resonance (SPR). ISVDs T043800179 (SEQ ID NO: 99), T043800180 (SEQ ID NO: 100), T043800181 (SEQ ID NO: 101), T043800182 (SEQ ID NO: 102), T043800185 (SEQ ID NO: 105), T043800187 ((SEQ ID NO: 107), T043800190 (SEQ ID NO: 110), T043800196 (SEQ ID NO: 116), T043800198 (SEQ ID NO: 118) and T043800200 (SEQ ID NO: 120) were tested. Wildtype ISVDs T043800178 (SEQ ID NO: 98) and T043800189 (SEQ ID NO: 109) were taken along as references.

EGFR (Sino Biological, Cat# LC14JA1103) was directly immobilized on Flow Cell 2 (FC) of a CM5 Biacore chip on a Biacore 8K(+) instrument: Chip activation was done by a 7 min injection of EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide, 200 mM, Sigma Aldrich Cat# 39391)/NHS (N-Hydroxysuccinimide, 50 mM, Sigma Aldrich Cat# 130672), EGFR was diluted to 4 µg/ml in 10 mM Acetate buffer, pH 4.5 and flowed over channels 1 to 8 of FC1 for immobilization, prior to a deactivation step with ethanolamine HCl (1M, Cytiva) for 7min. Flow rate during activation, immobilization and deactivation was set on 10 µL/min. An affinity determination was set up with a 6-point dilution series ranging between 2500 and 0,06 nM of ISVD. The various concentrations were tested in a Multi Cycle Kinetic Mode with a flow rate of 30 µL/min, 120 s association time and 600 s dissociation time in 1x HBS-EP+ buffer. Regeneration conditions after each interaction analysis was done by flowing 2 times 10mM Glycine pH 2.5 buffer for 30 s at a flow rate of 45 µL/min over the chip surface. Data analysis was performed using the Biacore insight evaluation software.

Results

Results of the affinity analysis can be found below in Table 6.

Table 6: affinity determination of ISVDs to EGFR (SPR)

ISVD ID	SEQ ID NO	Valency/ mutation in which ISVD	K_D (M)
T043800178 (wildtype)	98	Bi/N-term	2,30E-06
T043800179	99	Bi/N-term	3,40E-06
T043800180	100	Bi/N-term	2,20E-06
T043800181	101	Bi/N-term	3,20E-06
T043800182	102	Bi/N-term	6,00E-06
T043800185	105	Bi/N-term	2,50E-06
T043800187	107	Bi/N-term	3,60E-06

T043800189 (wildtype)	109	Bi/C-term	7,50E-06
T043800190	110	Bi/C-term	6,40E-05
T043800196	116	Bi/C-term	9,30E-06
T043800198	118	Bi/C-term	9,00E-06
T043800200	120	Bi/C-term	3,20E-05

As can be seen from Table 6, the affinity of the glycosylated ISVDs versus the wildtype reference ISVDs decreased by a maximum of 4-fold, with all ISVDs maintaining high affinity. This shows that the glycosylated ISVDs maintain their high affinity for their target.

Example 9: Affinity analysis of ISVD/Human Serum Albumin interaction using Meso Scale Discovery

The affinity of the ISVDs to HSA was further determined by Meso Scale Discovery (MSD). ISVDs T043800056 (SEQ ID NO: 36), T043800057 (SEQ ID NO: 37), T043800060 (SEQ ID NO: 40), T043800064 (SEQ ID NO: 44), T043800065 (SEQ ID NO: 45), T043800066 (SEQ ID NO: 46), T043800067 (SEQ ID NO: 47), T043800068 (SEQ ID NO: 48), T043800070 (SEQ ID NO: 50), T043800071 (SEQ ID NO: 51), T043800072 (SEQ ID NO: 52), T043800074 (SEQ ID NO: 54), T043800075 (SEQ ID NO: 55), T043800076 (SEQ ID NO: 56), T043800089 (SEQ ID NO: 57), T043800093 (SEQ ID NO: 61) and T043800096 (SEQ ID NO: 64) were tested. Wildtype ISVDs T043800055 (SEQ ID NO: 35) and T043800098 (SEQ ID NO: 66) were taken along as references.

Human Serum Albumin (HSA, Sigma-Aldrich, Cat# A8763000) was biotinylated using NHS-LC-Biotin (ThermoFisher, Cat# 21336) according to the instructions of the manufacturer with an average degree of labelling of 1. The biotinylated HSA was captured at a concentration of 1 µg/mL on an MSD GOLD 96-well Small Spot Streptavidin SECTOR Plate (MSD, Cat# L45SA-1). Subsequently, 25 µL of pre-equilibrated mixes (2 hours at room temperature) containing a fixed concentration of 100 pM of the to-be-tested ISVD compound with HSA that ranged from 1,13 pM to 10 µM, respectively (23 dilutions, 1/3 dilution factor) was added to the plate. The plate was allowed to incubate for 10 minutes to capture free ISVD compound before washing with 3x 150 µL PBS + 0.05% Tween-20. During the final detection step 25 µL of an Sulfo-tag-labelled anti-ISVD antibody was added at a concentration of 2 µg/ml and allowed to incubate for 1 hour, followed by a final wash of 150 µL with 1x PBS + 0.05% Tween-20. After the addition of 150 µL MSD read buffer (Meso Scale Diagnostics, cat# R92TG-1) the plate was read on an MSD QuickPlex SQ120 reader. Data was analysed using a Four Parameter Logistic (4PL) fit in GraphPad Prism 8.

Results

Results of the affinity analysis can be found below in Table 7.

