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Dennler, P. et al., 'Transglutaminase-Based Chem-Enzymatic Conjugation Approach Yields Homogenous Antibody-Drug Conjugates', Bioconjugate Chem., (2014-02-03), vol. 25, pages 569-578, doi: 10.1021/bc400574z.
Mindt, T. et al., 'Modification of Different IgG1 Antibodies via Glutamine and Lysine using Bacteria and human Tissue Transglutaminase', Bioconjugate Chem., (2008), vol. 19, pages 271-278, doi: 10.1021/bc700306n.
Josten, A. et al., 'Use of microbial transglutaminase for the enzymatic biotinylation of antibodies', Journal of Immunological Methods, (2000), vol. 240, pages 47-54.
Deonarain, M. et al., 'Emergin formats for next-generation antibody drug conjugates', Expert Opinion of Drug Discovery, (2015), vol. 10, no. 5, pages 463-481, doi:10.1517/17460441.2015.1025049.

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(54) Title: LYSINE CONJUGATED IMMUNOGLOBULINS

(57) Abstract: Provided herein are conjugated immunoglobulins and methods for generating conjugated immunoglobulins using a microbial transglutaminase.

WO 2017/213267 A1

Description

Title of Invention: LYSINE CONJUGATED IMMUNOGLOBULINS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/348,410, filed on June 10, 2016, the entire contents of which are incorporated herein by reference.

[0002] SEQUENCE LISTING

The instant application contains a Sequence Listing submitted currently herewith, and which is hereby incorporated by reference in its entirety.

Technical Field

[0003] Provided herein are lysine conjugated immunoglobulins and methods of creating the same.

Background Art

[0004] The utility of monoclonal antibodies extends from basic research to therapeutic and diagnostic applications. The ability to conjugate antibodies to functional agents extends their functionality even further. The manufacture of conjugated antibodies usually involves conjugation of a linker, drug, or other functional agent to reactive lysine cysteine residues on the heavy (HC) and light (LC) chains of a monoclonal antibody (mAb). See Deonarain, et al., "Emerging formats for next-generation antibody drug conjugates", Expert Opinion in Drug Discovery (2015), 10(5): 463-481. Lysine conjugation is typically mediated by succinimide (NHS)-based or isothiocyanate-based chemistry. Cysteine-based conjugation requires partial reduction of the antibody to break some of the interchain disulfide bonds, thereby creating free thiol side chains. Thiol-reactive functional agents can then react with the free thiol groups on the antibody to generate antibody-drug conjugates (ADCs). Both of these methods result in modification of multiple lysines or cysteines leading to heterogeneous mixtures of ADCs with a distribution of drug-to-antibody (DAR) ratios and drug modifications at random positions.

[0005] A recent push to utilize site-specific conjugation technologies as a way to produce a homogeneous ADC product with a defined DAR has yielded several methods including engineering unpaired cysteines, incorporation of non-natural amino acids, and site-specific enzymatic modification. While these methods produce homogeneous products, they each have their disadvantages. Cysteine-based conjugation requires an added step to remove a capping cysteine, glutathione, or even a light chain from the unpaired cysteine. See, e.g., Junutula, et al., "Site-Specific Conjugation of a Cytotoxic

Drug to an Antibody Improves Therapeutic Index", Nature Biotechnology, (2008) 26:925-932; Chen, et al., "Charge-based Analysis of Antibodies with Engineered Cysteines", MAbs (2009) 1(6): 563-571; Gomez, et al., "Effect of temperature, pH, dissolved oxygen, and hydrolysate on the formation of triple light chain antibodies in cell culture" Biotechnol Progress (2010), 26: 1438-1445. Further, serum instability of maleimide-based chemistry currently used for cysteine-based conjugates has been demonstrated raising concerns for loss in potency or off-target toxicity. Alley, et al., "Contribution of Linker Stability to the Activities of Anticancer Immunoconjugates", Bioconjugate Chemistry (2008) 19(3): 759-765; Shen, et al., "Conjugation site modulates the in vivo stability and therapeutic activity of antibody-drug conjugates", Nature Biotechnology (2012) 30: 184-189. Incorporation of non-natural amino acids requires expression in either a genetically modified cell-based or cell-free system. Hallam, et al., "Unnatural Amino Acids in Novel Antibody Conjugates", Future Med. Chem. (2014) 6(11): 1309-1324. Further, the presence of an unnatural amino acid could trigger an immunogenic response in patients. Site-specific enzymatic modifications, however, could potentially utilize a native, wild-type amino acid in the antibody sequence, thereby minimizing the chance for immunogenicity. Further, the post-translational bonds typically formed by protein-modifying enzymes are very stable.

[0006] Site-specific enzymatic modification of proteins has been explored using a family of proteins called transglutaminases that catalyze the formation of a stable isopeptide bond between the γ-carboxyamide group (acyl donor) of a glutamine and the ε-amino group (acyl acceptor) of a lysine (see FIG. 1) (see, e.g., Yokoyama, et al., "Properties and Applications of Microbial Transglutaminase", Appl. Microbiol. Biotech. (2004) 64: 47-454; Strop, "Versatility of Microbial Transglutaminase", Bioconjugate Chemistry, (2014) 25(5): 855-862; Kieliszek et al., "Microbial Transglutaminase and its Application in the Food Industry", Folia Microbiol (2014) 59:241-250). Recently, several groups have explored utilizing transglutaminase as a means to produce ADCs (see, e.g., Josten et al., "Use of Microbial Transglutaminase for the Enzymatic Biotinylation of Antibodies", J. Immunol Methods, (2000) 240:47-54; Mindt et al., "Modification of Different IgG1 Antibodies via Glutamine and Lysine Using Bacterial and Human Tissue Transglutaminase", Bioconjugate Chemistry (2008) 19(1): 271-278); Jeger, et al., "Site-specific and stoichiometric modification of antibodies by bacterial transglutaminase" Angew. Chem. Int. Ed. Engl. (2010) 49: 9995-9997; Strop et al., "Location Matters: Site of Conjugation Modulates Stability and Pharmacokinetics of Antibody Drug Conjugates", Chem Biol (2013) 20(2):161-167; Dennler et al., "Transglutaminase-Based Chemo-Enzymatic Conjugation Approach Yields Homogeneous Antibody-Drug Conjugates", Bioconjugate Chemistry (2014) 25(3): 569-578;

Siegmund, et al., "Locked by Design: A Conformationally Constrained Transglutaminase Tag Enables Efficient Site-Specific Conjugation", Angew. Chem. Int. Ed. Engl. (2015) 54(45):13420-13424). Transglutaminases are found in organisms ranging from bacteria through humans that are structurally and functionally related, yet each is involved in specific cellular processes. A microbial transglutaminase (microbial transglutaminase) isolated from the bacterium Streptomyces mobaraensis has been used extensively throughout the food industry to crosslink proteins together for various applications. Besides its low manufacturing cost, it is an attractive conjugation technique due to its ability to function under a wide range of pH, salt, and temperature conditions.

[0007] Despite over two decades of research, the substrate specificity of microbial transglutaminase has not been clearly defined. In general, glutamines or lysines on exposed loops with hydrophobic or positively charged adjacent residues tend to be preferred. See, Taguchi et al., "Substrate specificity analysis of microbial transglutaminase using proteinaceous protease inhibitors as natural model substrates", J. Biochem. (2000) 128:415-425; Sugimura et al., "Identification of preferred substrate sequences of microbial transglutaminase from Streptomyces mobaraensis using a phage-displayed peptide library", Arch. Biochem. Biophys. (2008) 477:379-383; Tagami et al., "Substrate specificity of microbial transglutaminase as revealed by three-dimensional docking simulation and mutagenesis", Protein Eng. Des. Sel. (2009) 22:747-752. The context of the acyl donor glutamine has been found to be more critical than the acyl acceptor lysine. See, e.g., Ohtsuka et al., "Substrate specificities of microbial transglutaminase for primary amines", J. Agric. Food Chem. (2000) 48: 6230-6233; Ohtsuka et al., "Comparison of substrate specificities of transglutaminases using synthetic peptides as acyl donors", Biosci. Biotechnol. Biochem. (2000) 64: 2608-2613; Gundersen et al., "Microbial transglutaminase displays broad acyl-acceptor substrate specificity", Appl. Microbiol. Biotechnol. (2013) 98:219-230. Indeed, a minimal acyl donor substrate requires an N-terminal N-carboxybenzyloxl (CBZ) group followed by a glutamine and a C-terminal glycine (CBZ-L-glutaminylglycine or Z-Gln-Gly) while the minimal acyl acceptor is ammonia.

[0008] Due to a lower specificity for the acyl acceptor amine by microbial transglutaminase, research thus far has been focused mainly on transamidation of antibody glutamine residues. See, Josten et al., Mindt et al., Jeger et al., Strop et al., Dennler et al., and Siegmund et al., referenced above. Contrary to an earlier report (Josten et al. 2000) describing mTGase-mediated biotinylation of an antibody using acyl acceptor substrates, several groups recently showed little or no mTGase modification of wild-type antibodies by the same or similar substrates. These data confirmed that despite the abundance of solvent-exposed glutamines, none were in the proper context to be

transamidated by mTGase (Mindt et al. 2008; Jeger et al. 2010; Strop et al. 2013).

[0009] It has also been speculated that utilizing an amine donor-based substrate to transamidate a lysine may yield a heterogeneous ADC product due to multiple reactive lysines on the surface of an IgG (Josten et al. 2000; Jeger et al. 2010). Human IgG is comprised of an average of 80 lysines, of which 80-90% are predicted to be solvent exposed (Gautier et al., "Lysine Conjugated Properties in Human IgGs Studied by Integrating High-Resolution Native Mass Spectrometry and Bottom-Up Proteomics", Proteomics (2015) 15(16):2756-2765; data not shown), and the C-terminal codon of IgG_1, IgG_2, IgG_3, and IgG_4 is a lysine (Ellison et al., DNA (1981) 1:11-18; Ellison et al.. ("Ellison et al.. 2"), Proc. Nat. Acad. Sci. USA, (1982) 79:1984-1988; Ellison et al., Nucleic Acid Res. (1982) 10:4071-4079). However, serum-derived IgG lacks the lysine (Wang et al., J. Immunol. (1980) 125:1048-1054; Edelman et al., Proc Natl Acad. Sci. USA (1969) 63:78-85; Frangione et al., Biochemistry (1980) 19:4304-4308; Pink et al., Biochem. J. (1970) 117:33-47). The same has been observed for IgD (White et al., Science (1985) 228:733-737; Lin et al., Proc. Natl. Acad. Sci. USA, (1981) 78:504-508; Shinoda et al., Proc. Natl. Acad. Sci. USA (1981) 78:785-789). Recombinant expression of IgG1 in HEK293 and CHO cells also results in a protein lacking the C-terminal Lys447 (Ellison et al.; Harris et al., Eur. J. Biochem. (1990) 194:611-620; Harris, J. Chromatogr. A (1995) 705:129-134; Dick et al., Biotechnol. Bioeng. (2008) 100:1132-1143).

[0010] To date, those of ordinary skill in the art thought that utilizing an amine donor-based substrate to transamidate a lysine may yield a heterogeneous ADC product due to the plethora of reactive lysines on the surface of an IgG (Josten et al. and Jeger et al.) and, thus, use of an amine donor-based substrate to transamidate lysine residues on immunoglobulins has been discouraged.

[0011] Thus, there exists a need for site-specific enzymatic modifications of immunoglobulins to create conjugates which have a predictable rate of conjugation. This will allow for creation of ADCs with a relatively homologous DAR.

Citation List

Non Patent Literature

[0012] NPL 1: Deonarain, et al., "Emerging formats for next-generation antibody drug conjugates", Expert Opinion in Drug Discovery (2015), 10(5): 463-481

NPL 2: Junutula, et al., "Site-Specific Conjugation of a Cytotoxic Drug to an Antibody Improves Therapeutic Index", Nature Biotechnology, (2008) 26:925-932

NPL 3: Chen, et al., "Charge-based Analysis of Antibodies with Engineered Cysteines", MAbs (2009) 1(6): 563-571

NPL 4: Gomez, et al., "Effect of temperature, pH, dissolved oxygen, and hydrolysate

on the formation of triple light chain antibodies in cell culture" Biotechnol Progress (2010), 26: 1438-1445

NPL 5: Alley, et al., "Contribution of Linker Stability to the Activities of Anticancer Immunoconjugates", Bioconjugate Chemistry (2008) 19(3): 759-765

NPL 6: Shen, et al., "Conjugation site modulates the in vivo stability and therapeutic activity of antibody-drug conjugates", Nature Biotechnology (2012) 30: 184-189

NPL 7: Hallam, et al., "Unnatural Amino Acids in Novel Antibody Conjugates", Future Med. Chem. (2014) 6(11): 1309-1324

NPL 8: Yokoyama, et al., "Properties and Applications of Microbial Transglutaminase", Appl. Microbiol. Biotech. (2004) 64: 47-454

NPL 9: Strop, "Versatility of Microbial Transglutaminase", Bioconjugate Chemistry, (2014) 25(5): 855-862

NPL 10: Kieliszek et al., "Microbial Transglutaminase and its Application in the Food Industry", Folia Microbiol (2014) 59:241-250

NPL 11: Josten et al., "Use of Microbial Transglutaminase for the Enzymatic Biotinylation of Antibodies", J. Immunol Methods, (2000) 240:47-54

NPL 12: Mindt et al., "Modification of Different IgG1 Antibodies via Glutamine and Lysine Using Bacterial and Human Tissue Transglutaminase", Bioconjugate Chemistry (2008) 19(1): 271-278

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NPL 14: Strop et al., "Location Matters: Site of Conjugation Modulates Stability and Pharmacokinetics of Antibody Drug Conjugates", Chem Biol (2013) 20(2):161-167

NPL 15: Dennler et al., "Transglutaminase-Based Chemo-Enzymatic Conjugation Approach Yields Homogeneous Antibody-Drug Conjugates", Bioconjugate Chemistry (2014) 25(3): 569-578

NPL 16: Siegmund, et al., "Locked by Design: A Conformationally Constrained Transglutaminase Tag Enables Efficient Site-Specific Conjugation", Angew. Chem. Int. Ed. Engl. (2015) 54(45):13420-13424

NPL 17: Taguchi et al., "Substrate specificity analysis of microbial transglutaminase using proteinaceous protease inhibitors as natural model substrates", J. Biochem. (2000) 128:415-425

NPL 18: Sugimura et al., "Identification of preferred substrate sequences of microbial transglutaminase from Streptomyces mobaraensis using a phage-displayed peptide library", Arch. Biochem. Biophys. (2008) 477:379-383

NPL 19: Tagami et al., "Substrate specificity of microbial transglutaminase as revealed by three-dimensional docking simulation and mutagenesis", Protein Eng. Des. Sel. (2009) 22:747-752

NPL 20: Ohtsuka et al., "Substrate specificities of microbial transglutaminase for primary amines", J. Agric. Food Chem. (2000) 48: 6230-6233
NPL 21: Ohtsuka et al., "Comparison of substrate specificities of transglutaminases using synthetic peptides as acyl donors", Biosci. Biotechnol. Biochem. (2000) 64: 2608-2613
NPL 22: Gundersen et al., "Microbial transglutaminase displays broad acyl-acceptor substrate specificity", Appl. Microbiol. Biotechnol. (2013) 98:219-230
NPL 23: Gautier et al., "Lysine Conjugated Properties in Human IgGs Studied by Integrating High-Resolution Native Mass Spectrometry and Bottom-Up Proteomics", Proteomics (2015) 15(16):2756-2765
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NPL 26: Ellison et al., Nucleic Acid Res. (1982) 10:4071-4079
NPL 27: Wang et al., J. Immunol. (1980) 125:1048-1054
NPL 28: Edelman et al., Proc Natl Acad. Sci. USA (1969) 63:78-85
NPL 29: Frangione et al., Biochemistry (1980) 19:4304-4308
NPL 30: Pink et al., Biochem. J. (1970) 117:33-47
NPL 31: White et al., Science (1985) 228:733-737
NPL 32: Lin et al., Proc. Natl. Acad. Sci. USA, (1981) 78:504-508
NPL 33: Shinoda et al., Proc. Natl. Acad. Sci. USA (1981) 78:785-789
NPL 34: Harris et al., Eur. J. Biochem. (1990) 194:611-620
NPL 35: Harris, J. Chromatogr. A (1995) 705:129-134
NPL 36: Dick et al., Biotechnol. Bioeng. (2008) 100:1132-1143

Summary of Invention

[0013] The instant invention surprisingly discloses that, while no modification of wild-type immunoglobulin lysines by microbial transglutaminase was observed, when an engineered lysine residue was introduced into the immunoglobulin, or antigen-binding portion thereof, microbial transglutaminase was able to utilize the engineered lysine residue as an acyl acceptor. Surprisingly, conjugation of the engineered lysine residue using microbial transglutaminase leads to site-specific and predictable incorporation of conjugated functional agents. Moreover, engineering lysine residues into the constant region has a wide applicability to any immunoglobulin, or antigen-binding portion thereof, regardless of its variable regions and binding specificity.

[0014] In one aspect, disclosed herein is a method for generating a conjugated immunoglobulin, the method comprising contacting an immunoglobulin, or antigen-

binding portion thereof, with a microbial transglutaminase and a functional agent comprising an acyl donor substrate and wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, wherein the acyl donor substrate comprises a glutamine residue, and wherein the functional agent is a therapeutic agent or a diagnostic agent, wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate on the functional agent, thereby generating the conjugated immunoglobulin.

[0015] In another aspect, disclosed herein is a method for generating a conjugated immunoglobulin, the method comprising i) contacting an immunoglobulin, or antigen-binding portion thereof, with a microbial transglutaminase and an acyl donor substrate, wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue and wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, and wherein the acyl donor substrate comprises a glutamine residue and a reactive group, wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate, and ii) conjugating a functional agent to the reactive group of the acyl donor substrate, wherein the functional agent is a therapeutic agent or a diagnostic agent, thereby generating the conjugated immunoglobulin. In one embodiment, the engineered lysine residue is present in a heavy chain. In one embodiment, the engineered lysine residue is present in a heavy chain constant region. In one embodiment, the engineered lysine residue is present in a light chain. In another embodiment, the the engineered lysine residue is present in a light chain constant region. In one embodiment, the engineered lysine residue is present in a kappa light chain chain. In one embodiment, the engineered lysine residue is present in a kappa light chain constant region. In one embodiment, the engineered lysine residue is present in a lambda light chain. In one embodiment, the engineered lysine residue is present in a lambda light chain constant region. In one embodiment, the engineered lysine residue is present in a variable region. In one embodiment, the engineered lysine residue is not present in a variable region.

[0016] In one embodiment, the reactive group of the acyl donor substrate is conjugated to the functional agent by click chemistry.

[0017] In one embodiment, the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of: Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223

(T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, or Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof. In one embodiment, the heavy chain (before mutation) comprises an amino acid sequence set forth as SEQ ID NO:18. In another embodiment, the kappa light chain (before mutation) comprises an amino acid sequence set forth as SEQ ID NO:19. In another embodiment, the lambda light chain (before mutation) comprises a sequence set forth as SEQ ID NO:20.

[0018] In one embodiment, the heavy chain further comprises an amino acid residue which has been added to its C-terminus at position 448, and wherein said amino acid residue is not proline or an acidic amino acid residue. In a further embodiment, the amino acid residue which has been added to the C-terminus at position 448 is leucine.

[0019] In one embodiment, the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof.

[0020] In one embodiment, the immunoglobulin is a fragment-antigen binding (Fab'), and wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of: Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Proline 228 (P228K), Proline 230 (P230K), and Glutamic acid 233 (E233K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof. In another embodiment, the Fab' comprises the entire hinge region. In another embodiment, the Fab' comprises a truncated hinge region.

[0021] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, further comprises a second engineered lysine residue, wherein the second engineered lysine residue is a second lysine residue insertion or a second natural amino acid residue which has been mutated to a lysine residue, and wherein the microbial transglutaminase conjugates the second engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate. In another embodiment, the natural amino acid residue which has been mutated to the engineered lysine residue is Serine 136 (S136K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the second engineered lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof. In another embodiment, the natural amino acid residue which has been mutated to the engineered lysine residue is Threonine 135 (T135K) on a heavy chain of

the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the second engineered lysine residue is Leucine 201 (L201K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof. In yet another embodiment, the natural amino acid residue which has been mutated to the engineered lysine residue is Threonine 135 (T135K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the second engineered lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof. In a further embodiment, the heavy chain further comprises an amino acid residue which has been added to its C-terminus at position 448, and wherein said amino acid residue is not proline or an acidic amino acid residue. In one further aspect, the amino acid residue which has been added to the C-terminus at position 448 is leucine.

[0022] In one embodiment, the immunoglobulin further comprises a third engineered lysine residue, wherein the third engineered lysine residue is a third lysine residue insertion or a third natural amino acid residue which has been mutated to a lysine residue, and wherein the microbial transglutaminase conjugates the third engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate. In one embodiment, the first natural amino acid residue which has been mutated to the engineered lysine residue is Serine 136 (S136K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, the second natural amino acid residue which has been mutated to the second engineered lysine residue is Asparagine 297 (N297K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the third natural amino acid residue which has been mutated to the third engineered lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof.

[0023] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, further comprises a fourth engineered lysine residue, wherein the fourth engineered lysine residue is a fourth lysine residue insertion or a fourth natural amino acid residue which has been mutated to a lysine residue, and wherein the microbial transglutaminase conjugates the fourth engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate. In another embodiment, the first natural amino acid residue which has been mutated to the engineered lysine residue is Serine 136 (S136K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, the second natural amino acid residue which has been mutated to the second engineered lysine residue is Asparagine 297 (N297K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, the third natural amino acid residue which has been mutated to the third en-

gineered lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and the fourth natural amino acid residue which has been mutated to a fourth engineered lysine residue is Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof.

[0024] In one embodiment, the amino acid residue after the engineered lysine residue is not proline or an acidic amino acid residue. In another embodiment, the amino acid residue before the engineered lysine residue is not an acidic amino acid residue. In another embodiment, mutating the amino acid residue after the engineered lysine residue to any amino acid other than proline or an acidic amino acid residue and mutating the amino acid residue before the engineered lysine residue to any amino acid other than an acidic amino acid residue, provide the optimal sequence for an engineered acyl acceptor lysine site.

[0025] In one embodiment, the amino acid residue before the engineered lysine residue is a non-acidic amino acid residue insertion, or a natural acidic amino acid residue which has been mutated to a non-acidic amino acid residue. In one embodiment, the amino acid residue after the engineered lysine residue is an amino acid residue insertion, wherein the amino acid residue insertion is a non-acidic amino acid residue insertion and a non-proline residue insertion, or a natural acidic amino acid residue or a natural proline residue, which has been mutated to a non-acidic amino acid residue and a non-proline residue. In one embodiment, the non-acidic amino acid residue is lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, proline, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan. In one embodiment, the non-acidic amino acid residue insertion is an lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, proline, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan insertion. In one embodiment, the non-acidic amino acid and non-proline residue is lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan. In one embodiment, the natural acidic amino acid residue is aspartic acid or glutamic acid.

[0026] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a heavy chain which further comprises at least one amino acid residue which has been added to its C-terminus at position 448, and wherein said at least one amino acid residue is not proline or an acidic amino acid residue. In a further embodiment, the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

[0027] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a light chain which comprises an insertion of one to four additional amino

acids after cysteine 214, wherein the lysine residue insertion is a lysine residue which has been inserted after the one to four additional amino acids, and wherein a leucine residue has been inserted after the lysine residue. In a further embodiment, the insertion of one to four additional amino acids after cysteine 214, the lysine residue which has been inserted after the one to four additional amino acids, and the leucine residue which has been inserted after the lysine residue comprises a sequence selected from the group consisting of: GKL, GGKL, GGSKL, and GGSGKL. In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a light chain which comprises an insertion of the sequence GGSGKL after cysteine 214.

[0028] In one embodiment, the functional agent comprising the acyl donor substrate is according to one Formulae (I) or (II):

$(Z)_m$-Gln-$(L)_n$-(Y) (I)

(Y)-$(L)_n$-Gln-$(Z)_m$ (II)

[0029] wherein Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue; Gln is a glutamine amino acid residue; each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue; m is an integer from 0 to 5; n is an integer from 0 to 5; and Y is a functional agent.

[0030] In one embodiment, the functional agent comprising the acyl donor substrate is according to formula (I), and wherein Z is a CBZ group; wherein L is a polyethylene glycol moiety (PEG) (-O($(CH_2)_2$)-), ethyl amine (-NH($(CH_2)_2$)-) or propyl amine (-NH($(CH_2)_3$)-); and wherein n is 0, 1, 2 or 3. In one embodiment, L is a polyethylene glycol moiety (PEG). In another embodiment, L comprises one or more amino acids and a polyethylene glycol moiety (PEG). In another embodiment, the functional agent comprising the acyl donor substrate is according to formula (I), wherein Z is a CBZ group, and wherein L is an amino acid. In one embodiment, L is Gly; m is 1; and n is 1. In another embodiment, the functional agent comprising the acyl donor substrate is according to formula (II), wherein Z is a CBZ group; m is 1; n is 2, 3 or 4; and at least one L is Gly; and at least one L is a PEG moiety. In a further embodiment, the functional agent comprising the acyl donor substrate is according to formula (II), wherein Z is a CBZ group; m is 1; n is 4; one L is Gly and the remaining three L groups are each PEG moieties. In another embodiment, the functional agent Y is auristatin F.

[0031] In one embodiment, the acyl donor substrate is according to one Formulae (III) or (IV):

$(Z)_m$-Gln-$(L)_n$-(X) (III)

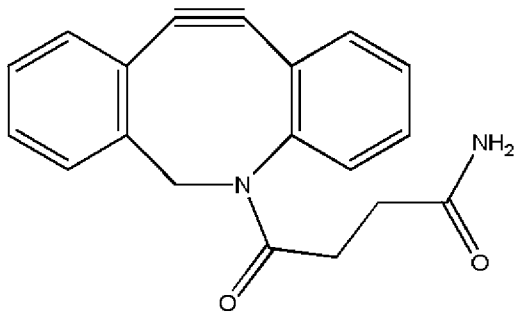
$(X)-(L)_n-Gln-(Z)_m$ (IV)

wherein Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue; Gln is a glutamine amino acid residue; each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue; m is an integer from 0 to 5; n is an integer from 0 to 5; and X is a reactive group.

[0032] In one embodiment, L is a polyethylene glycol moiety (PEG). In another embodiment, when n is 2-5, at least one L comprises one or more amino acids and another L is a polyethylene glycol (PEG) moiety. In one embodiment, the acyl donor substrate is according to formula (III), and wherein Z is a CBZ group; wherein L is a polyethylene glycol moiety (PEG) ($-O((CH_2)_2)-$), ethyl amine ($-NH((CH_2)_2)-$) or propyl amine ($-NH((CH_2)_3)-$); and wherein n is 0, 1, 2 or 3. In another embodiment, the acyl donor substrate is according to formula (III), wherein Z is a CBZ group, and wherein L is an amino acid. In one embodiment, L is Gly; n is 1; and m is 1. In another embodiment, the acyl donor substrate is according to formula (IV), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is Gly.

[0033] In another embodiment, X is a reactive group selected from the group consisting of (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN),

[Chem.1]



(dibenzocyclooctyne; DBCO), trans-cyclooctene (TCO), azido (N_3), alkyne, tetrazine methylcyclopropene, norbornene, hydrazide/hydrazine, and aldehyde.

[0034] In one embodiment, the therapeutic agent is an antibody or antigen-binding portion thereof, a chemotherapeutic agent, a drug agent, a radioactive agent, a cytotoxic agent, an antibiotic, a small molecule, a nucleic acid, or a polypeptide. In another embodiment, the diagnostic agent is a fluorophore, a fluorescent dye, a radionuclide, or an enzyme.

[0035] In one embodiment, the microbial transglutaminase is from Streptomyces mobaraensis.

[0036] In one embodiment, the immunoglobulin, or antigen-binding portion thereof is an

IgG_1 immunoglobulin, or antigen-binding portion thereof. In another embodiment, the immunoglobulin, or antigen-binding portion thereof, is an IgG_2, IgG_3, or IgG_4 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the immunoglobulin is an IgA_1, an IgA_2, or an IgM immunoglobulin. In one embodiment, the immunoglobulin, or antigen-binding portion thereof, is an IgD or IgE, immunoglobulin, or antigen-binding portion thereof.

[0037] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, is a human immunoglobulin, or antigen-binding portion thereof or a humanized immunoglobulin, or antigen-binding portion thereof. In one embodiment, the immunoglobulin, or antigen-binding portion thereof, is a chimeric immunoglobulin or a non-human immunoglobulin, or antigen-binding portion thereof.

[0038] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises two heavy chains and two light chains. In one embodiment, there is no intramolecular cross-linking, i.e., no disulfide bond(s), between the two heavy chains of the immunoglobulin, or antigen-binding portion thereof.

[0039] In one embodiment, the ratio of functional agent to immunoglobulin, or antigen-binding portion thereof, is 1:1 to 200:1 or 1:1 to 100:1.

[0040] In another aspect, described herein is a conjugated immunoglobulin comprising an immunoglobulin, or antigen-binding portion thereof and a functional agent, wherein the immunoglobulin comprises an engineered lysine residue and wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, the functional agent comprises an acyl donor substrate, wherein the acyl donor substrate comprises a glutamine residue, and the functional agent is a therapeutic agent or a diagnostic agent, wherein the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, is conjugated to the glutamine residue of the acyl donor substrate of the functional agent. In one embodiment, the engineered lysine residue is present in a heavy chain. In one embodiment, the engineered lysine residue is present in a heavy chain constant region. In one embodiment, the engineered lysine residue is present in a light chain. In another embodiment, the the engineered lysine residue is present in a light chain constant region. In one embodiment, the engineered lysine residue is present in a kappa light chain chain. In one embodiment, the engineered lysine residue is present in a kappa light chain constant region. In one embodiment, the engineered lysine residue is present in a lambda light chain. In one embodiment, the engineered lysine residue is present in a lambda light chain constant region. In one embodiment, the engineered lysine residue is present in a variable region. In one embodiment, the engineered lysine residue is not present in a variable region.

[0041] In another aspect, described herein is a conjugated immunoglobulin comprising an

immunoglobulin, or antigen-binding portion thereof and a functional agent, wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue and wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, the engineered lysine residue is conjugated to a glutamine residue on an acyl donor substrate, wherein the acyl donor substrate further comprises a reactive group, the reactive group is conjugated to a functional agent, wherein the functional agent is a therapeutic agent or a diagnostic agent. In one embodiment, the engineered lysine residue is present in a heavy chain. In one embodiment, the engineered lysine residue is present in a heavy chain constant region. In one embodiment, the engineered lysine residue is present in a light chain. In another embodiment, the the engineered lysine residue is present in a light chain constant region. In one embodiment, the engineered lysine residue is present in a kappa light chain chain. In one embodiment, the engineered lysine residue is present in a kappa light chain constant region. In one embodiment, the engineered lysine residue is present in a lambda light chain. In one embodiment, the engineered lysine residue is present in a lambda light chain constant region. In one embodiment, the engineered lysine residue is present in a variable region. In one embodiment, the engineered lysine residue is not present in a variable region.

[0042] In one embodiment, the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of: Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, or Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof. In one embodiment, the heavy chain (before mutation) comprises an amino acid sequence of SEQ ID NO:18. In one embodiment, the kappa light chain (before mutation) comprises an amino acid sequence of SEQ ID NO:19. In one embodiment, the lambda light chain (before mutation) comprises an amino acid sequence of SEQ ID NO:20.

[0043] In one embodiment, the heavy chain further comprises an amino acid residue which has been added to its C-terminus at position 448, and wherein said amino acid residue is not proline or an acidic amino acid residue. In a further embodiment, the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

[0044] In another embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a light chain which comprises at least one amino acid residue that has been added to the C-terminus after position 214, wherein the at least one amino acid residue

is GGSGKL (glycine glycine serine glycine lysine leucine). In another embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a light chain which further comprises at least one amino acid residue which has been added to the C-terminus after position 214, wherein the at least one amino acid residue is GGSGKL. In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a light chain which comprises an insertion of the sequence GGSGKL after cysteine 214.

[0045] In one embodiment, the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof.

[0046] In one embodiment, the immunoglobulin, or antigen-binding portion thereof is a fragment-antigen binding (Fab), and wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of: Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Proline 228 (P228K), Proline 230 (P230K), and Glutamic acid 233 (E233K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof. In another embodiment, the Fab comprises the entire hinge region. In another embodiment, the Fab comprises a truncated hinge region.

[0047] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, further comprises a second natural amino acid residue which has been mutated to a second lysine residue, and wherein the microbial transglutaminase conjugates the second lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate. In another embodiment, the first natural amino acid residue which has been mutated to the lysine residue is Serine 136 (S136K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the second lysine residue is Serine 202 (S202K). In another embodiment, the natural amino acid residue which has been mutated to the engineered lysine residue is Threonine 135 (T135K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the second engineered lysine residue is Leucine 201 (L201K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof. In yet another embodiment, the natural amino acid residue which has been mutated to the engineered lysine residue is Threonine 135 (T135K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the second engineered lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof.

[0048] In a further embodiment, the heavy chain further comprises at least one amino acid

residue which has been added to its C-terminus at position 448, and wherein the at least one amino acid residue is not proline or an acidic amino acid residue. In one embodiment, the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

[0049] In one embodiment, the immunoglobulin further comprises a third natural amino acid residue which has been mutated to a third lysine residue, and wherein the microbial transglutaminase conjugates the third lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate. In another embodiment, the first natural amino acid residue which has been mutated to the lysine residue is Serine 136 (S136K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, the second natural amino acid residue which has been mutated to the second lysine residue is Asparagine 297 (N297K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the third natural amino acid residue which has been mutated to the third lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof.

[0050] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, further comprises a fourth natural amino acid residue which has been mutated to a fourth lysine residue, and wherein the microbial transglutaminase conjugates the fourth lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate. In another embodiment, the first natural amino acid residue which has been mutated to the lysine residue is Serine 136 (S136K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, the second natural amino acid residue which has been mutated to the second lysine residue is Asparagine 297 (N297K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, the third natural amino acid residue which has been mutated to the third lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and the fourth natural amino acid residue which has been mutated to a fourth lysine residue is Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof.