Table 7: affinity determination of ISVDs to HSA (MSD)

ISVD ID	SEQ ID NO	Valency/ mutation in which ISVD	K_D (M)
T043800055 (wildtype)	35	Tri / N-term	3,10E-09
T043800056	36	Tri / N-term	1,60E-09
T043800057	37	Tri / N-term	2,00E-09
T043800060	40	Tri / N-term	2,30E-09
T043800064	44	Tri / N-term	4,30E-10
T043800065	45	Tri / N-term	5,90E-10
T043800066	46	Tri / N-term	1,60E-09
T043800067	47	Tri / middle	5,7E-09
T043800068	48	Tri / middle	4,2E-09
T043800070	50	Tri / middle	4,7E-09
T043800071	51	Tri / middle	4,9E-09
T043800072	52	Tri / middle	4,2E-09
T043800074	54	Tri / middle	5,8E-09
T043800075	55	Tri / middle	5,4E-09
T043800076	56	Tri / middle	2,9E-09
T043800089	57	Tri / N-term	8,8E-10
T043800093	61	Tri / N-term	4,5E-10
T043800096	64	Tri / middle	5,10E-09
T043800098 (wildtype)	66	Tri / middle	2,1E-09

As can be seen from Table 7 , the affinity of the glycosylated ISVDs versus the wildtype reference ISVDs is comparable. This shows that the glycosylated ISVDs maintain their high affinity for their target.

Example 10: Affinity analysis of ISVD/TNFα interaction using Meso Scale Discovery

The affinity of the ISVDs to TNFα was determined by Meso Scale Discovery (MSD). T043800002 (SEQ ID NO: 20), T043800003 (SEQ ID NO: 21), T043800004 (SEQ ID NO: 22). T043800005 (SEQ ID NO: 23),

T043800008 (SEQ ID NO: 26), T043800012 (SEQ ID NO: 30) and T043800016 (SEQ ID NO: 34) were tested. The wildtype ISVD T04380001 (SEQ ID NO: 19) was used as reference.

Human TNFα (Bio-techne, Cat# 210-TA) was biotinylated using NHS-LC-Biotin (ThermoFisher, Cat# 21336) according to the instructions of the manufacturer with an average degree of labelling of 1. The biotinylated TNFα was captured at a concentration of 0.5 µg/mL on an MSD GOLD 96-well Small Spot Streptavidin SECTOR Plate (MSD, Cat# L45SA-1). Subsequently, a 25 µL of pre-equilibrated mixes (24 hours at room temperature) containing a fixed concentration of 12.8 pM of the to-be-tested ISVD compound with TNFα that ranged from 78 fM to 10 µM, respectively (23 dilutions, 1/10 dilution factor from 1 to 0,01 µM; 1/1.8 dilution factor from 10 nM to 78 fM) was added to the plate. The plate was allowed to incubate for 10 minutes to capture free ISVD compound before washing with 3x 150 µL PBS + 0.05% Tween-20. During the final detection step 25 µL of an Sulfo-tag-labeled anti-ISVD antibody was added at a concentration of 2 ug/ml and allowed to incubate for 1 hour, followed by a final wash of 150 µL with 1x PBS + 0.05% Tween-20. After the addition of 150 µL MSD read buffer, the plate was read on an MSD QuickPlex SQ120 reader. Data was analysed using a Four Parameter Logistic (4PL) fit in GraphPad Prism 8.

Results

Results of the affinity analysis can be found below in Table 8.

Table 8: affinity determination of ISVDs to TNF (MSD)

ISVD ID	SEQ ID NO	Valency/ mutation in which ISVD	K_D (M)
T04380001 (wildtype)	19	Tri / C-term	1,6E-11
T043800002	20	Tri / C-term	1,3E-11
T043800004	22	Tri / C-term	3,4E-11
T043800005	23	Tri / C-term	1,2E-11
T043800008	26	Tri / C-term	1,5E-11
T043800012	30	Tri / C-term	1,6E-11
T043800016	34	Tri / C-term	3,4E-12

As can be seen from Table 8, the affinity of the glycosylated ISVDs versus the wildtype reference ISVD is comparable. This shows that the glycosylated ISVDs maintain their high affinity for their target.

Example 11: Glycan profiling of the ISVD glycoproteins

The pattern of the glycans on the ISVD glycoproteins T043800002 (SEQ ID NO: 20), T043800004 (SEQ ID NO: 22), T043800005 (SEQ ID NO: 23), T043800006 (SEQ ID NO: 24), T043800008 (SEQ ID NO: 26), T043800012 (SEQ ID NO: 30), and T043800016 (SEQ ID NO: 34) was further analyzed by LC-MS.

Samples for glycan analysis were prepared per the RapiFluor-MS (Waters) protocol. Samples were diluted 9:31 using 2.1:1 acetonitrile:dimethylformamide (Waters) prior to analysis. Samples were analyzed on a 6545XT LC-QTOF, alongside RapiFluor-MS performance test standard (Waters) utilizing a linear gradient from 25% 50 mM ammonium formate, pH 4.4 (Waters) in water (Fisher) to 56% acetonitrile (Thermo Scientific) at 0.4 mL/min over 35 minutes with the column (Glycan BEH Amide, Waters) held at 60°C. Data was analyzed using Genedata Expressionist.

Results

Examples of glycan patterns of the ISVD glycoproteins on LC-QTOF are shown in Figures 1 and 2. All ISVD glycoproteins showed a similar pattern of glycan species; typically containing a high proportion of sialic acid containing glycans such as G1FS1, G2FS1, and G2FS2, in addition to neutral glycans such as G0F, G1F, and G2F. The amount of the glycan species varied slightly between ISVD glycoproteins. Sialylated species with one or two sialic acids were most abundantly present.