[0051] In one embodiment, the amino acid residue after the engineered lysine residue is not proline or an acidic amino acid residue. In another embodiment, the amino acid residue before the engineered lysine residue is not an acidic amino acid residue. In another embodiment, mutating the amino acid residue after the engineered lysine residue to any amino acid other than proline or an acidic amino acid residue and mutating the amino acid residue before the engineered lysine residue to any amino acid other than an acidic amino acid residue, provide the optimal sequence for an engineered acyl acceptor lysine site.

[0052] In one embodiment, the amino acid residue before the engineered lysine residue is a

non-acidic amino acid residue insertion, or a natural acidic amino acid residue which has been mutated to a non-acidic amino acid residue. In one embodiment, the amino acid residue after the engineered lysine residue is an amino acid residue insertion, wherein the amino acid residue insertion is a non-acidic amino acid residue and a non-proline residue insertion, or a natural acidic amino acid residue or a natural proline residue, which has been mutated to a non-acidic amino acid residue and a non-proline residue. In a further embodiment, the non-acidic amino acid residue is lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, proline, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan. In another further embodiment, the non-acidic amino acid residue insertion is an lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, proline, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan insertion. In yet another further embodiment, the non-acidic amino acid and non-proline residue is lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan. In yet another further embodiment, the natural acidic amino acid residue is aspartic acid or glutamic acid.

[0053] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a heavy chain which further comprises at least one amino acid residue which has been added to its C-terminus at position 448, and wherein the at least one amino acid residue is not proline or an acidic amino acid residue. In a further embodiment, the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

[0054] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises a light chain which comprises an insertion of one to four additional amino acids after cysteine 214, wherein the lysine residue insertion is a lysine residue which has been inserted after the one to four additional amino acids, and wherein a leucine residue has been inserted after the lysine residue. In a further embodiment, the insertion of one to four additional amino acids after cysteine 214, the lysine residue which has been inserted after the one to four additional amino acids, and the leucine residue which has been inserted after the lysine residue comprise a sequence selected from the group consisting of: GKL, GGKL, GGSKL, and GGSGKL.

[0055] In one embodiment, the functional agent comprising the acyl donor substrate is according to one Formulae (I) or (II):

$(Z)_m$-Gln-$(L)_n$-(Y) (I)

(Y)-$(L)_n$-Gln-$(Z)_m$ (II)

[0056] wherein Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue; Gln is a glutamine amino acid residue; each L is independently a straight or branched linker

from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue; m is an integer from 0 to 5; n is an integer from 0 to 5; and Y is a functional agent.

[0057] In one embodiment, the functional agent comprising the acyl donor substrate is according to formula (I), and wherein Z is a CBZ group; wherein L is a polyethylene glycol moiety (PEG) (-O($(CH_2)_2$)-), ethyl amine (-NH($(CH_2)_2$)-) or propyl amine (-NH($(CH_2)_3$)-); and wherein n is 0, 1, 2 or 3. In another embodiment, the functional agent comprising the acyl donor substrate is according to formula (I), wherein Z is a CBZ group, and wherein L is an amino acid. In one embodiment, L is Gly; m is 1; and n is 1. In one embodiment, the functional agent comprising the acyl donor substrate is according to formula (II), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is Gly. In one embodiment, L is a polyethylene glycol moiety (PEG). In another embodiment, L comprises one or more amino acids and a polyethylene glycol moiety (PEG). In another embodiment, the functional agent Y is auristatin F.

[0058] In one embodiment, the acyl donor substrate is according to one Formulae (III) or (IV):

$(Z)_m$-Gln-$(L)_n$-(X) (III)

(X)-$(L)_n$-Gln-$(Z)_m$ (IV)

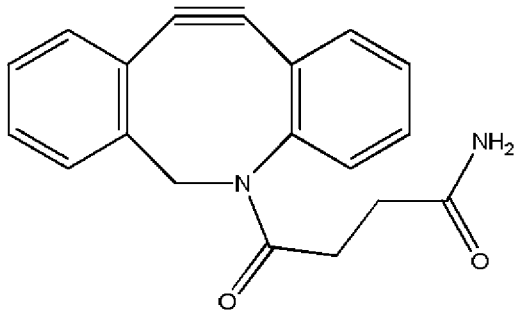
[0059] wherein Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue; Gln is a glutamine amino acid residue; each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue; m is an integer from 0 to 5; n is an integer from 0 to 5; and X is a reactive group.

[0060] In one embodiment, the acyl donor substrate is according to formula (III), and wherein Z is a CBZ group; wherein L is a polyethylene glycol moiety (PEG) (-O($(CH_2)_2$)-), ethyl amine (-NH($(CH_2)_2$)-) or propyl amine (-NH($(CH_2)_3$)-); and wherein n is 0, 1, 2 or 3. In another embodiment, the acyl donor substrate is according to formula (III), wherein Z is a CBZ group, and wherein L is an amino acid. In one embodiment, L is Gly; m is 1; and n is 1. In another embodiment, the acyl donor substrate is according to formula (IV), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is Gly. In one embodiment, L is a polyethylene glycol moiety (PEG). In another embodiment, when n is 2-5, then at least one L comprises one or more amino acids and one or more L comprises a polyethylene glycol moiety (PEG).

[0061] In one embodiment, X is a reactive group selected from the group consisting of

(1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN),

[Chem.2]



(dibenzocyclooctyne; DBCO), trans-cyclooctene (TCO), azido (N_3), alkyne, tetrazine methylcyclopropene, norbornene, hydrazide/hydrazine, and aldehyde.

[0062] In one embodiment, the therapeutic agent is an antibody or antigen-binding portion thereof, a chemotherapeutic agent, a drug agent, a radioactive agent, a cytotoxic agent, an antibiotic, a small molecule, nucleic acid, or a polypeptide. In another embodiment, the diagnostic agent is a fluorophore, a fluorescent dye, a radionuclide, or an enzyme.

[0063] In one embodiment, the microbial transglutaminase is from Streptomyces mobaraensis.

[0064] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, is an IgG_1 immunoglobulin, or antigen-binding portion thereof. In another embodiment, the immunoglobulin, or antigen-binding portion thereof, is an IgG_2, IgG_3, or IgG_4 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the immunoglobulin, or antigen-binding portion thereof, is an IgA_1, an IgA_2, or an IgM immunoglobulin, or antigen-binding portion thereof. In one embodiment, the immunoglobulin, or antigen-binding portion thereof, is an IgD or IgE, immunoglobulin, or antigen-binding portion thereof.

[0065] In one embodiment, the immunoglobulin, or antigen-binding portion thereof is a human immunoglobulin, or antigen-binding portion thereof, or a humanized immunoglobulin, or antigen-binding portion thereof. In one embodiment, the immunoglobulin, or antigen-binding portion thereof, is a chimeric immunoglobulin or a non-human immunoglobulin, or antigen-binding portion thereof.

[0066] In one embodiment, the immunoglobulin, or antigen-binding portion thereof, comprises two heavy chain and two light chains. In one embodiment, there is no intramolecular cross-linking between the two heavy chains of the immunoglobulin, or antigen-binding portion thereof.

[0067] In one embodiment, the ratio of functional agent to immunoglobulin, or antigen-binding portion thereof is 1:1 to 200:1 or 1:1 to 100:1.

[0068] In one embodiment, the functional agent is an antibody, or antigen-binding portion thereof, and wherein the immunoglobulin, or antigen-binding portion thereof, and the

functional agent bind the same antigen or bind different antigens.

[0069] In another aspect, described herein is a nucleic acid encoding a conjugatable immunoglobulin. In another aspect, described herein is a plasmid comprising a nucleic acid. In another embodiment, described herein is an isolated cell comprising a plasmid.

[0070] In another aspect, described herein is a pharmaceutical composition comprising a conjugated immunoglobulin and a pharmaceutically acceptable carrier.

[0071] In one aspect, described herein is a conjugated immunoglobulin produced by any of the methods described herein. In one embodiment, the method further comprises a step of purifying the immunoglobulin conjugated to the glutamine residue of the acyl donor substrate before conjugating the functional agent to the reactive group of the acyl donor substrate. In one embodiment, the purifying step comprises size-based methods, such as chromatography or diafiltration. In another embodiment, the purifying step includes charge-based separation, such as anion exchange or cation exchange chromatography. In another embodiment, the purifying step comprises an affinity-based step, such as Protein A or Protein G chromatography and hydrophobic interaction chromatography (HIC).

[0072] The summary, as well as the following detailed description, is further understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosed methods, and conjugated immunoglobulins, there are shown in the drawings exemplary embodiments; however, the methods and conjugated immunoglobulins are not limited to the specific embodiments disclosed. In the drawings:

Brief Description of Drawings

[0073] [fig.1]FIG. 1 shows a transglutaminase reaction, wherein the transglutaminase catalyzes the formation of an isopeptide bond between an acyl donor glutamine and an acyl acceptor lysine with release of an ammonia molecule.

[fig.2]FIG. 2 shows the structures of exemplary Z-Gln-Gly acyl-donor substrates.

[fig.3]FIG. 3 shows possible routes to synthesize exemplary Z-Gln-Gly acyl-donor substrates.

[fig.4]FIGS. 4A, 4B, and 4C, show solvent exposed lysines (stick representation) in human IgG_1 Fab and Fc crystal structures; (FIG. 4A) Fab VH-CH1 and Vκ-Cκ, (FIG. 4B) Fab VH-CH1 and Vλ-Cλ, and (FIG. 4C) Fc CH2 and CH3 were determined using Discovery Studio 4.5 with a 1.4 Å probe radius.

[fig.5]FIGS. 5A, 5B, 5C, 5D, 5E, and 5F, show ESI-MS analysis of antibodies contacted with an acyl donor and microbial transglutaminase. Antibodies were contacted with 50-fold molar excess Z-Gln-Gly-CAD-biotin and 1U/mL microbial transglutaminase overnight at 37°C. Following IdeS digestion and reduction, the LC, Fd, and Fc masses were determined by ESI-MS.

[fig.6]FIG. 6 shows sequences of human IgG_1, kappa, and lambda constant domains. Solvent exposed constant domain lysines based on the crystal structures 1FC1 (Fcγ) , 4F3F (CH1 and Cκ), and 4HK0 (Cλ) are in bold; lysines within loops are underlined. The constant domains are numbered according the EU numbering system.

[fig.7-1]FIG. 7-1 and FIG. 7-2 show the conjugation levels of purified CH1 and hinge mutant mAbs screened for transamidation by incubating mTGase with Z-Gln-Gly-CAD-biotin overnight at 37°C. Biotinylated mAbs were detected by an ELISA as detailed in the Material and Methods and the relative fluorescent units (RFUs) were analyzed. The masses of the HC and LC were analyzed by ESI-MS (data not shown) and the percentage of conjugation was determined.

[fig.7-2]FIG. 7-1 and FIG. 7-2 show the conjugation levels of purified CH1 and hinge mutant mAbs screened for transamidation by incubating mTGase with Z-Gln-Gly-CAD-biotin overnight at 37°C. Biotinylated mAbs were detected by an ELISA as detailed in the Material and Methods and the relative fluorescent units (RFUs) were analyzed. The masses of the HC and LC were analyzed by ESI-MS (data not shown) and the percentage of conjugation was determined.

[fig.8]FIG. 8 shows the conjugation levels of CH1, CH2, CH3 and hinge mutant mAbs previously identified as having the highest conjugation efficiency. MAbs were incubated with Z-Gln-Gly-CAD-biotin and mTGase at 37°C overnight. The samples were digested with IdeS, reduced, and analyzed by ESI-MS, and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined. The DAR was determined by dividing the Δmass by the mass of Z-Gln-Gly-CAD-biotin

[fig.9]FIG. 9 shows the conjugation effeiciency of mAbs with multiple acyl acceptor cytes with various acyl donors. MAbs were incubated with Z-Gln-Gly-N3, Z-Gln-Gly-PEG2-BCN, or Z-Gln-Gly-PEG2-AuF and mTGase at 37°C overnight. The masses of the reduced LCs were analyzed by LC-MS (data not shown), and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined.

[fig.10]FIG. 10 shows the alignment of IgG Fc isotypes. The primary amino acid sequences of human IgG1, 2, 3, and 4 Fc are aligned and differences are highlighted. IgG1 residues Met252, Asn297, and Pro445 are indicated by an X.

[fig.11]FIG. 11 shows Fab's with an acyl acceptor were conjugated to the Q295 and/or Q297 acyl donor site in the mAb mutant. Antibody 01-N297Q was incubated with Fab' lysine mutants and mTGase overnight at 37°C. The samples were reduced and analyzed by SDS-PAGE using a 4-12% Bis-Tris polyacrylamide gel. The mass of the FabCH1-HC dimer is approximately 75 kDa and the FabCH1-HC-FabCH1 trimer is approximately 100 kDa.

[fig.12]FIG. 12 illustrates engineered acyl acceptor sites located through the mAb. The locations of residues engineered with acyl acceptor cites were highlighted in the (A)

Fab (4F3F) HC and LC and (B) Fc (1FC1).

Description of Embodiments

DETAILED DESCRIPTION

[0074] The disclosed methods and conjugated immunoglobulins may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed methods and conjugated immunoglobulins are not limited to the specific embodiments described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed methods or conjugated immunoglobulins.

[0075] Unless specifically stated otherwise, any description as to a possible mechanism or mode of action or reason for improvement is meant to be illustrative only, and the disclosed methods and conjugated immunoglobulins are not to be constrained by the correctness or incorrectness of any such suggested mechanism or mode of action or reason for improvement.

[0076] Throughout this text, the descriptions refer to conjugated immunoglobulins and methods of generating the same. Where the disclosure describes or claims a feature or embodiment associated with a conjugated immunoglobulin, such a feature or embodiment is equally applicable to the methods of generating the same. Likewise, where the disclosure describes or claims a feature or embodiment associated with a method of generating a conjugated immunoglobulin, such a feature or embodiment is equally applicable to the conjugated immunoglobulin.

[0077] Reference to a particular numerical value includes at least that particular value, unless the context clearly dictates otherwise. When a range of values is expressed, another embodiment includes from the one particular value and/or to the other particular value. Further, reference to values stated in ranges include each and every value within that range. All ranges are inclusive and combinable.

[0078] When values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment.

[0079] It is to be appreciated that certain features of the disclosed methods and conjugated immunoglobulins which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed methods and conjugated immunoglobulins that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

[0080] As used herein, the singular forms "a," "an," and "the" include the plural.

[0081] Various terms relating to aspects of the description are used throughout the speci-

fication and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0082] The term "about" when used in reference to numerical ranges, cutoffs, or specific values is used to indicate that the recited values may vary by up to as much as 10% from the listed value. Thus, the term "about" is used to encompass variations of ± 10% or less, variations of ± 5% or less, variations of ± 1% or less, variations of ± 0.5% or less, or variations of ± 0.1% or less from the specified value.

[0083] In general, the term "engineered" refers to the manipulation of nucleic acid or polypeptide molecules by synthetic means (e.g., by recombinant techniques, in vitro peptide synthesis, by enzymatic or chemical coupling of peptides, or by other methods commonly used in the art).

[0084] As used herein, the term "engineered amino acid residue" refers to a non-naturally-occurring amino acid residue within the context of a sequence. As used herein, the term "engineered nucleic acid residue" refers to a non-naturally occurring nucleic acid residue within the context of a sequence. These terms include, for example, a polypeptide sequence or nucleic acid sequence that comprises one or more amino acid or nucleotide changes, including additions, deletions or substitutions, relative to the corresponding naturally occurring polypeptide sequence or nucleic acid sequence, wherein such changes were introduced by recombinant DNA techniques. For instance, an "engineered lysine residue" is a lysine residue that did not exist in the corresponding naturally occurring, or wild-type, polypeptide sequence and was introduced into the polypeptide, either by mutating an existing amino acid residue, or by the insertion of a lysine residue, when sequences of the wild type and the ngineered version are aligned. An engineered amino acid residue is not a synthetic amino acid residue. For instance, an engineered lysine residue is not a synthetic lysine residue.

[0085] In one embodiment, an "engineered amino acid residue" is an amino acid residue insertion. For example, an amino acid residue or residues may be inserted in between two naturally occurring amino acid residues. In another embodiment, an "engineered amino acid residue" is a naturally occurring amino acid residue which has been mutated to a different amino acid residue. For example, an engineered lysine residue may be a naturally-occurring amino acid residue (e.g., histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, or tyrosine) which has been mutated, e.g., using recombination DNA techniques, to a lysine residue. Those skilled in the art can readily generate engineered polypeptide sequence useful according to this aspect of the invention. Engineered polypeptide sequences may be produced by any means, including, for example, peptide, polypeptide, or protein

synthesis.

[0086] As used herein, the term "insertion" or "addition" refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, as compared to the naturally occurring molecule (e.g., the wild-type sequence). The insertion or addition of one or more amino acid residues can take place in-between internal amino acid residues. Alternatively, an insertion may occur at the N-terminus of the amino acid sequence. Alternatively, an insertion may occur at the C-terminus of the amino acid sequence.