Example 12: Bis-Mannose 6-Phosphate conjugation

The ISVD glycoproteins T043800005 (SEQ ID NO: 23), T043800002 (SEQ ID NO: 20), T043800124 (SEQ ID NO: 73), T043800125 (SEQ ID NO: 74), T043800126 (SEQ ID NO: 75), T043800127 (SEQ ID NO: 76), T043800128 (SEQ ID NO: 77), and T043800129 (SEQ ID NO: 78) were used in conjugation with bis-Mannose 6-Phosphate (bis-M6P).

Conjugation of bis-Mannose 6-Phosphate (internally produced) containing glycan to the glycosylated ISVDs was completed by first oxidizing the ISVD with sodium periodate (Sigma) at concentrations of 2.5 mg/mL and 20 mM, respectively, in 100 mM sodium acetate (Sigma), pH 5.6, at 4°C for 30 minutes with gentle shaking. The reaction was then quenched with 3% glycerol (Sigma) at 4°C for 15 minutes. The ISVD

was purified via molecular weight cut-off filter (Millipore) into 100 mM sodium acetate, pH 5.6 by centrifuging for a minimum of five exchanges.

After protein concentration was determined via Stunner (Unchained Labs), the oxidized ISVD was conjugated with bis-M6P glycan in 100 mM sodium acetate, pH 5.6. The reaction was allowed to proceed at room temperature for at least 16 hours with gentle shaking. The ISVD was then purified via molecular weight cut-off filter into PBS by centrifuging for a minimum of five exchanges. The resulting ISVD concentration and polydispersity was then determined via Stunner. Intact mass LC-QTOF and MALDI-TOF were then completed to analyze the conjugates (protocols described here below).

Protocol intact mass analysis LC-QTOF

Samples for intact mass analysis were diluted to 1 mg/mL before adding 20 mM DTT (final concentration, Sigma) and incubated at 37°C for 30 minutes. Samples were then analyzed on a 6545XT LC-QTOF (Agilent) utilizing a linear gradient from 75% water + 0.1% formic acid (Fisher) to 60% acetonitrile + 0.1% formic acid (Fisher) at 0.5 mL/min over 7.5 minutes with the column (PLRP-S, Agilent) held at 60°C. Data was analyzed using Expressionist (Genedata).

Protocol MALDI-TOF analysis

Samples for MALDI-TOF were first brought to 1 mg/mL with sample diluent, 0.1% formic acid (Sigma) in water, before being mixed 1:1 with matrix solution, 50% acetonitrile and 1% formic acid in water. Matrix diluted samples were each spotted 3 times on a MALDI target plate (Bruker) and allowed to dry at room temperature. Sample replicates were then analyzed on a MALDI-TOF instrument (Bruker) with data analyzed via FlexAnalysis software (Bruker).

Results

MALDI-TOF analysis of the conjugates with T043800005 can be seen in Figure 3. Conjugates with one and two bis-M6P per ISVD are visible. An average degree of labeling (DoL) of 0.9 bis-M6P per ISVD was obtained.

MALDI-TOF analysis of the conjugates with T043800002 can be seen in Figure 4. Conjugates with one, two and three bis-M6P per ISVD are visible. An average degree of labeling (DoL) of 2.1 bis-M6P per ISVD was obtained.

Example 13: PROTAC Conjugation

The ISVD glycoprotein T043800005 (SEQ ID NO: 23) was further conjugated to an alkoxyamine-DBCO linker followed by PROTAC. Conjugation of PROTAC BRD4 Degrader-5-CO-PEG3-N3 (PROTAC, MedChemExpress) to the glycosylated ISVD T04380005 was completed by first oxidizing the ISVD with sodium periodate at concentrations of 2.5 mg/mL and 20 mM, respectively, in 100 mM sodium acetate, pH 5.6. The oxidation was allowed to proceed at 4°C for 30 minutes with gentle shaking before being quenched with 3% glycerol at 4°C for 15 minutes. The ISVD was then purified via molecular weight cut-off filter into 100 mM sodium acetate, pH 5.6 by centrifuging for a minimum of five exchanges.

After protein concentration was determined via Stunner, the ISVD was then coupled with Aminooxy-PEG2-bis-PEG3-DBCO (linker, Conju-Probe) at final concentrations of 2.5 mg/mL for both materials in 100 mM sodium acetate, pH 5.6. The reaction was allowed to proceed at room temperature for at least 16 hours with gentle shaking. The ISVD was then purified via molecular weight cut-off filter into PBS by centrifuging for a minimum of five exchanges.

After purification protein concentration was determined via Stunner, and then the ISVD was conjugated with PROTAC at final concentrations of 2.5 and 1.8 mg/mL, respectively, in PBS. The reaction was allowed to proceed at room temperature for at least 16 hours with gentle shaking. The ISVD was then purified via molecular weight cut-off filter into PBS, by centrifuging for a minimum of five exchanges. The resulting ISVD concentration and polydispersity was then determined via Stunner. SDS-PAGE, protocol shown below, and MALDI-TOF protocol described in Example 12, were then completed to analyze the conjugations.

Protocol SDS-PAGE

Samples for SDS-PAGE were first diluted to 1 mg/mL in Phosphate Buffered Saline (PBS, Gibco). The samples were then incubated at 70°C for 10 minutes. Following incubation, samples were mixed with 4X NuPAGE LDS Sample buffer (Invitrogen) and water before being loaded onto a 4-12% bis-tris NuPAGE gel (Invitrogen) alongside PageRuler standard ladder (Thermo Scientific). The gel was run in an Xcell Surelock cell with Powerease power supply (Invitrogen) with MES running buffer (Invitrogen) utilizing NuPAGE gel settings. After program completion, gels were extracted from the cassette and placed in InstantBlue stain

(Abcam) for 15 minutes. Gels were then visualized in a ChemiDoc Imager and Image Lab software (Bio-Rad).

Results

Per SDS-PAGE, conjugation with the PROTAC was observed at a DoL (degree of labelling) of 0.42.

Example 14: PEGylation

The ISVD glycoprotein T043800005 (SEQ ID NO: 23) was further conjugated to an alkoxyamine functionalized PEG. Conjugation of 2 kDa Aminooxy-PEG (PEG, BroadPharm) to the glycosylated ISVD T043800005 was completed by first oxidizing the ISVD with sodium periodate at concentrations of 2.5 mg/mL and 20 mM, respectively, in 100 mM sodium acetate, pH 5.6, at 4°C for 30 minutes with gentle shaking. The reaction was then quenched with 3% glycerol at 4°C for 15 minutes. The ISVD was then purified via molecular weight cut-off filter into 100 mM sodium acetate, pH 5.6 by centrifuging for a minimum of five exchanges.

After protein concentration was determined via Stunner, the ISVD was PEGylated at concentrations of 2.5 mg/mL and 7.5 mg/mL, respectively, in 100 mM sodium acetate, pH 5.6. The reaction was allowed to proceed at room temperature for at least 16 hours with gentle shaking. The ISVD was then purified via molecular weight cut-off filter into PBS by centrifuging for a minimum of five times. The resulting ISVD concentration and polydispersity was then determined via Stunner. SDS-PAGE, protocol described in Example 13, and MALDI-TOF, protocol described in Example 12, were then completed to analyze the conjugations.

Results

Per SDS-PAGE, conjugation with PEG was observed at a DoL of 0.55.

Example 15: TNF-α internalization mediated by an ISVD glycovariant

Materials & methods

Materials

Biotinylated TNF was purchased from R&D systems, while Alexa Fluor™ 647 labeled streptavidin was acquired from Thermo Fisher Scientific. Except for bisM6P glycan that was produced in house, all other chemical reagents were purchased from Millipore Sigma unless otherwise specified.

Expression and purification of ISVD glycovariant

A trivalent ISVD glycovariant, T043800005 (SEQ ID NO: 23), was engineered by introducing an N-glycosylation site with R19N and the mutant was generated using site-directed mutagenesis. The ISVD construct was produced from Chinese hamster ovary (CHO) cells. The protocols are as described in Examples 1 and 4.

Site-specific ISVD conjugation with bisM6P glycan

The conjugation was performed based on what is known in the art. Briefly, the anti-TNF ISVD was oxidized with 20 mM sodium periodate in sodium phosphate buffer (pH 7.2) on ice for 30 minutes. The oxidation reaction was protected from light and quenched with glycerol (3% v/v) for 15 minutes. The oxidized ISVD was buffer exchanged into 100 mM sodium acetate (pH 5.6) by 5 rounds of ultrafiltration through Amicon® ultra centrifugal filters. The desalted ISVD was then reacted with bisM6P glycan at 30-fold molar excess overnight at room temperature. The un-conjugated free glycan was removed by ultrafiltration using the same protocol as for the oxidized ISVD.

ISVD characterization

The ISVD and ISVD conjugates were characterized using LC-MS intact protein analysis. The assay was performed by partially reducing ISVDs or ISVD conjugates at 0.1 mg/mL with 20 mM dithiothreitol at 37 °C for 30 minutes. The reaction was quenched with 0.1% trifluoroacetic acid before the reduced samples were run on an Agilent 1290/6545XT Q-ToF UPLC/MS system.

The UPLC-MS was run using an Agilent PLRP column (2.1 mm x 50 mm, 5 µm) at 55 °C with a mobile phase A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) with m/z range 100-9000 Da. The data acquired were processed using Expressionist 16.5 software. The most intense charge states in each spectrum were used for deconvolution using MaxEnt algorithm (resolution: 1.0 Da, mass range 20–200 kDa).

The molecular weights of ISVDs and ISVD bisM6P conjugates were measured using MALDI-TOF MS to determine the number of copies of bisM6P glycan per ISVD. The analysis was run on a Bruker Autoflex III. The intact protein mass was determined on a target plate spotted with ISVD or conjugate samples mixed with sinapinic acid matrix using linear positive mode. The data for each sample was acquired in triplicate. The number of bisM6P glycans conjugated per ISVD was calculated by subtracting the molecular weight of ISVD from that of the conjugate before dividing the difference by the molecular weight of the glycan.

Size-exclusion ultra performance liquid chromatography (SEC-UPLC) was performed on a Water ACQUITY UPLC H-class PLUS Bio System. ISVDs or bisM6P-conjugated ISVDs (about 5 µg) were separated on a Superdex 200 increase 10/300 gl column at room temperature with a flow rate of 0.3 mL/minute under isocratic conditions using PBS (pH 7.2) as the mobile phase.

Characterization of protein of interest (POI) internalization by flow cytometry

Jurkat cells or K562 cells (ATCC) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/mL of amphotericin B, 55 µM 2-Mercaptoethanol, and 10 mM HEPES (all from Gibco). They were plated at 1 x 10^5 cells/well in U-bottom 96-well plates.

Biotinylated recombinant human TNF-α and streptavidin labeled with Alexa Fluor™ 647 were sequentially added at final concentration of 50 and 100 nM, respectively. The ISVD T043800005 or bisM6P-conjugated ISVD was subsequently included at various concentrations between 0.78-50 nM.

After the cells were cultured at 37 °C for 1 or 4 hours, they were washed twice with cold phosphate buffered saline (PBS) (pH 7.2), then flow cytometry was conducted to assess fluorescence of TNF complex containing Alexa Fluor™.