[0087] The term "natural amino acid," "natural amino acid residue," "naturally occurring amino acid" or "naturally occurring amino acid residue" refers to naturally occurring amino acids which typically occur within the context of a wild-type polypeptide sequence. In other words, a "natural amino acid" has not been mutated or changed in any way to differ from the amino acid residue present in the parent sequence, e.g., naturally occurring sequence. A "natural amino acid" includes amino acids at any position within the polypeptide sequence (e.g., internal amino acid residues) and also any amino acid at the N-terminus or the C-terminus of the polypeptide sequence.

[0088] The term "acyl donor substrate" refers to a group with a terminal acyl group on it. Preferably, the "acyl donor substrate" comprises a glutamine residue. An acyl donor substrate may optionally contain a further reactive group. In a first embodiment, the acyl donor substrate is covalently connected to a functional agent. In a second embodiment, the acyl donor substrate is not connected to a functional agent. In one embodiment, the acyl donor substrate comprises a glutamine residue and a reactive group. In another embodiment, the acyl donor substrate comprises one or more linkers, as described further herein. In any of the above embodiments, there is optionally a linker between the acyl donor substrate and the functional agent or between the acyl donor substrate and the reactive group.

[0089] The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains. The term "antibody", as used herein, also refers to any antigen-binding portion, mutant, variant, or derivative of an immunoglobulin molecule, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art and nonlimiting embodiments of which are discussed herein. In one embodiment, the antibody is a humanized antibody. In another embodiment, the antibody is a human antibody. In another embodiment, the antibody is a chimeric antibody. In another embodiment, the antibody is a non-human antibody.

[0090] In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The

heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass.

[0091] The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a Fab' fragment, a Fab containing hinge region not linked by disulfide via either mild reduction or by mutating or deleting cysteines; (iii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iv) a Fd fragment consisting of the VH and CH1 domains; (v) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (vi) a dAb fragment (Ward et al., (1989) Nature 341:544-546, Winter et al., PCT publication WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vii) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al. (1993)

Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

[0092] "Acidic Amino Acid" refers to an amino acid exhibiting a negative charge at physiological pH. Genetically encoded acidic amino acids include aspartic acid (Asp; D) and glutamic acid (Glu; E).

[0093] "Non-Acidic Amino Acid" refers to an amino acid which is not an acidic amino acid. Non-acidic amino acids include arginine (Arg; R), histidine (His; H), lysine (Lys; K), serine (Ser; S); threonine (Thr; T), asparagine (Asn; N), glutamine (Gln; Q), cysteine (Cys; C), glycine (Gly; G), proline (Pro; P), alanine (Ala; A), valine (Val; V); isoleucine (Ile; I), leucine (Leu; L), methionine (Met; M), phenylalanine (Phe; F), tyrosine (Tyr; Y), and tryptophan (Trp; W).

[0094] "Basic Amino Acid" refers to an amino acid exhibiting a positive charge at physiological pH. Genetically encoded basic amino acids include histidine (His; H), lysine (Lys, K), and arginine (Arg; R).

[0095] As used herein, the term "biological sample" refers to a sample obtained from a subject, including sample of biological tissue or fluid origin obtained in vivo or in vitro. Such samples can be, but are not limited to, body fluid (e.g., blood, blood plasma, serum, milk, spinal fluid, ascites, or urine), organs, tissues, fractions, and cells isolated from mammals including, humans. Biological samples also may include sections of the biological sample including tissues (e.g., sectional portions of an organ or tissue). Biological samples may also include extracts from a biological sample, for example, an antigen from a biological fluid (e.g., blood or urine).

[0096] The term "click chemistry" refers to particular reactions for protein synthesis and/or conjugation which are high yield, highly-selective, reliable and clean. See, e.g., King et al., "Developments in the Field of Bioorthagonal Bond Forming Reactions - Past and Present Trends", Bioconjug. Chem., (2014) 25(5): 825-839; McKay et al., "Click Chemistry in Complex Mixtures: Bioorthagonal Bioconjugation", Chem. Biol., (2014) 21(9): 1075-1101.

[0097] The term "chimerized," "chimeric," "chimeric antibody" and like terms refer to an immunoglobulin comprising a heavy chain variable region and light chain variable region, i.e., antigen-binding region, from one source or species and at least a portion of a heavy chain constant region and light chain constant region derived from a different source or species. These portions may be joined together chemically by conventional techniques (e.g., synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques (e.g., DNA encoding the protein portions of the chimeric antibody may be expressed to produce a contiguous polypeptide chain). Other forms of

"chimeric immunoglobulins" encompassed by the present disclosure are those in which the class or subclass has been modified or changed from that of the original immunoglobulin (also referred to as "class-switched immunoglobulins"). Throughout the disclosure, chimeric immunoglobulins are designated "xi." Herein, "chimeric immunoglobulin" and like terms refer to the sequence of the immunoglobulin rather than the process used to generate the antibody.

[0098] As used herein, "functional agent" refers to an agent having therapeutic, diagnostic, or other functional property(ies). In one embodiment, a functional agent may be a therapeutic agent. In another embodiment, a functional agent may be a diagnostic agent. Functional agents may be large molecules or small molecules. Large molecule functional agents include, but are not limited to, an antibody and antigen-binding portions thereof. Small molecule functional agents include, but are not limited to, chemotherapeutic agents, cytotoxic agents, antibiotics, other organic compounds which may regulate biological process (e.g., drugs), and polypeptides.

[0099] The term "humanized," "humanized immunoglobulin" and like terms refer to immunoglobulins in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR of an immunoglobulin of different specificity as compared to that of the parent immunoglobulin. For the most part, humanized immunoglobulins are human immunoglobulins (recipient immunoglobulin) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor immunoglobulin) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized immunoglobulins may comprise residues that are not found in the recipient immunoglobulin or in the donor immunoglobulin. These modifications are made to further refine immunoglobulin performance. In general, the humanized immunoglobulin will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized immunoglobulin can optionally also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. See, e.g., Riechmann, L., et al., Nature 332 (1988) 323-327; and Neuberger, M. S., et al., Nature 314 (1985) 268-270. Herein, "humanized immunoglobulin" and like terms refer to the sequence of the immunoglobulin rather than the process used to generate the immunoglobulin.

[0100] The term "diagnostic agent" refers to a compound which may be useful for in vivo imaging studies such as CT, MRI and X-ray and/or in vitro imaging studies. Non-

limiting examples of diagnostic agents include a fluorophore, a fluorescent dye, a radionuclide, and an enzyme.

[0101] The term "donor immunoglobulin" refers to a non-human immunoglobulin that contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to the humanized immunoglobulin, and thereby provides the humanized immunoglobulin with the antigenic specificity and neutralizing activity characteristic of the donor immunoglobulin.

[0102] The term "recipient immunoglobulin" refers to an immunoglobulin heterologous to the donor immunoglobulin, which provides the amino acid sequences of its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the humanized immunoglobulin. The recipient immunoglobulin may be derived from any mammal. In preferred embodiments, the recipient immunoglobulin is non-immunogenic in humans. Preferably the recipient immunoglobulin is a human immunoglobulin.

[0103] "Humanizing" refers to a process of generating a humanized immunoglobulin and includes any process for generating humanized immunoglobulins having the above characteristics, including, but not limited to, in silico humanization, engineering species/host CDRs into human immunoglobulins, substituting framework region residues of a chimeric immunoglobulin to match a corresponding human framework region, etc.

[0104] "Immunoglobulin," as used herein, refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes including the kappa and lambda light chains and the alpha, gamma, delta, epsilon and mu heavy chains. Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH_2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH - terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). "Immunoglobulins" include: (a) immunoglobulin polypeptides, i.e., polypeptides of the immunoglobulin family that contain an antigen binding site that specifically binds to a specific antigen, including all immunoglobulin isotypes (IgG, IgA, IgE, IgM, IgD, and IgY), classes (e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, IgA_2), subclasses, and various monomeric and polymeric forms of each isotype, unless otherwise specified; and (b) conservatively substituted variants of such immunoglobulin polypeptides that immunospecifically bind to the antigen. Immunoglobulins are generally described in, for example, Harlow & Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988).

[0105] The term "microbial transglutaminase" refers to a type of transferase that catalyzes an acyl transfer reaction. A preferred embodiment comprises the use of a microbial transglutaminase to catalyze an acyl transfer reaction between a first moiety containing a glutamine residue (acyl donor) and a second moiety containing a primary amine group (acyl acceptor). It is preferable that the reactive glutamine residue is solvent exposed.

[0106] One form of immunoglobulin disclosed herein constitutes the basic structural unit of an antibody. For example, an antibody can include a tetramer and consist of two identical pairs of immunoglobulin chains, each pair having one light chain and one heavy chain. Generally, in each pair, the light chain and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions.

[0107] In addition to antibodies, immunoglobulins may exist in a variety of other forms including, for example: antigen-binding fragments or portions of a full-length immunoglobulin, such as Fv, Fab, $(Fab')_2$ and Fv fragments; and alternative antibody formats such as single chain immunoglobulins (scFV and scFab), diabodies, triabodies, tetrabodies, linear antibodies, and multispecific antibodies, to name a few. See, for example, James D. Marks, Antibody Engineering, Chapter 2, Oxford University Press (1995) (Carl K. Borrebaeck, Ed.).

[0108] In one embodiment, an immunoglobulin may comprise an Fab fragment. In another embodiment, an immunoglobulin may comprise a CH3 domain. In another embodiment, an immunoglobulin may comprise a heavy chain.

[0109] As used herein, the term "immunospecifically" refers to the ability of an immunoglobulin to specifically bind to an antigen against which the immunoglobulin was generated and not specifically bind to other peptides or proteins. An immunoglobulin that immunospecifically binds to an antigen against which the immunoglobulin was generated may not bind to other polypeptides or proteins, or may bind to other polypeptides or proteins with a lower binding affinity than the antigen against which the immunoglobulin was generated as determined by, for example, immunoassays, BIAcore, or other assays known in the art. An immunoglobulin binds immunospecifically to an antigen against which the immunoglobulin was generated when it binds to the antigen with a higher binding affinity than to any cross-reactive antigen as determined using experimental techniques, such as, but not limited to, radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISAs) (See, for example, Paul, ed., Fundamental Immunology, 2nd ed., Raven Press, New York, pages 332-336 (1989) for a discussion regarding antibody specificity.).

[0110] "Linker," as used herein, refers to a spacer, which may be a straight or branched chain, for connecting an immunoglobulin (through an acyl donor substrate) to a functional agent or a reactive group. Such linkers may be cleavable (e.g., acid labile or

protease cleavable) or non-cleavable. In one embodiment, a linker is a polyethylene glycol (PEG) moiety. In another embodiment, a linker comprises one or more amino acids and a polyethylene glycol moiety (PEG).

[0111] The term "monoclonal antibody" refers to an antibody that is derived from a single cell clone, including any eukaryotic or prokaryotic cell clone, or a phage clone, and not the method by which it is produced. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope. The term "monoclonal antibody" is not limited to antibodies produced through hybridoma technology.

[0112] "Native" refers to the wild type immunoglobulin sequence from the species in which the immunoglobulin is derived.

[0113] As used herein, "percent identity" and like terms is used to describe the sequence relationships between two or more nucleic acids, polynucleotides, proteins, or polypeptides, and is understood in the context of and in conjunction with the terms including: (a) reference sequence, (b) comparison window, (c) sequence identity and (d) percentage of sequence identity.

(a) A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, exemplary lengths of the reference polypeptide sequence include at least about 16 amino acids, at least about 20 amino acids, at least about 25 amino acids, at least about 35 amino acids, at least about 50 amino acids, or at least about 100 amino acids. For nucleic acids, exemplary length of the reference nucleic acid sequence include at least about 50 nucleotides, at least about 60 nucleotides, at least about 75 nucleotides, at least about 100 nucleotides, or at least about 300 nucleotides, or any integer thereabout or therebetween.

(b) A "comparison window" includes reference to a contiguous and specified segment of a polynucleotide or polypeptide sequence, wherein the polynucleotide or polypeptide sequence may be compared to a reference sequence and wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions, substitutions, or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions, substitutions, or deletions) for optimal alignment of the two sequences. Exemplary comparison windows can be at least 20 contiguous nucleotides or amino acids in length, and optionally may be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a misleadingly high similarity to a reference sequence due to inclusion of gaps in the polynucleotide or polypeptide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

(c) Methods of alignment of sequences for comparison are well known in the art.

Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math., 2: 482, 1981; by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol., 48: 443, 1970; by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA, 8: 2444, 1988; by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, Calif., GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 7 Science Dr., Madison, Wis., USA; the CLUSTAL program is well described by Higgins and Sharp, Gene, 73: 237-244, 1988; Corpet, et al., Nucleic Acids Research, 16:881-90, 1988; Huang, et al., Computer Applications in the Biosciences, 8:1-6, 1992; and Pearson, et al., Methods in Molecular Biology, 24:7-331, 1994. The BLAST family of programs which may be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, Current Protocols in Molecular Biology, Chapter 19, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York, 1995. New versions of the above programs or new programs altogether will undoubtedly become available in the future, and may be used with the present disclosure.

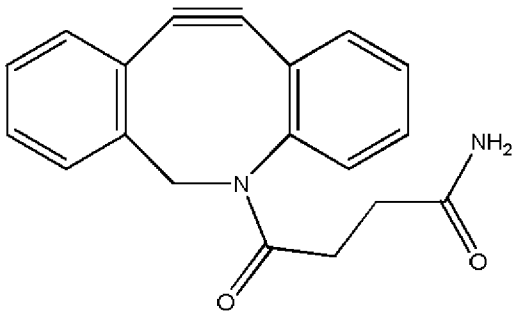
(d) "Percent identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions, substitutions, or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions, substitutions, or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0114] "Pharmaceutically effective amount" refers to an amount of an immunoglobulin that treats a subject.

[0115] "Pharmaceutically acceptable carrier" refers to components of a pharmaceutical formulation for an immunoglobulin as described herein for administration to a subject. For example, a pharmaceutically acceptable carrier may be a liposome-based, lipid-based and/or nano-particle-based.

[0116] The term "reactive group" as used here in refers to a chemical functional group which may react to other compounds, such as functional agents, to form at least one covalent bond. In one embodiment, reactive groups are reactive in click chemistry coupling reactions. Non-limiting examples of reactive groups include (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN),

[Chem.3]



(dibenzocyclooctyne; DBCO), trans-cyclooctene (TCO), azido (N_3), alkyne, tetrazine methylcyclopropene, norbornene, hydrazide/hydrazine, and aldehyde.

[0117] The term "subject" as used herein refers to a human or non-human organism. Thus, the methods, immunoglobulins, and conjugated immunoglobulins described herein are applicable to both human and veterinary diseases and conditions. Subjects can be "patients," i.e., living humans or non-human organisms that are receiving medical care for a disease or condition, or humans or non-human organisms with no defined illness who are being investigated for signs of pathology or presence/absence of a particular condition.

[0118] "Substituting" refers to the replacement of one amino acid residue for another. "Substituting" includes, for example, missense mutations in one or more DNA base pairs encoding the amino acid residue or engineering the protein to exchange one amino acid with another.

[0119] As used herein, "treating" and like terms refer to reducing the severity and/or frequency of disease symptoms, eliminating disease symptoms and/or the underlying cause of said symptoms, reducing the frequency or likelihood of disease symptoms and/or their underlying cause, and improving or remediating damage caused, directly or indirectly, by disease.

[0120] The term "therapeutic agent" means a large or small molecule which may be administered to a subject in need thereof to treat a condition. Therapeutic agents may be administered to treat, or prevent the onset, slow the progression, or to ameliorate one or more symptoms of a medical condition in subjects suffering from the same. Therapeutic agents include, but are not limited to, an antibody or antigen-binding portion thereof, a chemotherapeutic agent, a radioactive agent, a cytotoxic agent, an antibiotic, etc. In one embodiment, the therapeutic agent is a small molecule. In

another embodiment, the therapeutic agent is a polypeptide.

[0121] As used herein "90% identical to" encompasses at least 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to the reference item (e.g., a biological sequence).

[0122] The following abbreviations are used throughout the disclosure: antibody drug conjugates (ADCs); drug-to-antibody ratio (DAR); frame work region (FR); complementary determining region (CDR); auristatin F (AuF); variable heavy region (VH); variable light region (VL); variable kappa (Vκ); gamma constant region (Cγ); kappa constant region (Cκ); monoclonal antibody (mAb); lysine at amino acid position 447 of the heavy chain of the immunoglobulin, as numbered using the EU numbering system (Lys447).

Generation of conjugated immunoglobulins

[0123] Disclosed herein are methods for generating a conjugated immunoglobulin, the methods comprising: contacting an immunoglobulin, or an antigen-binding portion thereof, with a microbial transglutaminase and a functional agent comprising an acyl donor substrate, a) wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, b) wherein the acyl donor substrate comprises a glutamine residue, and c) wherein the functional agent is a therapeutic agent or a diagnostic agent, wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate on the functional agent, thereby generating the conjugated immunoglobulin.

[0124] Also disclosed herein are methods for generating a conjugated immunoglobulin, the methods comprising: i) contacting an immunoglobulin, or antigen-binding portion thereof, with a microbial transglutaminase and an acyl donor substrate, a) wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, b) wherein the acyl donor substrate comprises a glutamine residue and a reactive group, wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate, and ii) conjugating a functional agent to the reactive group of the acyl donor substrate, wherein the functional agent is a therapeutic agent or a diagnostic agent, thereby generating the conjugated immunoglobulin.

[0125] Conjugation can be performed by dissolving a functional agent comprising an acyl

donor substrate in a dissolution solution and contacting the dissolved functional agent with the immunoglobulin, or antigen-binding portion thereof, and microbial transglutaminase in a conjugation buffer. Conjugation may also be performed by dissolving a acyl donor substrate in a dissolution solution and contacting the acyl donor substrate with the immunoglobulin, or antigen-binding portion thereof, and microbial transglutaminase in a conjugation buffer.

[0126] For aqueous-insoluble functional agents and acyl donor substrates, suitable dissolution solutions include organic, water-miscible solvents such as dimethylsulfoxide (DMSO). For aqueous-soluble functional agents and acyl donor substrates, suitable dissolution solutions include, but are not limited to, water or buffered aqueous solutions, such as phosphate-buffered saline, pH 7.2 (1 x PBS) or DPBS.

[0127] Suitable concentrations of the functional agent or the acyl donor substrate include from about 10 μM to about 800 mM, from about 10 mM to about 100 mM, from about 25 mM to about 100 mM, from about 40 mM to about 100 mM, from about 55 mM to about 100 mM, from about 70 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 75 mM, from about 10 mM to about 60 mM, from about 10 mM to about 50 mM, from about 10 mM to about 40 mM, or from about 10 mM to about 30 mM.

[0128] In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 10 μM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 25 μM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 50 μM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 100 μM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 250 μM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 500 μM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 750 μM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 1 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 10 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 20 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 30 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 40 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 50 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 60 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be

about 70 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 80 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 90 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 100 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 150 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 200 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 250 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 300 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 350 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 400 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 450 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 500 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 550 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 600 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 650 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 700 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 750 mM. In some embodiments, the concentration of the functional agent or the acyl donor substrate can be about 800 mM.

[0129] Suitable concentrations of immunoglobulin include from about 0.1 mg/ml to about 100 mg/ml, from about 0.5 mg/ml to about 20 mg/ml, from about 1 mg/ml to about 20 mg/ml, from about 5 mg/ml to about 20 mg/ml, from about 10 mg/ml to about 20 mg/ml, from about 0.1 mg/ml to about 15 mg/ml, from about 0.1 mg/ml to about 12 mg/ml, from about 0.1 mg/ml to about 10 mg/ml, from about 0.1 mg/ml to about 5 mg/ml, or from about 0.1 mg/ml to about 2 mg/ml, from about 10 mg/ml to about 30 mg/ml, from about 20 mg/ml to about 45 mg/ml, from about 35 mg/ml to about 50 mg/ml, from about 45 mg/ml to about 60 mg/ml, from about 50 mg/ml to about 75 mg/ml, from about 60 mg/ml to about 85 mg/ml or from about 80 mg/ml to about 100 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 0.1 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 0.5 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 1 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 2 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 5 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 10 mg/ml. In

some embodiments, the concentration of immunoglobulin can be about 15 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 20 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 25 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 30 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 35 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 40 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 45 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 50 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 55 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 60 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 65 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 70 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 75 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 80 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 85 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 90 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 95 mg/ml. In some embodiments, the concentration of immunoglobulin can be about 100 mg/ml.

[0130] Suitable ratios of a functional agent or an acyl donor substrate:immunoglobulin include from about 1:1 to 100:1. In one embodiment, the ratio of functional agent to acyl donor substrate:immunoglobulin is about 25:1 to about 75:1. In another embodiment, the ratio of functional agent to acyl donor substrate:immunoglobulin is about 40:1 to about 60:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 1:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 2:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 3:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 4:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 5:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 6:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 7:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 8:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 9:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 10:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 11:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 12:1.

In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 13:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 14:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 15:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 16:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 17:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 18:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 19:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 20:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 25:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 30:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 35:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 40:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 45:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 50:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 60:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 70:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 80:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 90:1. In some embodiments, the ratio of a functional agent or an acyl donor substrate:immunoglobulin can be 100:1.

[0131] The contacting can be performed in a number of suitable conjugation buffers including, for example, DPBS, 1xPBS, pH 7.2, sodium phosphate, potassium phosphate, sodium borate, Tris, and HEPES, to name a few. The concentration of conjugation buffer include from about 5 mM to about 2 M, from about 5 mM to about 1 M, from about 5 mM to about 500 mM, from about 5 mM to about 100 mM, from about 10 mM to about 100 mM, from about 20 mM to about 100 mM, from about 30 mM to about 100 mM, from about 45 mM to about 100 mM, from about 60 mM to about 100 mM, from about 75 mM to about 100 mM, from about 10 mM to about 90 mM, from about 10 mM to about 75 mM, from about 10 mM to about 60 mM, from about 10 mM to about 45 mM, or from about 10 mM to about 30 mM. In some embodiments, the concentration of the conjugation buffer can be about 10 mM. In some embodiments, the concentration of the conjugation buffer can be about 20 mM. In

some embodiments, the concentration of the conjugation buffer can be about 30 mM. In some embodiments, the concentration of the conjugation buffer can be about 40 mM. In some embodiments, the concentration of the conjugation buffer can be about 50 mM. In some embodiments, the concentration of the conjugation buffer can be about 60 mM. In some embodiments, the concentration of the conjugation buffer can be about 70 mM. In some embodiments, the concentration of the conjugation buffer can be about 80 mM. In some embodiments, the concentration of the conjugation buffer can be about 90 mM. In some embodiments, the concentration of the conjugation buffer can be about 100 mM. In some embodiments, the concentration of the conjugation buffer can be about 250 mM. In some embodiments, the concentration of the conjugation buffer can be about 500 mM. In some embodiments, the concentration of the conjugation buffer can be about 750 mM. In some embodiments, the concentration of the conjugation buffer can be about 1 M. In some embodiments, the concentration of the conjugation buffer can be about 1.25 M. In some embodiments, the concentration of the conjugation buffer can be about 1.5 M. In some embodiments, the concentration of the conjugation buffer can be about 1.75 M. In some embodiments, the concentration of the conjugation buffer can be about 2 M.

[0132] The conjugation buffer can further include sodium chloride. Suitable concentrations of sodium chloride include from about 0 mM to about 2 M, from about 0 mM to about 1 M, from about 1 M to about 2 M, from about 500 mM to about 1.5 M, from about 25 mM to about 500 mM, from about 50 mM to about 500 mM, from about 75 mM to about 500 mM, from about 100 mM to about 500 mM, from about 150 mM to about 500 mM, from about 200 mM to about 500 mM, from about 250 mM to about 500 mM, from about 300 mM to about 500 mM, from about 350 mM to about 500 mM, from about 400 mM to about 500 mM, from about 0 mM to about 400 mM, from about 0 mM to about 350 mM, from about 0 mM to about 300 mM, from about 0 mM to about 250 mM, from about 0 mM to about 200 mM, from about 0 mM to about 150 mM, from about 0 mM to about 100 mM, from about 0 mM to about 50 mM, or from about 0 mM to about 25 mM. In some embodiments, the concentration of sodium chloride can be about 25 mM. In some embodiments, the concentration of sodium chloride can be about 50 mM. In some embodiments, the concentration of sodium chloride can be about 75 mM. In some embodiments, the concentration of sodium chloride can be about 100 mM. In some embodiments, the concentration of sodium chloride can be about 150 mM. In some embodiments, the concentration of sodium chloride can be about 200 mM. In some embodiments, the concentration of sodium chloride can be about 250 mM. In some embodiments, the concentration of sodium chloride can be about 300 mM. In some embodiments, the concentration of sodium chloride can be about 350 mM. In some embodiments, the concentration of sodium

chloride can be about 400 mM. In some embodiments, the concentration of sodium chloride can be about 500 mM. In some embodiments, the concentration of sodium chloride can be about 750 mM. In some embodiments, the concentration of sodium chloride can be about 1 M. In some embodiments, the concentration of sodium chloride can be about 1.25 M. In some embodiments, the concentration of sodium chloride can be about 1.5 M. In some embodiments, the concentration of sodium chloride can be about 1.75 M. In some embodiments, the concentration of sodium chloride can be about 2 M.

[0133] The pH of the conjugation buffer can be from about 4 to about 9. In some embodiments, the pH of the conjugation buffer can be about 5 to about 8. In another embodiment, the pH of the conjugation buffer can be about 6 to about 7. In some embodiments, the pH of the conjugation buffer can be about 4. In some embodiments, the pH of the conjugation buffer can be about 4.5. In some embodiments, the pH of the conjugation buffer can be about 5. In some embodiments, the pH of the conjugation buffer can be about 5.5. In some embodiments, the pH of the conjugation buffer can be about 6.0. In some embodiments, the pH of the conjugation buffer can be about 6.5. In some embodiments, the pH of the conjugation buffer can be about 6.6. In some embodiments, the pH of the conjugation buffer can be about 6.7. In some embodiments, the pH of the conjugation buffer can be about 6.8. In some embodiments, the pH of the conjugation buffer can be about 6.9. In some embodiments, the pH of the conjugation buffer can be about 7.0. In some embodiments, the pH of the conjugation buffer can be about 7.1. In some embodiments, the pH of the conjugation buffer can be about 7.2. In some embodiments, the pH of the conjugation buffer can be about 7.3. In some embodiments, the pH of the conjugation buffer can be about 7.4. In some embodiments, the pH of the conjugation buffer can be about 7.5. In some embodiments, the pH of the conjugation buffer can be about 7.6. In some embodiments, the pH of the conjugation buffer can be about 7.7. In some embodiments, the pH of the conjugation buffer can be about 7.8. In some embodiments, the pH of the conjugation buffer can be about 7.9. In some embodiments, the pH of the conjugation buffer can be about 8.0. In some embodiments, the pH of the conjugation buffer can be about 8.1. In some embodiments, the pH of the conjugation buffer can be about 8.2. In some embodiments, the pH of the conjugation buffer can be about 8.3. In some embodiments, the pH of the conjugation buffer can be about 8.4. In some embodiments, the pH of the conjugation buffer can be about 8.5. In some embodiments, the pH of the conjugation buffer can be about 9.

[0134] To facilitate solubility of a functional agent or an acyl donor substrate in the conjugation buffer, a final concentration of organic, water-miscible solvent in the conjugation buffer may be from about 0% to about 20%, from about 2% to about 20%, from about 5% to about 20%, from about 8% to about 20%, from about 11% to about

20%, from about 16% to about 20%, from about 0% to about 18%, from about 0% to about 15%, from about 0% to about 12%, from about 0% to about 10%, from about 0% to about 8%, from about 0% to about 6%, or from about 0% to about 2%.

[0135] The conjugation buffer can further comprise propylene glycol to facilitate solubility of the thiol-reactive compound in the conjugation buffer. Suitable concentrations of propylene glycol include from about 1% to about 50%, from about 20% to about 50%, from about 30% to about 50%, from about 40% to about 50%, from about 10% to about 40%, from about 10% to about 30%, or from about 10% to about 20%. In some embodiments, the concentration of propylene glycol can be about 1% or about 5%. In some embodiments, the concentration of propylene glycol can be about 10%. In some embodiments, the concentration of propylene glycol can be about 20%. In some embodiments, the concentration of propylene glycol can be about 30%. In some embodiments, the concentration of propylene glycol can be about 40%. In some embodiments, the concentration of propylene glycol can be about 50%.

[0136] The conjugation buffer can further comprise a non-ionic detergent to facilitate solubility of the conjugated immunoglobulin in the conjugation buffer. Exemplary non-ionic detergents include, but are not limited to, polysorbate-20 or polysorbate-80. Suitable concentrations of non-ionic detergent include from about 0% to about 1%, from about 0.1% to about 1%, from about 0.3% to about 1%, from about 0.5% to about 1%, from about 0.7% to about 1%, from about 0% to about 0.8%, from about 0% to about 0.6%, from about 0% to about 0.4%, or from about 0% to about 0.2%. In some embodiments, the concentration of non-ionic detergent can be about 0.1%. In some embodiments, the concentration of non-ionic detergent can be about 0.2%. In some embodiments, the concentration of non-ionic detergent can be about 0.3%. In some embodiments, the concentration of non-ionic detergent can be about 0.4%. In some embodiments, the concentration of non-ionic detergent can be about 0.5%. In some embodiments, the concentration of non-ionic detergent can be about 0.6%. In some embodiments, the concentration of non-ionic detergent can be about 0.7%. In some embodiments, the concentration of non-ionic detergent can be about 0.8%. In some embodiments, the concentration of non-ionic detergent can be about 0.9%. In some embodiments, the concentration of non-ionic detergent can be about 1.0%.

[0137] The contacting can be performed for about 30 minutes to about 48 hours, for about 1 hour to about 48 hours, for about 2 hours to about 24 hours, for about 24 hours to about 48 hours, for about 30 hours to about 48 hours, for about 36 hours to about 48 hours, for about 42 hours to about 48 hours, for about 2 hours to about 42 hours, for about 2 hours to about 36 hours, for about 2 hours to about 30 hours, for about 2 hours to about 24 hours, for about 2 hours to about 18 hours, for about 2 hours to about 12 hours, about 30 minutes to about 1 hour, about 30 minutes to about 2 hours, or for

about 2 hours to about 6 hours. In some embodiments, the contacting can be performed for about 30 minutes. In some embodiments, the contacting can be performed for about 1 hour. In some embodiments, the contacting can be performed for about 1.5 hours. In some embodiments, the contacting can be performed for 2 hours. In some embodiments, the contacting can be performed for 6 hours. In some embodiments, the contacting can be performed for 12 hours. In some embodiments, the contacting can be performed for 18 hours. In some embodiments, the contacting can be performed for 24 hours. In some embodiments, the contacting can be performed for 30 hours. In some embodiments, the contacting can be performed for 36 hours. In some embodiments, the contacting can be performed for 42 hours. In some embodiments, the contacting can be performed for 48 hours.

[0138] The temperature of the contacting can be from about 4°C to about 50°C, from about 18°C to about 37°C, from about 20°C to about 37°C, from about 22°C to about 37°C, from about 24°C to about 37°C, from about 26°C to about 37°C, from about 28°C to about 37°C, from about 30°C to about 37°C, from about 32°C to about 37°C, from about 34°C to about 37°C, from about 18°C to about 34°C, from about 18°C to about 32 °C, from about 18°C to about 30°C, from about 18°C to about 28°C, from about 18°C to about 26°C, or from about 18°C to about 24°C. In some embodiments, the contacting can be performed at 4°C. In some embodiments, the contacting can be performed at 18° C. In some embodiments, the contacting can be performed at 20°C. In some embodiments, the contacting can be performed at 22°C. In some embodiments, the contacting can be performed at 24°C. In some embodiments, the contacting can be performed at 26°C. In some embodiments, the contacting can be performed at 28°C. In some embodiments, the contacting can be performed at 30°C. In some embodiments, the contacting can be performed at 32°C. In some embodiments, the contacting can be performed at 34°C. In some embodiments, the contacting can be performed at 37°C. In some embodiments, the contacting can be performed at 50°C.

[0139] Unincorporated functional agent or acyl donor substrate can be separated from the conjugated immunoglobulin by desalting chromatography using a number of suitable resins including, but not limited to, G-25 resin, G-50 resin, Biogel P10, or other resins with exclusion limits of ranges 5,000-10,000 Da. Chromatography can be performed in column format or spin-column format, depending on scale. Suitable buffers for desalting include, for example, DPBS, 1xPBS, sodium phosphate, potassium phosphate, sodium borate, Tris, or HEPES-based buffers may substitute for 1x PBS.

[0140] In a first embodiment, the functional agent comprising an acyl donor substrate which comprises a glutamine residue conjugated to the engineered lysine residue via the acyl donor substrate. In this first embodiment, the functional agent is combined with the acyl donor substrate prior to conjugation with the immunoglobulin by reacting the

reactive group on the acyl donor substrate with the functional agent. In a second embodiment, the acyl donor substrate comprising a glutamine residue and a reactive group is first conjugated to the immunoglobulin, and then the reactive group is joined to a functional agent.

[0141] The acyl donor substrates can comprise a linker, "L". Linkers can be non-cleavable linkers or cleavable linkers. Exemplary linkers include, for example, disulfide containing linkers, acetal-based linkers, and ketal-based linkers. In some aspects, the linker can be a non-cleavable linker. Suitable non-cleavable linkers include, but are not limited to, one or more amino acid, polyethylene glycol (PEG) or an alkyl. In some embodiments, the linker can comprise PEG. In some aspects, the linker can be a cleavable linker. Suitable cleavable linkers include, for example, valine-citrulline-para aminobenzyl. In some aspects, the linker can be a disulfide containing linker. In some aspects, the linker can be an acetal-based linker. In some aspects, the linker can be a ketal-based linker. A linker may also be one or more amino acids, alone or in combination with another linker such as one or more PEG groups.