Characterization of POI degradation by Western blotting

K562 cells or Jurkat cells were plated at 1 x 10^5 cells/well in U-bottom 96 well plates. Sequentially, recombinant human TNF-α (50 nM, Peprotech) and the anti-TNF ISVD (T043800005) or bisM6P-conjugated ISVD at final concentrations of 25 nM were added.

After the cells were cultured at 37 °C for 2 hours, they were washed twice with media and lysed with RIPA buffer (Boston BioProducts). Some cells were kept in culture for an additional 4 or 24 hours in the presence of DMSO or 100 nM Bafilomycin A1 (InvivoGen).

At each timepoint, the cells were washed twice with cold PBS and lysed with RIPA buffer. Western blotting to detect TNF-α or β-actin was conducted with prepared lysates using anti-TNF (clone D5G9, Cell Signaling Technology) followed by anti-rabbit detection module (ProteinSimple) or anti-β-actin (clone 8H10D10, Cell Signaling Technology) followed by anti-mouse detection module (ProteinSimple) using Jess (ProteinSimple).

To measure TNF-α in the supernatant of media after internalization, K562 cells (1 x 10^5 cells/well) were treated with 50 nM of TNF-α and 25 nM of ISVD or bisM6P-conjugated ISVD at 37 °C.
During the incubation period, media supernatants were collected at different timepoints (24, 48 and 72 hours), and Western blotting was performed with the ISVD or conjugate as mentioned above.

Results

The glycosylated ISVDs contained one copy of bisM6P per ISVD as was determined by MALDI-TOF MS.

The ISVD conjugate containing approximately 1 bisM6P attached to a single introduced N-glycosylation site efficiently induced TNF-α internalization in Jurkat cells or K562 cells (Figure 5).

The bisM6P-conjugated ISVD construct showed dose-dependent TNF-α internalization after incubation for 1 and 4 hours while reduced internalization was observed at a high ISVD concentration of 50 nM, likely due to the hook effect (Figure 6).

This was further confirmed by Western Blotting analysis of the cell lysate (Figure 7A) and the supernatant (Figure 7B). The amount of internalized TNF-α was already quite low at 4 hours and was not detected at 22 hours after incubation. The addition of bafilomycin A1 significantly delayed TNF-α degradation at 4 and 22 hours, suggesting that TNF-α degradation through bisM6P-conjugated ISVDs occurs in lysosomes. The amount of TNF-α remaining in cell culture media was also determined. Although there was little change in TNF-α levels in culture supernatant derived from human K562 cells treated with the control anti-TNF ISVD alone, the amount of TNF-α was significantly reduced during incubation of cells with bisM6P-conjugated ISVDs from 24 to 72 hours.

This shows that TNF-α internalization mediated by an ISVD glycovariant with one copy of bisM6P is effective, which suggests that a single bisM6P on an ISVD is sufficient for inducing internalization and degradation of a soluble POI in the lysosome.

The following table shows sequences disclosed herein:

ISVD ID	SEQ ID NO	Sequence
ALB00606	1	EVQLVESGGGVVQPNGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSS
ALB00607	2	EVQLVESGGGVVQPGGSLNLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSS
ALB00608	3	EVQLVESGGGVVQPGGSLRLSCAASNFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSS
ALB00609	4	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGNDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQG TLVTVSS
ALB00610	5	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSNGT LVTVSS
ALB00611	6	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT NVTVSS
ALB00612	7	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSTNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSS
ALB00613	8	EVQLVESGGGVVQPNGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSS
ALB00614	9	EVQLVESGGGVVQPGGSLNLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSS
ALB00615	10	EVQLVESGGGVVQPGGSLRLSCAASNFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSS
ALB00616	11	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGNDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQG TLVTVSS

ALB00617	12	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSNGT LVTVSS
ALB00618	13	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT NVTVSS
ALB00619	14	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSTNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSS
ALB00620	15	EVNLTESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSS
ALB00621	16	EVNLTESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSS
ALB00622	17	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSS
ALB00623	18	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSS
T043800001	19	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTLVTVSS
T043800002	20	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVNLTESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTLVTVSS
T043800003	21	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLNESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPG KGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTI GGSLSRSSQGTLVTVSS
T043800004	22	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ

		GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPNGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTLVTVSS
T043800005	23	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLNLSCAASGFTFRSFGMSWVRQAPG KGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTI GGSLSRSSQGTLVTVSS
T043800006	24	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASNFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTLVTVSS
T043800007	25	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGNGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTI GGSLSRSSQGTLVTVSS
T043800008	26	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGNDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTI GGSLSRSSQGTLVTVSS
T043800009	27	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFNISRDNSKNTLYLQMNSLRPEDTALYYCTI GGSLSRSSQGTLVTVSS

T043800010	28	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNNKTTLYLQMNSLRPEDTALYYCTI GGSLSRSSQGTLVTVSS
T043800011	29	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSNSSQGTLVTVSS
T043800012	30	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSNGTLVTVSS
T043800013	31	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTNVTVSS
T043800014	32	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTLVNVSS
T043800015	33	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK

		GPEWVSSISGSGSDTLYADSVKGRFTISRDNSNNTLYLQMNSLRPEDTALYYCTI GGSLSRSSQGTLVTVSS
T043800016	34	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSTNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTLVTVSS
T043800055	35	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800056	36	EVQLVESGGGVVQPNGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMY WVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPE DTALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAP GKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYC ARSPSGFNRGQGTLVTVSS
T043800057	37	EVQLVESGGGVVQPGGSLNLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMY WVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPE DTALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAP GKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYC ARSPSGFNRGQGTLVTVSS
T043800058	38	EVQLVESGGGVVQPGGSLRLSCAASNFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMY WVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPE DTALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAP GKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYC ARSPSGFNRGQGTLVTVSS