[0142] The acyl donor substrate comprising a glutamine residue can be present in, part of, or attached to, a functional agent. Suitable functional agents include, for example, fluorophores, fluorescent dyes, polypeptides, immunoglobulins, antibiotics, nucleic acids, radionuclides, chemical linkers, small molecules, chelators, lipids, nucleic acids (such as DNA or RNA) and drugs. In some aspects, the functional agent can comprise a fluorophore. In some aspects, the functional agent can comprise a fluorescent dye. In some aspects, the functional agent can comprise a polypeptide. In some aspects, the functional agent can comprise an immunoglobulin. In some aspects, the functional agent can comprise an antibiotic. In some aspects, the functional agent can comprise a nucleic acid (such as DNA or RNA). In some aspects, the functional agent can comprise a radionuclide. In some aspects, the functional agent can comprise a small molecule. In some aspects, the functional agent can comprise a chelator (for example, DOTA, CHX-A"-DTPA, NOTA, among others). In some aspects, the functional agent can comprise a lipid. In some aspects, the functional agent can comprise a drug. In some aspects, the functional agent can comprise a combination of any of the above listed functional agents.

[0143] The acyl donor substrate (i.e., a first acyl donor substrate) can be bound to a second acyl donor substrate or linker, the second acyl donor substrate or linker being bound to a second immunoglobulin having a second heavy chain variable region and a second light chain variable region, the second heavy chain variable region having an engineered lysine residue. For example, the first acyl donor substrate and the second acyl donor substrate can have a first and second chemical linker as the first and second functional agents, respectively. The first and second chemical linkers can be bound to

each other by a number of suitable means including, for example, by click chemistry.

[0144] In one embodiment, the functional agent comprising an acyl donor substrate is according to one of formulae (I) or (II):

$(Z)_m$-Gln-$(L)_n$-(Y) (I)

(Y)-$(L)_n$-Gln-$(Z)_m$ (II)

wherein Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue; Gln is a glutamine amino acid residue; each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue; m is an integer from 0 to 5; n is an integer from 0 to 5; and Y is a functional agent.

[0145] In another embodiment, the acyl donor substrate is according to one of formulae (III) or (IV):

$(Z)_m$-Gln-$(L)_n$-(X) (III)

(X)-$(L)_n$-Gln-$(Z)_m$ (IV)

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue; Gln is a glutamine amino acid residue; each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue; m is an integer from 0 to 5; n is an integer from 0 to 5; and X is a reactive group.

[0146] In one embodiment, Z is a CBZ group. In another embodiment, Z is an amino acid residue.

[0147] In one embodiment, L is an amino acid residue. In one embodiment, n is 2-5, and each L is independently an amino acid residue. In another embodiment, L is a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted. In another embodiment, L is a polyethylene glycol (PEG) moiety. In another embodiment, n is 2-5, and one or more L comprises one or more amino acids and one or more additional L groups comprises a polyethylene glycol moiety (PEG).

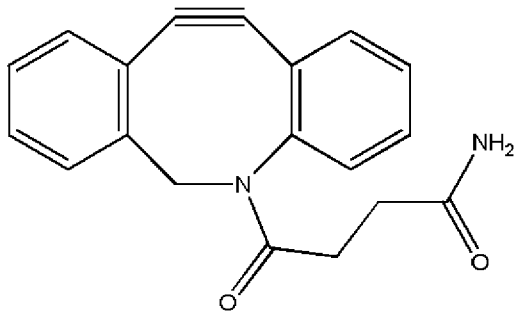
[0148] In one embodiment, m is 0. In another embodiment, m is 1. In another embodiment, m is 2. In another embodiment, m is 3. In another embodiment, m is 4. In another embodiment, m is 5.

[0149] In one embodiment, n is 0. In another embodiment, n is 1. In another embodiment, n

is 2. In another embodiment, n is 3. In another embodiment, n is 4. In another embodiment, n is 5.

[0150] In one embodiment, X is (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN). In another embodiment, X is

[Chem.4]



(dibenzocyclooctyne; DBCO). In another embodiment, X is trans-cyclooctene (TCO). In another embodiment, X is azido (N_3). In another embodiment, X is alkyne. In another embodiment, X is tetrazine methylcyclopropene. In another embodiment, X is norbornene. In another embodiment, X is hydrazide/hydrazine. In another embodiment, X is aldehyde.

[0151] In one embodiment, for an acyl donor substrate according to formula (I), Z is a CBZ group; L is a polyethylene glycol moiety (PEG) (-O((CH_2)$_2$)-), ethyl amine (-NH((CH_2)$_2$)-) or propyl amine (-NH((CH_2)$_3$)-); and n is 0, 1, 2 or 3.

[0152] In another embodiment, the acyl donor substrate is according to formula (I), wherein Z is a CBZ group, and L is an amino acid. In one embodiment, L is Gly. In one aspect of this embodiment, m is 1, and n is 1.

[0153] In one embodiment, the acyl donor substrate is according to formula (II), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is a Gly.

[0154] In another embodiment, the functional agent Y is auristatin F.

[0155] In one embodiment, for an acyl donor substrate according to formula (III), Z is a CBZ group; L is a polyethylene glycol moiety (PEG) (-O((CH_2)$_2$)-), ethyl amine (-NH((CH_2)$_2$)-) or propyl amine (-NH((CH_2)$_3$)-); and n is 0, 1, 2 or 3.

[0156] In another embodiment, the acyl donor substrate is according to formula (III), wherein Z is a CBZ group, and L is an amino acid. In one embodiment, L is Gly. In one aspect of this embodiment, m is 1, and n is 1.

[0157] In one embodiment, the acyl donor substrate is according to formula (IV), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is a Gly. In another embodiment, the functional agent Y is auristatin F.

[0158] The disclosed methods can be performed on a humanized immunoglobulin, or antigen-binding portion thereof. Thus, in some embodiments, the immunoglobulin, or antigen-binding portion thereof can be a humanized immunoglobulin, or antigen-

binding portion thereof.

[0159] The disclosed methods can be performed on a human immunoglobulin, or antigen-binding portion thereof. Thus, in some embodiments, the immunoglobulin, or antigen-binding portion thereof can be a human immunoglobulin, or antigen-binding portion thereof. In another embodiment, the immunoglobulin, or antigen-binding portion thereof can be a non-human immunoglobulin, or antigen-binding portion thereof.

[0160] In one embodiment, the disclosed methods can be performed on an IgG_1, IgG_2, IgG_3 or IgG_4 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgG_1 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgG_2 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgG_3 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgG_4 immunoglobulin, or antigen-binding portion thereof.

[0161] In one embodiment, the disclosed methods can be performed on an IgA_1, IgA_2, or IgM immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgA_1 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgA_2 immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgM immunoglobulin, or antigen-binding portion thereof. In one embodiment, the IgA or IgM immunoglobulin, or antigen-binding portion thereof has a tail piece. In another embodiment, the IgA or IgM immunoglobulin, or antigen-binding portion thereof has the tail piece removed.

[0162] In one embodiment, the method is performed on an IgD or IgE immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgD immunoglobulin, or antigen-binding portion thereof. In one embodiment, the method is performed on an IgE immunoglobulin, or antigen-binding portion thereof.

[0163] For the methods described herein, in one embodiment, the microbial transglutaminase is from Actinomadura sp. T-2, Bacillus circulans BL32, Bacillus subtilis spores, Corynebacterium ammoniagenes, Corynebacterium glutamicum, Enterobacter sp. C2361, Providencia sp. C1112, Streptoverticillium mobaraense (aka Streptomyces mobaraensis), Streptomyces platensis M5218, Streptomyces hygroscopicus, Streptomyces lividans, Streptomyces lividans JT46/pAE053, Streptomyces lydicus, Streptomyces platensis, Streptomyces sioyansis, Streptoverticillium griseocarneum, Streptoverticillium ladakanum NRRL-3191, Streptoverticillium sp. s-8112, or Streptococcus suis. In one embodiment, the microbial transglutaminase is from Streptomyces mobaraensis.

[0164] For the methods described herein, in one embodiment, the transglutaminase is isolated from a plant selected from the group consisting of Medicago sativa, Beta

vulgaris, Helianthus tuberosus, Zea mays, Glycine max, Arabidopsis thaliana, Nicotiana tabacum, Chlamydomonas reinhardtii, Dunaliella salina, Oryza sativa, and Rosmarinus officinalis L.

[0165] For the methods described herein, in one embodiment, the transglutaminase is mammalian and is isolated from Transglutaminase 1 thru 7 and Factor XIII.

[0166] In one embodiment, the transglutaminase is at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a microbial transglutaminase described herein. In one embodiment, the transglutaminase is at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the microbial transglutaminase is from Streptomyces mobaraensis. Transglutaminase enzymes can be purchased from Ajinomoto(R) or Zedira (Product number T001). In another embodiment, the transglutaminase is purified. In another embodiment, the transglutaminase is recombinantly expressed and subsequently purified using methods known to one of ordinary skill in the art.

[0167] In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 0.1 units/mL to about 250 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 1 unit/mL to about 25 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 1 unit/mL to about 25 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 0.1 unit/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 0.5 unit/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 1 unit/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 5 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 10 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 15 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 20 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 25 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 50 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 75 units/mL. In one embodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 100 units/mL. In one em-

bodiment, the transglutaminase enzyme is present in the methods described herein in a concentration of about 150 units/mL, 200 units/mL, or 250 units/mL.

[0168] For the methods provided herein, in one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 200:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 100:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 25:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 20:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 15:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 10:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 9:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 8:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 7:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 6:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 5:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 4:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 3:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 2:1. In one embodiment, the ratio of functional agent to immunoglobulin is about 1:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 10:1 to about 100:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 50:1 to about 200:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 50:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 100:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 1:1 to about 150:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 50:1 to about 100:1. In one embodiment, the ratio of functional agent to immunoglobulin is from about 100:1 to about 200:1.

[0169] In one embodiment, the ratio of functional agent to immunoglobulin is about 20:1. In one embodiment, the ratio of functional agent to immunoglobulin is known and is consistently reproducible by following the methods disclosed herein. In some embodiments, the ratio of a functional agent:immunoglobulin is about 1:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 2:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 3:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 4:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 5:1. In some em-

bodiments, the ratio of a functional agent:immunoglobulin is about 6:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 7:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 8:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 9:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 10:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 11:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 12:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 13:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 14:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 15:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 16:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 17:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 18:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 19:1. In some embodiments, the ratio of a functional agent:immunoglobulin is about 20:1. The ratio of functional agent to immunoglobulin, as used herein, is calculated based on an average of the conjugation ratio of the functional agent to an immunoglobulin in a pool of antibodies in a composition.

[0170] In embodiments of the present invention, acyl acceptor sites are engineered in a immunoglobulin, or antigen-binding portion thereof, by either insertion of a lysine between two natural amino acid residues, or by a the substitution of a natural amino acid with lysine substitution. Engineered lysine residues may be located at one or more positions throughout the sequence of an immunoglobulin. The optimal context for an acyl acceptor site is not only position-dependent, but also requires that the amino acid residue adjacent to and immediately before the engineered lysine not be an acidic amino acid residue; and that the amino acid residue adjacent to and immediately after the engineered lysine not be an acidic amino acid residue or a proline residue. MTGase conjugation technology can utilize these engineered sites to conjugate a variety of acyl donor-containing functional agents for making antibody drug conjugates (ADCs), bispecific antibodies, immunotoxins, or other mAb-protein complexes. In another embodiment, combining multiple engineered acyl acceptor sites, the theoretical drug-to-antibody ratio (DAR) increases for each engineered acyl acceptor site. Increasing the DAR of an ADC results in delivery of more functional agents into a subject per monoclonal antibody, which allows for defined DARs, better product homogeneity, and lower patient dosing.

[0171] In embodiments provided herein, wherein at least two additional engineered lysine residues are present, the ratio of functional agent to immunoglobulin is increased based on the number of additional engineered lysine residues. For example, wherein two en-

gineered lysine residues are present, resulting in an antibody with four transamidation sites and a ratio of functional agent to immunoglobulin of about 2:1 to about 4:1. As another example, wherein three engineered lysine residues are present, resulting in an antibody with six transamidation sites and a ratio of functional agent to immunoglobulin of about 2:1 to about 6:1.

Conjugated immunoglobulins

[0172] Also disclosed herein are conjugated immunoglobulins comprising any of the immunoglobulins or antigen-binding portions thereof disclosed herein, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, and is conjugated to a functional agent comprising an acyl donor substrate, wherein the acyl donor substrate comprises a glutamine residue. Additional embodiments include conjugated immunoglobulins comprising any of the immunoglobulins or antigen-binding portions thereof disclosed herein, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue, and is conjugated to an acyl donor substrate, wherein the acyl donor substrate comprises a glutamine residue and a reactive group, wherein the reactive group can be reacted with a functional agent after the conjugation of the acyl donor substrate to the immunoglobulin, or antigen-binding portion thereof.

[0173] In one embodiment, the amino acid residues flanking the engineered lysine are also engineered, e.g., mutated to optimize the sequence for an engineered acyl acceptor lysine site on an immunoglobulin. For example, the amino acid residue adjacent to and immediately after the engineered lysine residue (e.g., amino acid position +1) may be mutated to any amino acid residue other than proline or an acidic amino acid residue. The amino acid residue adjacent to and immediately before the engineered lysine residue (e.g., amino acid position -1) may be mutated to any amino acid residue other than an acidic amino acid residue.

[0174] In one embodiment, the amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) may be mutated to lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is glycine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is lysine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is alanine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is valine. In one embodiment, the mutated

amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is leucine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is isoleucine. In one embodiment, the mutated amino acid residue adjacent to and immediately after to the engineered lysine (amino acid position +1) is methionine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is phenylalanine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is tyrosine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is tryptophan. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is serine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is threonine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is cysteine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is asparagine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is glutamine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is histidine. In one embodiment, the mutated amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is arginine. In one embodiment, wherein the amino acid residue adjacent to and immediately after the engineered lysine (amino acid position +1) is not proline, aspartic acid, or glutamic acid.

[0175] In one embodiment, the amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) may be mutated to lysine, arginine, histidine, serine, threonine, asparagine, glutamine, cysteine, glycine, proline, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is glycine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is lysine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is alanine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is valine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is leucine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is

isoleucine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is methionine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is phenylalanine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is tyrosine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is tryptophan. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is serine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is threonine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is cysteine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is asparagine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is glutamine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is histidine. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is proline. In one embodiment, the mutated amino acid residue adjacent to and immediately before the engineered lysine (amino acid position -1) is arginine. In one embodiment, wherein the amino acid residue adjacent to and immediately before the engineered lysine (amino acid position +1) is not aspartic acid or glutamic acid.

[0176] In some embodiments, the immunoglobulin can be humanized. In other embodiments, the immunoglobulin is human. In another embodiment, the immunoglobulin is chimeric.

[0177] The acyl donor substrate comprising a glutamine residue and a reactive group can also comprise a linker, "L". Likewise, the functional agents which contain an acyl donor substrate comprising a glutamine residue can have a linker between the functional agent and the acyl donor substrate portion of the molecule. Linkers can be non-cleavable linkers or cleavable linkers. Exemplary linkers include, for example, disulfide containing linkers, acetal-based linkers, and ketal-based linkers. In some aspects, the linker can be a non-cleavable linker. Suitable non-cleavable linkers include, but are not limited to, polyethylene glycol (PEG) or an alkyl. In some embodiments, the linker can comprise PEG. In some aspects, the linker can be a cleavable linker. Suitable cleavable linkers include, for example, valine-citrulline-para aminobenzyl. In some aspects, the linker can be a disulfide containing linker. In some

aspects, the linker can be an acetal-based linker. In some aspects, the linker can be a ketal-based linker.

[0178] The conjugated immunoglobulins of the invention comprise a functional agent. Suitable functional agents include, for example, a therapeutic agent or a diagnostic agent. Suitable functional agents include, for example, fluorophores, fluorescent dyes, polypeptides, immunoglobulins, antibiotics, nucleic acids, radionuclides, chemical linkers, small molecules, chelators, lipids, and drugs. In some aspects, the functional agent can comprise a fluorophore. In some aspects, the functional agent can comprise a fluorescent dye. In some aspects, the functional agent can comprise a polypeptide. In some aspects, the functional agent can comprise an immunoglobulin. In some aspects, the functional agent can comprise an antibiotic. In some aspects, the functional agent can comprise a nucleic acid (such as DNA or RNA). In some aspects, the functional agent can comprise a radionuclide. In some aspects, the functional agent can comprise a small molecule. In some aspects, the functional agent can comprise a chelator (for example, DOTA, CHX-A"-DTPA, NOTA, among others). In some aspects, the functional agent can comprise a lipid. In some aspects, the functional agent can comprise a drug. In some aspects, the functional agent can comprise a combination of any of the above listed functional agents.

[0179] Accordingly, the disclosed conjugated immunoglobulins include, but are not limited to, immunoglobulin-fluorophore conjugates, immunoglobulin-fluorescent dye conjugates, immunoglobulin-polypeptide conjugates, immunoglobulin-immunoglobulin conjugates, immunoglobulin-antibiotic conjugates, immunoglobulin-nucleic acid conjugates, immunoglobulin-radionuclide conjugates, immunoglobulin-chemical linker conjugates, immunoglobulin-small molecule conjugates, immunoglobulin-chelator conjugates, immunoglobulin-lipid conjugates, and immunoglobulin-drug conjugates.