T043800059	39	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG NGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQG TLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYW VRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPED TALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPG KGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCA RSPSGFNRGQGTLVTVSS
T043800060	40	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGNDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQG TLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYW VRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPED TALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPG KGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCA RSPSGFNRGQGTLVTVSS
T043800061	41	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFNISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQG TLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYW VRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPED TALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPG KGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCA RSPSGFNRGQGTLVTVSS
T043800062	42	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNNKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800063	43	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSNSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800064	44	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSNGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK

		GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800065	45	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSNNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800066	46	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSTNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800067	47	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPNGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800068	48	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLNLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800069	49	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASNFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800070	50	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGNGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRP EDTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSL RLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISR DNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS

T043800071	51	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGNDTLYADSVKGRFTISRDNSKNTLYLQMNSLRP EDTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSL RLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISR DNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800072	52	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFNISRDNSKNTLYLQMNSLRP EDTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSL RLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISR DNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800073	53	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNNKTTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800074	54	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSNSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800075	55	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSNGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800076	56	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSNNTLYLQMNSLRP EDTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSL RLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISR DNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800089	57	EVNLTESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG

		GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800090	58	EVQLNESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSIS GSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMY WVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPE DTALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAP GKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYC ARSPSGFNRGQGTLVTVSS
T043800091	59	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVNLTESGGGVVQPGGSLRLSCAASGFTFRSFGMSW VRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPED TALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRL SCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800092	60	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT NVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYW VRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPED TALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPG KGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCA RSPSGFNRGQGTLVTVSS
T043800093	61	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVNVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYW VRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPED TALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPG KGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCA RSPSGFNRGQGTLVTVSS
T043800094	62	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLNESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800095	63	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTNVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR

		LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800096	64	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVNVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800097	65	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSTNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800098	66	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800118	67	AVNLTESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS
T043800119	68	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVNVSS
T043800120	69	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNANNTVDLQMNSLKPEDTAIYYCAAAAGSTW YGTLYEYDAWGQGTLVTVSS
T043800121	70	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNATNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS
T043800122	71	AVQLNESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS
T043800123	72	AVQLVESGGGSVQANGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS
T043800124	73	AVQLVESGGGSVQAGGSLNLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS
T043800125	74	AVQLVESGGGSVQAGGSLRLTCAASNRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS

T043800126	75	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGNSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS
T043800127	76	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFNISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTW YGTLYEYDAWGQGTLVTVSS
T043800128	77	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGNGTLVTVSS
T043800129	78	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTNVTVSS
T043800130	79	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSS
T043800131	80	EVQLNESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSS
T043800133	81	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGNITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRG QGTLVTVSS
T043800134	82	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFNISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSS
T043800135	83	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTNVTVSS
T043800136	84	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVNVSS
T043800137	85	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNATNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSS
T043800138	86	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSS
T043800139	87	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVNVSS
T043800143	88	EVQLVESGGGVVQPNGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS
T043800144	89	EVQLVESGGGVVQPGGSLNLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS

T043800145	90	EVQLVESGGGVVQPGGSLRLSCTASNFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS
T043800146	91	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDNTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS
T043800147	92	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFNISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS
T043800148	93	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGNGTLVTVSS
T043800149	94	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTNVTVSS
T043800150	95	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS
T043800159	96	NVSLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSS
T043800161	97	DVNLTESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS
T043800178	98	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800179	99	AVNLTESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800180	100	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVNVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800181	101	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNANNTVDLQMNSLKPEDTAIYYCAAAAGSTW YGTLYEYDAWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCA ASGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKN TLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800182	102	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNATNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAA

		SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800183	103	AVQLNESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800184	104	AVQLVESGGGSVQANGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800185	105	AVQLVESGGGSVQAGGSLNLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800186	106	AVQLVESGGGSVQAGGSLRLTCAASNRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800187	107	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGNSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTLVTVSSGGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800188	108	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY GTLYEYDAWGQGTNVTVSSGGGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800189	109	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800190	110	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVNLTESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800191	111	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVNVSS

T043800192	112	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNANNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800193	113	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNATNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800194	114	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLNESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800195	115	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQANGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800196	116	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLNLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800197	117	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASNRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800198	118	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGNSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800199	119	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFNISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800200	120	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGNGTLVTVSS
T043800201	121	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT

		LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTNVTVSS
T043800202	122	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800203	123	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVNVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800204	124	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSNNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800205	125	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSTNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800206	126	EVQLNESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800207	127	EVQLVESGGGVVQPNGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800208	128	EVQLVESGGGVVQPGGSLNLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800209	129	EVQLVESGGGVVQPGGSLRLSCTASNFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800210	130	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDNTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF

		RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800211	131	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFNISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800212	132	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGNGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800213	133	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTNVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800214	134	DVNLTESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800215	135	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800216	136	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVNVSS
T043800217	137	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSNNTVYLQMNSLRPEDT ALYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800218	138	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSTNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800219	139	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLNESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS

T043800220	140	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPNGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800221	141	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLNLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800222	142	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASNFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800223	143	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDNTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800224	144	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFNISRDNSKNTVYLQMNSLRPEDT ALYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800225	145	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGNGTLVTVSS
T043800226	146	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTNVTVSS
T043800227	147	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSDVNLTESGGGVVQPGGSLRLSCTASGFTFSTADMGWF RQAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTA LYYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800228	148	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFNISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTW YGTLYEYDAWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCA ASGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKN TLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800229	149	AVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGIS WRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWY

		GTLYEYDAWGNGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAA SGFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNT LYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
5GS linker	150	GGGGS
9GS linker	151	GGGGSGGGS
15GS linker	152	GGGGSGGGGSGGGGS
35GS linker	153	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
VTVSS$(X)_{10}$	154	VTVSSXXXXXXXXXX
C-terminus sequence	155	VKVSS
C-terminus sequence	156	VQVSS
C-terminus sequence	157	VTVKS
C-terminus sequence	158	VTVQS
C-terminus sequence	159	VKVKS
C-terminus sequence	160	VKVQS
C-terminus sequence	161	VQVKS
C-terminus sequence	162	VQVQS
Ala-extension	163	VTVSSA
Ala-extension	164	VKVSSA
Ala-extension	165	VQVSSA
Ala-extension	166	VTVKSA
Ala-extension	167	VTVQSA
Ala-extension	168	VKVKSA
Ala-extension	169	VKVQSA
Ala-extension	170	VQVKSA
Ala-extension	171	VQVQSA
VTVSS$(X)_1$	172	VTVSSX
VTVSS$(X)_2$	173	VTVSSXX
VTVSS$(X)_3$	174	VTVSSXXX
VTVSS$(X)_4$	175	VTVSSXXXX
VTVSS$(X)_5$	176	VTVSSXXXXX
T043800899	177	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGSITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSS
T043800901	178	NVSLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS

T043800902	179	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG NDSTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSS
T043800969	180	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDNWSQGTLVTVSS
T043800970	181	NVSLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWFRQAPGKEREFVSGISW RGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPEDTAIYYCAAAAGSTWYG TLYEYDAWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAAS GFTFRSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTL YLQMNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800971	182	NVSLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG IDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800972	183	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSNVSLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800973	184	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSNVSLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFR QAPGKGREFVARISGIDGTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTAL YYCRSPRYADQWSAYDYWGQGTLVTVSS
T043800974	185	NVSLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800975	186	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSNVSLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSW VRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPED TALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRL SCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800976	187	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSNVSLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK

		GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSRSSQGTLVTVSS
T043800977	188	EVQLVESGGGVVQPGGSLRLSCTASGFTFSTADMGWFRQAPGKGREFVARISG NDSTTYYDEPVKGRFTISRDNSKNTVYLQMNSLRPEDTALYYCRSPRYADQWSA YDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTF RSFGMSWVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQ MNSLRPEDTALYYCTIGGSLSRSSQGTLVTVSS
T043800978	189	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWNGSSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDAWGQGTLVTVSS
T043800979	190	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG NGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQG TLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYW VRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPED TALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPG KGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCA RSPSGFNRGQGTLVTVSS
T043800980	191	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSTNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800981	192	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSTNTLYLQMNSLRPE DTALYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800982	193	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSGGGGSGGGSAVQLVESGGGSVQAGGSLRLTCAASGRTSRSYGMGWF RQAPGKEREFVSGISWRGDSTGYADSVKGRFTISRDNAKNTVDLQMNSLKPED TAIYYCAAAAGSTWYGTLYEYDNWSQGTLVTVSS
T043800983	194	EVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGPEWVSSISG SGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIGGSLSNSSQGT LVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWV RQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDT ALYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSGGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGK

		GLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCAR SPSGFNRGQGTLVTVSS
T043800984	195	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMS WVRQAPGKGPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPE DTALYYCTIGGSLSNSSQGTLVTVSSGGGGSGGGSEVQLVESGGGVVQPGGSLR LSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRD NAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS
T043800985	196	EVQLVESGGGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEIN TNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESG GGVVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLRPEDTALYYCARSPSGFNRGQGTLVTVSS GGGGSGGGSEVQLVESGGGVVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGK GPEWVSSISGSGSDTLYADSVKGRFTISRDNSKNTLYLQMNSLRPEDTALYYCTIG GSLSNSSQGTLVTVSS

CLAIMS

1. Polypeptide comprising or (essentially) consisting of a heavy-chain immunoglobulin single variable domain (ISVD), wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 15, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

2. Polypeptide according to claim 1, wherein said glycosylation acceptor site is an N-glycosylation site.

3. Polypeptide according to claim 1 or 2, wherein said glycosylation acceptor site is contained in an NXT or NXS motif, wherein X can be any amino acid, and wherein the asparagine residue of the NXT or NXS motif is the glycosylation acceptor site.

4. Polypeptide according to any one of claims 1-3, wherein said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 19, 1, 26, 53, 55, 68, 73, 75, 105,108, and 110, according to Kabat numbering.

5. Polypeptide according to any one of claims 1-3, wherein said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 19, 26, 55, 73, 105, and 108, according to Kabat numbering.

6. Polypeptide according to any one of claims 1-3, wherein said glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 19, 1, 26, 53, 55, 68, 73, 75, 102, 105, 108, and 110, according to Kabat numbering.

7. Polypeptide according to claim 6, wherein said polypeptide is a monovalent polypeptide that comprises or (essentially) consists of one ISVD.

8. Polypeptide comprising or (essentially) consisting of one ISVD, wherein said ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

9. Polypeptide according to claim 8, wherein the glycosylation acceptor site is present at an amino acid position selected from the amino acid positions 19, 1, 26, 53, 55, 68, 73, 75, 102, 105, 108, and 110, according to Kabat numbering.

10. Polypeptide according to any one of claims 1-3, wherein said polypeptide comprises at least two ISVDs.

11. Polypeptide according to claim 10, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 15, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

12. Polypeptide according to claim 10, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 26, 53, 55, 68, 73, 75, 105, 108 and 110, according to Kabat numbering.

13. Polypeptide according to claim 10, wherein at least one of said at least two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 26, 55, 105, and 108, according to Kabat numbering.

14. Polypeptide according to claim 10, wherein said polypeptide is a bivalent polypeptide that comprises or (essentially) consists of two ISVDs.

15. Polypeptide according to claim 14, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 26, 53, 55, 68, 73, 75, 105, 108, and 110, according to Kabat numbering.

16. Polypeptide according to claim 14, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 26, 55, 105, and 108, according to Kabat numbering.

17. Polypeptide according to claim 14, wherein at least one of the two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 26, 55, 105, and 108, according to Kabat numbering.

18. Polypeptide comprising or (essentially) consisting of two ISVDs, wherein at least one of said two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 15, 26, 53, 55, 68, 73, 75, 76, 105, 108 and 110 according to Kabat numbering.

19. Polypeptide according to claim 18, wherein at least one of the two ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 26, 55, 105, and 108, according to Kabat numbering.

20. Polypeptide according to claim 18, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 26, 53, 55, 68, 73, 75, 105, 108, and 110, according to Kabat numbering.

21. Polypeptide according to claim 18, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 26, 55, 105, and 108, according to Kabat numbering.

22. Polypeptide comprising or (essentially) consisting of two ISVDs, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 26, 53, 55, 68, 73, 75, 105, 108 and 110, according to Kabat numbering.

23. Polypeptide comprising or (essentially) consisting of two ISVDs, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 26, 55, 105, and 108, according to Kabat numbering.

24. Polypeptide according to any one of claims 1-3, wherein said polypeptide comprises at least three ISVDs.

25. Polypeptide according to claim 24, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 15, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110, according to Kabat numbering.

26. Polypeptide according to claim 24, wherein least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 15, 26, and 105, according to Kabat numbering.

27. Polypeptide according to any one of claim 24-26, wherein said polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

28. Polypeptide according to claim 24, 25 or 27, wherein the N-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 15, 26, 55, 73, 75, 76, 105, 108, and 110, according to Kabat numbering.

29. Polypeptide according to claim 24, 25 or 27, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 15, 26, and 105, according to Kabat numbering.

30. Polypeptide according to claim 24, 25 or 27, wherein at least one of the at least three ISVDs that is neither at the C-terminal end nor at the N-terminal end comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 15, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

31. Polypeptide comprising or (essentially) consisting of at least three ISVDs, wherein at least one of said at least three ISVDs comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 15, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108 and 110 according to Kabat numbering.

32. Polypeptide according to claim 31, wherein the N-terminal ISVD comprises a glycosylation acceptor site at an amino acid position selected from the amino acid positions 19, 3, 15, 26, 55, 73, 75, 76, 105, 108 and 110, according to Kabat numbering.

33. Polypeptide according to claim 31, wherein the C-terminal ISVD comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 1, 3, 15, 26, and 105, according to Kabat numbering.

34. Polypeptide according to claim 31, wherein at least one of the at least three ISVDs that is neither at the C-terminal end nor at the N-terminal end comprises a glycosylation acceptor site present at an amino acid position selected from the amino acid positions 19, 3, 15, 26, 53, 55, 68, 73, 75, 76, 102, 105, 108, and 110, according to Kabat numbering.

35. Polypeptide according to any one of claims 31-34, wherein the polypeptide is a trivalent polypeptide that comprises or (essentially) consists of three ISVDs.

36. Polypeptide according to any one of claims 1-35 that is glycosylated at the glycosylation acceptor site with one or more glycans.

37. Polypeptide according to claim 36 wherein the glycan is selected from a terminal N-acetyl glucosamine (GlcNAc), a (terminal) mannose, a (terminal) sialic acid, a (terminal) galactose or a combination thereof.

38. Nucleotide sequence or nucleic acid encoding a polypeptide according to any one of claims 1-37.

39. Nucleotide sequence or nucleic acid according to claim 38, that is optimized for expression in a host cell or host organism that is capable of glycosylating the polypeptide encoded by the nucleotide sequence or nucleic acid.

40. Nucleotide sequence or nucleic acid according to claim 38 or 39, that is in the form of a construct or (expression) vector that can be expressed in a host cell or host organism that is capable of glycosylating the polypeptide encoded by the nucleotide sequence or nucleic acid.

41. Method for producing a polypeptide according to any one of claims 1-37, wherein the method comprises the step of:
    - Expressing the nucleotide sequence or nucleic acid according to any one of claims 38-40 in a suitable host cell or host organism,

    wherein the host cell or host organism is capable of glycosylating the expressed polypeptide.

42. Method for the conjugation of a moiety to the polypeptide of any one of claims 1-37, comprising the step of:

- Oxidation of one or more of the glycans present on the polypeptide, optionally using periodate oxidation; and
- Conjugation of the oxidized glycan to the moiety.

43. Method according to claim 42, wherein the moiety is selected from (bis-)mannose-6-phosphate, a PROTAC and a PEG moiety.

44. Conjugate comprising the polypeptide according to any one of claims 1-37 and a conjugated moiety, wherein the moiety is conjugated to the glycan.

45. Conjugate according to claim 44, wherein the moiety is selected from (bis-)mannose-6-phosphate, a PROTAC and a PEG moiety.

46. Composition comprising a polypeptide according to any one of claims 1-37, a polypeptide produced using the method of claim 42 or 43, or a conjugate according to claim 44 or 45.

47. The polypeptide according to any one of claims 1-37, the nucleotide sequence or nucleic acid according to any one of claims 38-40, the conjugate according to claim 44 or 45, or the composition according to claim 46 for use as a medicament.