[0180] Any of the immunoglobulins disclosed herein can be conjugated to any of the functional agents disclosed herein. For example, the conjugated immunoglobulin can comprise a fluorophore, fluorescent dye, polypeptide, immunoglobulin, antibiotic, nucleic acid, radionuclide, chemical linker, small molecule, chelator, lipid, or drug.

[0181] In some embodiments, the immunoglobulin can be conjugated to a small molecule antineoplastic agent, such as an auristatin. In some aspects, the functional agent can be auristatin F (AuF). Thus, the disclosed conjugated immunoglobulins include any of the above disclosed immunoglobulins conjugated to auristatin F (AuF-T135K conjugate).

Pharmaceutical compositions

[0182] Also provided herein are pharmaceutical compositions. In some embodiments, the pharmaceutical compositions can comprise any of the immunoglobulins disclosed herein. In some embodiments, the pharmaceutical compositions can comprise any of

the conjugated immunoglobulins disclosed herein. In one embodiment, the pharmaceutical composition comprises the conjugated immunoglobulin and a pharmaceutically acceptable carrier.

Nucleic acid molecules encoding conjugatable immunoglobulins and host cells comprising the same

[0183] Also provided herein are nucleic acid molecules encoding any of the conjugatable immunoglobulins or antigen-binding portions thereof disclosed herein. As an example, in one embodiment, the nucleic acid molecule encodes a conjugatable immunoglobulin comprising a heavy chain variable region and a light chain variable region and wherein the encoded conjugatable immunoglobulin comprises an engineered lysine residue, and wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue. The engineered lysine residue of the immunoglobulin or antigen-binding portions thereof, is conjugated to the glutamine residue of an acyl donor substrate, thereby generating the conjugated immunoglobulin.

[0184] Also disclosed are host cells comprising any of the disclosed nucleic acid molecules or plasmids that encode the conjugatable immunoglobulin or antigen-binding portions thereof. Suitable host cells include, but are not limited to, mammalian cells, bacterial cells, yeast cells, insect cells, to name a few.

[0185] The following examples are provided to further describe some of the embodiments disclosed herein. The examples are intended to illustrate, not to limit, the disclosed embodiments.

Examples

[0186]

Example 1

[0187] Materials & Methods

Site-Directed Mutagenesis

[0188] Mutations were generated using Stratagene's QuikChange XL according to the manufacturer's protocol. The desired mutations were confirmed by DNA sequencing.

Deletion Mutagenesis

[0189] HC fragments for Fab expression were made by PCR amplifying the HC leader sequence through the hinge that terminated at various 3' codons. The PCR fragments were cloned into a pcDNA3.1-based mammalian expression plasmid using an In-Fusion HD cloning kit according to the manufacturer's protocol (CLONTECH).

Transfection and stable cell line generation

[0190] For each milliliter of cells to be transfected with ExpiFectamine, 333.3 ng HC plasmid and 333.3 ng LC plasmid was contacted for 5 -10 min in 50 μL Opti-MEM

(ThermoFisher Scientific, Waltham, MA). Likewise, 2.67 μL ExpiFectamine was contacted in 50 μL Opti-MEM. The ExpiFectamine solution was added to the DNA mixture, and incubated for 20-30 min at room temperature. The DNA:ExpiFectamine mixture was added to the cells while swirling and incubated at 37°C, 8% CO_2, shaking at 125 rpm. The following day, 5 μL of enhancer 1 and 50 μL of enhancer 2 per mL of cells were added to the transfection with continued incubation for another 7-10 days.

[0191] Antibody-expressing stable pools were selected by adding 1 mL of transfectants to 14 mL DMEM in a T75 flask with 5 μg/mL blasticidin and 400 μg/mL zeocin (Invivogen, San Diego, CA) one to three days after transfection. After drug-resistant cells grew to confluency, the medium was replaced with FreeStyle 293 expression medium for 24 to 48 h. Cells were physically dislodged by tapping the flask (trypsinization resulted in low viability, data not shown) and were then seeded at $6x10^5$ cells/mL in 30 mL FreeStyle 293 expression medium in a 125-mL shake flask. Cultures were incubated at 37°C in 8% CO_2 with shaking at 125 rpm.

MAb and Fab production

[0192] Stably-transfected cell line pools were seeded at 0.6 to $1x10^6$ cells/mL in FreeStyle 293 expression medium. Cells were incubated at 37°C, 8% CO_2, shaking at 125 rpm. Two days after the culture reached a density of $1x10^6$ cells/mL, cultures were fed with final concentrations of 10 g/L Select Soytone (BD Biosciences, San Jose, CA), 5 mM valeric acid (Sigma Aldrich, St. Louis, MO), and 1:100 CD Lipid Concentrate (ThermoFisher Scientific, Waltham, MA). When the cell viability was less than 50% (7-10 days), the cultures were centrifuged for 1 h at 8000 rpm in a Beckman JLA8.1000 rotor. The supernatant was then filtered through a 0.2 μm PES filter and stored at 4°C or -20°C until purification.

MAb and Fab purification

[0193] MAbs were purified using one of two methods. For mAb and Fab supernatants less than 10 mL, affinity chromatography was performed using a batch purification method with protein A resin or anti-kappa resin, respectively. MAb and Fab supernatants greater than 25 mL were purified using pre-packed protein A or anti-kappa columns, respectively.

Batch purification

[0194] Prosep-vA High Capacity Protein A resin (Millipore, Billerica, MA) was equilibrated with DPBS, and 100 μL were added to 3 to 6 mL of sample. Following incubation at 4°C for 1 hour to overnight, the resin was washed three times with 1 mL DPBS and centrifuged at 18,000 x g for 30 s. The sample was eluted from the resin by addition of 400 μL 0.1 M Glycine, pH 2.9 followed by centrifugation at 18,000 x g for 30 s. The sample was neutralized with 40 μL of 1 M Tris, pH 8.0. The buffer was exchanged

using 0.5 mL Amicon Ultra, 10k cutoff filters (Millipore, Billerica, MA) by concentrating the sample to ~ 100 μL by centrifugation at 18,000 x g for 3 to 5 minutes. The concentrated sample was diluted in 400 μL DPBS, followed by centrifugation. The process was repeated a total of four times.

Column purification

[0195] A protein A or HiTrap KappaSelect column (GE Healthcare, Little Chalfont, UK) was equilibrated with 10 column volumes (CV) of 20 mM sodium phosphate, 10 mM EDTA, pH 7.2. The sample was then loaded, followed by washing unbound material with 10 CV of equilibration buffer. The sample was eluted using 5 CV of 0.1 M Glycine, pH 2.9. The fractions containing the mAb were pooled and dialyzed in DPBS using a MWCO 20K Slide-A-Lyzer (ThermoFisher Scientific, Waltham, MA).

Z-Gln-Gly substrate synthesis

[0196] Z-Gln-Gly was purchased from Bachem, and Z-Gln-Gly-CAD-biotin was purchased from ZEDIRA (FIG. 2).

Z-Gln-Gly-pentafluorophenyl ester (Z-Gln-Gly-PFP)

[0197] Synthesis was as described by Pasternack (Pasternack et al. 1997), with modifications (FIG. 3). Z-Gln-Gly (328.8 mg, 0.975 mmol) and pentafluorophenol (Sigma, 183.3 mg, 0.996 mmol) were dissolved in 10 mL N,N'-dimethylformamide (DMF). EDAC-HCl (Sigma, 201 mg, 1.04 mmol) was then added and the reaction was incubated at room temperature under N_2 for 2 hr. 100 mL of cold diethyl ether was added to the reaction and precipitated overnight at -80°C. The crude product was collected by centrifugation and re-crystallized from 20 mL 60°C methanol. The final product was rinsed with cold diethyl ether and dried over a stream of N_2. Final yield was 219.04 mg (44.7%). Electrospray ionization-mass spectrometry (ESI-MS) (direct infusion in 50% acetonitrile in 0.1% formic acid) m/z 504.0 ([M+H], 86%), 526.0 ([M+Na], 100%), 542.0 ([M+K], 22%).

Z-Gln-Gly-propyl azide (Z-Gln-Gly-N3)

[0198] Z-Gln-Gly-PFP (21.24 mg, 4.22 x 10^{-5} mol) and azidopropylamine (Click Chemistry Tools, 42.2 μL of a 0.91 M stock solution in DMF, 3.84 x 10^{-5} mol) were dissolved in 0.42 mL final volume of DMF. Reaction was stirred under N_2 overnight at room temperature. Product was purified by HPLC using a 0.1% formic acid in H_2O/0.1% formic acid in acetonitrile mobile phase. Product was dried in vacuo. Final yield was 10.7 mg (60.4%). ESI-MS (gradient purification) m/z 420.2 ([M+H], 100%), 442.1 ([M+Na], 32%).

Z-Gln-Gly-PEG2- bicyclononyne (Z-Gln-Gly-PEG2-BCN)

[0199] Z-Gln-Gly-PFP (18.4 mg, 3.66 x 10^{-5} mol) and N-[(1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-1,8-diamino-3,6-dioxaoct

ane (Sigma Aldrich) were dissolved in 0.37 mL final volume of DMF. Reaction was stirred under N_2 overnight at room temperature. Product was purified by HPLC using a 0.1% formic acid in H_2O/0.1% formic acid in acetonitrile mobile phase. Product was dried in vacuo. Final yield was 0.6 mg (2%). ESI-MS (gradient purification) m/z 688.2 ([M+H], 100%), 710.2 ([M+Na], 69%).

Z-Gln-Gly-PEG2-Auristatin F (Z-Gln-Gly-PEG2-AuF)

[0200] Z-Gln-Gly-PFP (22.2 mg, 4.37 x 10^{-5} mol) was dissolved in 0.85 mL DMF and 1,2-ethylenediamine (2.3 x 10^{-5} L, 3.5 x 10^{-4} mol) was added and mixed. Reaction was stirred under N_2 overnight at room temperature. Product was purified by HPLC using a 0.1% formic acid in H_2O/0.1% formic acid in acetonitrile mobile phase. Product was dried in vacuo. Final yield of Z-Gln-Gly-NH_2 was 3.8 mg (23%). ESI-MS (gradient purification) m/z 380.1 ([M+H], 100%). Z-Gln-Gly-NH_2 (3.8 mg, 1.01 x 10^{-5} mol) and NHS-PEG_2-AuF (10.3 mg, 1.03 x 10^{-5} mol) were dissolved in 0.2 mL DMF. Tri-ethylamine (14 μL, 1 x 10^{-4} mol) was added and reaction was incubated under N_2 overnight at room temperature. Half of the reaction was purified by HPLC using a 0.1% formic acid in H_2O/0.1% formic acid in acetonitrile mobile phase. Product was dried in vacuo. Final yield of CBZ-Gln-Gly-PEG_2-AuF was 3.8 mg (60%). ESI-MS (gradient purification) m/z 634.0 ($[M+H]^{2+}$,100%), 645.1($[M+Na]^{2+}$,45%). 1267.0 ([M+H], 16%).

Microbial transglutaminase reaction

[0201] MAbs ranging in concentrations from 100 μg/mL to 2.5 mg/mL were contacted with 785 μM Z-Gln-Gly-biotin (Zedira, Darmstadt, Germany), Z-Gln-Gly-N_3, Z-Gln-Gly-PEG_2-BCN, or Z-Gln-Gly-PEG_2-AuF with 1 U/mL microbial transglutaminase (Zedira, Darmstadt, Germany) in DPBS for at least 16 h at 37°C.

High-throughput mTGase assay

[0202] MAbs ranging in concentrations from 500 ng/mL to 10 μg/mL were incubated with 60 μM Z-Gln-Gly-biotin and 0.1 U/mL mTGase (Zedira) in DPBS for at least 16 h at 37°C. A 96-well plate was coated with 1 μg/mL goat-anti-human IgG Fcγ mAb (Jackson ImmunoResesearch) overnight at 4°C. After washing the plate, the overnight mTGase reactions were diluted 1:10 in 50 μL DPBS, added to the plate, and incubated for 1 h at 22°C. The plate was then washed, and 0.1 μg/mL of streptavidin-horseradish peroxidase (HRP) (Jackson ImmunoResesearch) was added to the wells. The plate was washed again, and streptavidin-HRP-bound biotinylated samples were quantitated in relative fluorescent units (RFUs) using QuantaBlue substrate (Thermo) according to the manufacturer's protocol.

Ultra-performance liquid chromatography (UPLC)/ESI-MS analysis of mAb conjugation

[0203] Purified antibodies were diluted to 1 mg/mL in DPBS (if below 1.0 mg/mL samples were left at original concentration). Reactions containing dimethylsulfoxide (DMSO) were desalted using a Zeba spin desalting column. The mAbs were then either deglycosylated using PNGase F (NEB) or digested into Fab'_2 and Fc fragments by IdeS (Promega). To deglycosylate the mAbs, G7 buffer (5 or 10 μL) and PNGase F (1 or 2 μL) were added to the mAb (50 or 100 μL). The reaction was incubated in a Discover microwave (CEM) for 2 cycles: 1.) microwave power 10 W, 37°C, 10 min, and then wait for 3-5 min; 2.) microwave power 2 W, 37°C, 10 min. A portion of the deglycosylated sample was reduced by adding dithiothreitol (DTT) to a final concentration of 20 mM, followed by incubation at 60°C for 3 min. To generate Fab'_2 and Fc fragments, 50U/μL of IdeS was added to 0.5 mg/mL of mAb and incubated at 37°C for 0.5-1 h. The IdeS samples were or were not reduced.

[0204] Samples were then analyzed using a Waters Acquity UPLC and Q-Tof Premier mass spectrometer. Samples (0.5-2 μg each) were injected onto a MassPrep micro desalting column at 65°C, eluted from the column with a 5 min equilibration in 95% of mobile phase A, a 10 min gradient (5-90% B), and a 10 min re-equilibration in 95% of mobile phase A, at 0.05 mL/min. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The Q-Tof mass spectrometer was run in positive ion, V-mode with detection in the range of 500-4000 m/z. The source parameters were as follows: capillary voltage, 2.25 kV (intact antibody)-2.50 kV (reduced antibody); sampling cone voltage, 65.0 V (intact antibody) or 50.0 V (reduced antibody); source temperature, 100°C; desolvation temperature, 250°C; desolvation gas flow, 550 L/hr. The protein peak was deconvoluted using the MassLynx MaxEnt 1 function.

Reverse phase liquid chromatography (LC)-MS

[0205] Samples were analyzed using reverse phase liquid chromatography. Samples containing 100 μL of ADC at the concentration of 1-2 mg/mL were reduced with 20 mM DTT at 60°C for 3 minutes. The samples were analyzed using Waters Alliance HPLC with SQD and PDA detectors. Each sample was injected onto a Proteomix RP-1000 column (5 μ, 4.6X150 mm, Sepax) at 65°C. For the LC and HC mutants, separation of the LC and HC occurred with a 3.0 min equilibration in 75% of mobile phase A (0.1% TFA in water) and a 27-minute gradient (25-55% mobile phase B [0.1% TFA in water]) at a flow rate of 1 mL/min.

[0206] The SQD mass spectrometer was run in positive ion, V-mode with detection in the range of 200-2000 m/z. Source parameters were as follows: capillary voltage, 3.20kV; sampling cone voltage, 40°C; source temperature, 150°C; desolvation temperature, 250°C; desolvation gas flow, 700 L/hr. Scan time, 1 second. The protein peak was deconvoluted by the MassLynx MaxEnt 1 function. The PDA detector was at 280nm.

[0207] The DAR was calculated based on the relative signal intensity of the unconjugated and conjugated LC and unconjugated and conjugated HC. Total DAR was calculated using the following equation: total DAR = (DAR LC+DAR HC) x 2.

Example 2

[0208]

Analysis of Solvent Exposed Lysines on IgG Antibodies

[0209] In order to further study the availability of solvent exposed lysines on IgG antibodies, the crystal structures of an IgG1-kappa Fab (4F3F), an IgG1-lambda Fab (4HK0), and IgG1 Fc (1FC1) were examined for potential acyl acceptor sites. As mTGase tends to prefer solvent-exposed substrate glutamines and lysines within loops (Spolaore et al. 2012), solvent exposed lysines were highlighted using Discovery Studio v4.5 with a 1.4 Å probe radius (FIG. 4). There are 7 solvent-exposed lysines in the Antibody 01 VH with 3 in turns or loops. As the number of lysines can vary between mAbs due to utilization of different germline variable region families and somatic hypermutation, the solvent exposure of lysines in the VH region of five other antibodies were also analyzed based on analogous positions of residues in the 4F3F structure. These VH regions potentially contain 1-5 solvent-exposed lysines with 1 or 2 present in a turn or loop. In the Antibody 01 Vκ there are 6 solvent exposed lysines and 4 are in loops or turns. The VK regions from four other antibodies potentially contain 3 to 5 solvent-exposed lysines with 2 in a loop or turn. Antibody 05 utilizes a lambda chain, and the solvent exposure of the lysines was determined using the crystal structure of 4HK0 based on sequence similarity of the light chain. Antibody 05 potentially has 2 solvent-exposed lysines in the Vλ domain with only 1 in a loop. The IgG1 constant domains have 23 solvent-exposed lysines with 13 in loops or turns (FIG. 6). The kappa constant region has 8 lysines with 5 in a turn or loop. The lambda has 6 solvent-exposed lysines with half in loops or turns. In total, the analyzed antibodies range from 42 to 50 solvent-exposed lysines in loops or turns per mAb.

[0210] To determine whether microbial transglutaminase can transamidate a native lysine residue on an IgG antibody, antibodies were incubated with Z-Gln-Gly-CAD-biotin and mTGase at 37°C overnight. The samples were digested with IdeS and reduced with DTT, and the masses of the LC, Fd, and Fc fragments were analyzed by mass spectrometry. Two mass peaks corresponding to the G0F (+1445 Da) and G1F (+1608 Da) glycoforms were observed for each Fc. Antibody 04 also contained an N-linked glycosylation site in VH and two glycan species, and G2FS and G2FS2 glycans were observed. All samples lacked the C-terminal lysine (128 Da), as evidenced by the -130 to -132 Da difference between the observed and theoretical mass for the Fc. Although there are 42-50 potential acyl acceptor lysines in the different antibodies, surprisingly

neither the HC nor the LC was modified by the acyl donor substrate (FIG. 7; Table 1).

[Table 1]

Table 1 – ESI-MS analysis of antibodies contacted with an acyl donor and microbial transglutaminase

ZQG-CAD-biotin +631 Da

	LC			Fd				Fc			
	Calculated	Observed	ΔMass	Calculated	Observed	Glycan	ΔMass	Calculated	Glycan	Observed	ΔMass
Antibody 02	23751	23750	-1	25673	25671		-2	25328	G0F	25198	-130
								25491	G1F	25360	-131
Antibody 03	23478	23478	0	26097	26097		0	25388	G0F	25258	-130
								25551	G1F	25420	-131
Antibody 01	23216	23213	-3	25072	25069		-3	25328	G0F	25198	-130
								25491	G1F	25359	-132
Antibody 04	23532	23530	-2	27566	27564	G2FS	-2	25296	G0F	25166	-130
				27857	27855	G2FS2	-2	25459	G1F	25327	-132
Antibody 05	22655	22653	-2	26340	26337		-3	25328	G0F	25198	-130
								25491	G1F	25360	-131
Antibody 06	23472	23470	-2	25383	25381		-2	25328	G0F	25198	-130
								25491	G1F	25359	-132

Table 1: The masses of the LC, Fd, and Fc were determined by ESI-MS. The theoretical mass of each fragment was determined by the amino acid sequence subtracted from the observed mass to determine the change in mass (Δmass). A Δmass of -128 Da is due to cleavage of Lys447. The Fc is glycosylated with one or two oligosaccharides, G0F or G1F.

Example 3

[0211]

Mutation of Natural Amino Acid Residues to Lysine to Create an Acyl Acceptor Site

[0212] Since there are no acyl acceptor sites in human IgG1 mAbs, lysine scanning mutagenesis was performed on Antibody 01 to identify regions that could be engineered to introduce an acyl acceptor site(s). In order to increase the chances of identifying novel acyl acceptor sites, mutagenesis was limited to solvent-exposed residues within the loops or turns of the constant regions of human IgG1 HC and kappa and lambda LCs (FIG. 6). Mutant mAbs were initially screened for transamidation using an ELISA-based assay by incubating mutants with mTGase and Z-Gln-Gly-CAD-biotin overnight at 37°C. MAbs were captured on an anti-Fcγ coated plate and biotinylated mAbs were detected by HRP-conjugated streptavidin. Wild-type Antibody 01 and Antibody 01-L (which is transamidated at Lys447) were included as negative and positive controls, respectively. The signal for the positive control Antibody 01-L was greater than 12,000 RFU and mutant mAbs ranged from ~1100 RFU to ~11,000 RFU (FIG. 7).

[0213] Transamidation of all CH1 and upper hinge mutants to Z-Gln-Gly was analyzed By ESI-MS to determine whether the percentage of conjugation correlates with the RFU

signal seen in the ELISA assay. Samples were incubated with mTGase and Z-Gln-Gly overnight at 37°C, and their masses were analyzed by ESI-MS. The RFU signal from the ELISA mostly correlated with the ESI-MS data where RFUs greater than 7000 corresponded to >70% conjugation of mutants S136K, D221K, T223K, and H224K (FIG. 7). The RFU signal for T135K and T225K was below 7000, but those mutants were 80.4% and 100% conjugated, respectively. In contrast, mutant G137K had an RFU of 8616, but only 38.9% was conjugated. Therefore, while a RFU signal >7000 corresponded to a high percentage of transamidation, there were a few false positives and false negatives. Rather than performing a low-throughput ESI-MS screen of the remaining mutants, a cutoff of >4000 RFU was used to pick CH2 and CH3 samples for analysis. Since only two kappa and no lambda samples were greater than 4000 RFU, samples greater than 3000 RFUs were also analyzed.

[0214] The transamidation reaction was performed by incubating Z-Gln-Gly-CAD-biotin and mTGase overnight at 37°C with CH2 mutants M252K, E283K, A287K, and N297K; CH3 mutants P343K, G385K, G420K, H433K, L443K, S444K, and P445K; kappa mutants D151K, L201K, S202K, and E213K; and lambda mutants V147K, Q187K, and E213K, and subsequent analysis was performed by ESI-MS (FIG. 7). Three additional CH1 mutants, S191K, S192K, and L193K, were analyzed by ESI-MS, but were not including in the initial ELISA screen. Of these mutants, the CH1 mutant L193K, CH2 mutant M252K, the CH3 mutant P445K, the kappa mutants L201K and S202K, and the lambda mutant E213K were greater than 70% conjugated (FIG. 7). The results for the CH2 mutant N297K were inconclusive due to very low signal.

[0215] The mutants identified as having the highest conjugation efficiency were re-analyzed in a single experiment using the Z-Gln-Gly-CAD-biotin substrate. The region of conjugation was confirmed by digesting the samples with IdeS and reduction prior to ESI-MS analysis to generate Fd (CH1 and hinge), Fc (CH2 and CH3), and LC fragments. Conjugation to N297K was again inconclusive due to low Fc signal despite high Fd and LC signals. Transamidation of samples all correlated to the domain containing the lysine mutation. T135K and P445K mutants were conjugated at 100%, and conjugation to M252K was nearly 100% (FIG. 8). The S136K and S221K mutations were greater than 80% conjugated. The other three hinge mutants had conjugation efficiencies of less than 50%. Both LC mutants were greater than 80% conjugated.

Example 4

[0216]

Inserting a Lysine Residue to Create an Acyl Acceptor Site

[0217] Residues Ser190 through Thr195 form a beta turn that connects beta strands E and F. Lysine scanning through this exposed area showed only one site - position 193 - is an

acceptable acyl donor site. This region forms an alpha helix, and it was possible that this secondary structure prevents transamidation in this region. To potentially disrupt the structure in this region, a lysine was inserted between Ser191 and Ser192, Ser192 and Leu193, or Leu193 and Gly194. Samples were incubated with mTGase and Z-Gln-Gly-CAD-biotin overnight at 37°C, and their masses were analyzed by ESI-MS. Insertion of a lysine between Ser191 and Ser192 or Ser192 and Leu193 was 100% transamidated, but not when inserted between Leu193 and Gly194 (Table 2).

[Table 2]

Table 2 – Transamidation of a lysine insertion

Z-Gln-Gly-CAD-biotin: +631Da

	sequence	Calculated	Observed	ΔMass	% conjugated
S191.K.S192	SS**K**SLGT	48937	49565	628	100.0%
S192.K.L193	SSS**K**LGT	48937	49565	628	100.0%
L193.K.G194	SSSL**K**GT	48937	48934	-3	0.0%

MAbs were incubated with Z-Gln-Gly-CAD-biotin and mTGase at 37°C overnight. The masses of the non-reduced mAbs were analyzed by ESI-MS (data not shown), and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined as above. The DAR was determined by dividing the Δmass by the mass of Z-Gln-Gly-CAD-biotin.

Example 5

[0218]

Transamidation Resulting From Addition of a Lysine to the LC C Terminus

[0219] It was previously demonstrated that the HC C-terminal Lys447 residue is a site of transamidation when cleavage of Lys447 is blocked by an additional C-terminal residue at position 448, see U.S. Provisional Application No. 62/269,138, filed on December 18, 2015, and PCT/US2016/067165 filed on December 16, 2016, the entire contents of each of which are expressly incorporated herein by reference. A lysine was engineered to the C terminus of the LC to determine whether a single C-terminal lysine extension is sufficient to act as an acyl acceptor site. In contrast to the native C-terminal lysine in the HC, a C-terminal lysine on the LC was not cleaved by a carboxypeptidase (Table 3). However, this engineered lysine was not an acyl acceptor site. An additional leucine was engineered to the C terminus of the engineered lysine (LC-KL), as a Lys-Leu motif at the C terminus of the HC is efficiently transamidated. However, LC-KL was not transamidated. The native C-terminal cysteine (Cys214) in the LC forms an interchain disulfide bond with the HC, and this region is buried in crystal structures of this region (data not shown). It could be that steric hindrance or lack of solvent exposure prevents transamidation. A single leucine was added between Cys214 and the lysine in an attempt to make the lysine more accessible to MTGase. Again, no cleavage was seen to the C-terminal lysine, and no transamidation was observed to the LK motif. However 9.1% of the LCs with the LKL motif were transamidated. To extend the lysine further away from the LC-HC interface, a Gly-Ser

linker was inserted between Cys214 and the lysine. This extension resulted in cleavage of a C-terminal lysine (GGSGK). Protecting cleavage of the lysine by addition of a C-terminal leucine (GGSGKL) resulted in the LCs being 77.8% transamidated.

[Table 3]

Table 3 - Transamindation of a C-terminal lysine addition to the LC

Z-Gln-Gly-CAD-biotin: +631Da

	Calculated	Observed	ΔMass	% conjugated
Antibody 01-LC-K	23340	23343	3	0.0%
	23340	23344	4	
Antibody 01-LC-KL	23453	23457	4	0.0%
Antibody 01-LC-LK	23453	23457	4	0.0%
Antibody 01-LC-LKL	23566	23569	3	9.1%
	23566	23570	4	
	23566	24201	635	
Antibody 01-LC-GGSGK	23598	23473	-125	0.0%
	23598	23474	-124	
Antibody 01-LC-GGSGKL	23711	23714	3	77.8%
	23711	24345	634	

MAbs were incubated with Z-Gln-Gly-CAD-biotin and mTGase at 37°C overnight. The masses of the reduced LCs were analyzed by ESI-MS, and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined as in (Table 1).

Example 6

[0220]

Analysis of Additional Acyl Donors

[0221] One utility of conjugations to an acyl acceptor on a mAb is for the manufacturing of site-specific ADCs. Conjugation of functional agents to a mAb could be achieved by one of two methods. First, a 2-step method would require mTGase conjugation of a lysine to an acyl donor synthesized with a reactive group such as BCN, DBCO, TCO, azido (N_3), alkyne, tetrazine, or maleimide. The second step involves conjugation of a functional agent to the reactive group using, for example, copper-free click chemistry or thiol-reactive chemistry. Z-Gln-Gly-N_3 is not available commercially; therefore aminopropyl-N_3 was added to the hydroxyl group of Z-Gln-Gly as detailed in the Methods section. Antibody 01 with lysine mutations in the HC or LC were incubated with Z-Gln-Gly-N3 or Z-Gln-Gly-PEG_2-BCN and mTGase as above. The samples were desalted and analyzed by LC-MS to determine addition of the substrate to the mAb. Z-Gln-Gly-N_3 was added efficiently (>75% conjugation or DAR 1.5) for most lysine substitutions (Table 4). The most permissible sites of transamidation were HC-S135K, HC-L193K, HC-D221K, HC-M252K, HC-N297K, HC-P445K, and LC-L201K. Mutants HC-T136K, HC-T223K, HC-T225K, LC-S202K, and LC-GGSGKL were all transamidated >75% with Z-Gln-Gly-CAD-biotin (FIG. 7), but not with Z-Gln-Gly-N_3. Therefore, not all acyl donors equally transamidate the same acyl acceptor site. This was also demonstrated by Z-Gln-Gly-PEG_2-BCN where no acyl acceptor site

was efficiently transamidated (Table 4). Contrary to Z-Gln-Gly-N_3, the percentage of transamidation by Z-Gln-Gly-PEG_2-BCN did not vary widely among the acyl acceptor sites. For example, there was a 32% difference in transamidation of T135K and S136K by Z-Gln-Gly-N_3, but only a 4% difference with Z-Gln-Gly-PEG_2-BCN.

[Table 4]

Table 4 – Transamidation with various acyl donors

HC Mutation	Z-Gln-Gly-N_3	Z-Gln-Gly-PEG_2-BCN	Z-Gln-Gly-PEG_2-AuF	DAR Species
T135K	68.1%	27.1%	51.4%	1
S136K	59.6%	23.1%	16.1%	1
L193K	81.5%	63.7%	23.3%	1
D221K	55.1%	31.5%	39.7%	1
	8.5%			2
T223K	48.7%	0.0%	ND	1
H224K	55.7%	17.3%	ND	1
T225K	34.1%	0.0%	ND	1
M252K	89.0%	15.6%	82.8%	1
N297K	27.6%	6.0%	46.5%	1
	41.3%	0.0%	30.4%	2
	23.7%	0.0%	4.5%	3
P445K	73.0%	49.6%	48.7%	1

LC Mutation	Z-Gln-Gly-N_3	Z-Gln-Gly-PEG_2-BCN	Z-Gln-Gly-PEG_2-AuF	DAP
L201K	100.0%	52.5%	92.0%	1
S202K	58.5%	0.0%	44.5%	1
GGSGKL	69.5%	0.0%	44.5%	1

MAbs were incubated with Z-Gln-Gly-N_3, Z-Gln-Gly-PEG_2-BCN, or Z-Gln-Gly-PEG_2-AuF and mTGase at 37°C overnight. The masses of the reduced mAbs were analyzed by LC-MS (data not shown), and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined as in (Table 1).

[0222] Interestingly, conjugation to D221K and N297K resulted in multiple conjugation sites in the HC. The D221K mutation is adjacent to Lys222, which is not typically an acyl acceptor site. Perhaps the presence of an adjacent lysine facilitates a low level of transamidation of Lys222. Of note, only the Z-Gln-Gly-N_3 substrate is conjugated at more than one site in the D221K mutant. The structure of Z-Gln-Gly-N_3 is smaller than the other substrates tested (FIG. 2), and it is likely that steric hindrence of the second conjugation site blocks its transamidation by the other substrates.

[0223] The N297K mutation removes the glycosylation site at Asn297. Aglycosylated mAbs adopt a different structure than the glycosylated forms. The N297Q mutation also results in an aglycosylated mAb and perturbs the structure in such a way that Gln295 is then transamidated by a variety of acyl acceptor substrates (Mindt, T.L. et al., 2008, Modification of different IgG1 antibodies via glutamine and lysine using bacterial and human tissue transglutaminase, Bioconjug. Chem 19:271-278; Jeger,S. et al., 2010, Site-specific and stoichiometric modification of antibodies by bacterial transglutaminase, Angew. Chem Int Ed Engl 49:9995-9997; Dennler, P.et al., 2014, Transglutaminase-based chemo-enzymatic conjugation approach yields homogeneous antibody-drug conjugates, Bioconjug. Chem 25:569-578). N297K likely changes the con-

firmation of the CH2 region resulting in native lysines now becoming acyl acceptors sites for Z-Gln-Gly-N_3.

[0224] A second conjugation method involves a single conjugation step whereby a functional agent is synthesized with an acyl donor group. This method was tested by synthesizing a Z-Gln-Gly group onto PEG_2-Auristatin F (Z-Gln-Gly-PEG_2-AuF). The Z-Gln-Gly-PEG_2-AuF was incubated with Antibody 01 HC and LC lysine mutants and mTGase overnight at 37°C. The samples were desalted and analyzed by LC-MS to determine addition of the substrate to the mAb. All HC mutants tested demonstrated various amounts of conjugation (Table 4). The efficiency of conjugation at certain sites did not correspond with the efficiency of conjugation seen with Z-Gln-Gly-N_3. Conjugation to Z-Gln-Gly-PEG_2-AuF was low for T135K, L193K, and D221K but high for Z-Gln-Gly-N_3. L201K was the most efficient with 92% of the LCs conjugated to Z-Gln-Gly-PEG_2-AuF. N297K again demonstrated multiple conjugation sites, but the efficiency was not as high as with Z-Gln-Gly-N_3.

Example 7

[0225]

Generating Multiple Acyl Acceptor Sites on a Single Antibody

[0226] A mutant mAb with one engineered acyl acceptor site yields a theoretical DAR of 2. By combining multiple engineered acyl acceptor sites, the theoretical DAR increases by 2 for each site. Increasing the drug load of an ADC results in delivery of more cytotoxic drugs into a target cell per mAb, which may allow for lower patient dosing. To determine whether multiple acyl acceptor sites could yield a mAb with a ratio of functional agent to immunoglobulin of greater than 2, mAbs were engineered to include the LC mutant L201K or S202K in combination with the CH1 mutations T135K or S136K, the CH1-CH2 mutations S136K-N297K, the CH1-CH2-CH3 mutations S136K-N297K-P445K, or the CH1-CH3 mutations T135K-L448 to yield mAbs with 4, 6, 8 or 6 acyl acceptor sites, respectively.

[0227] T135K-based samples were incubated with mTGase and Z-Gln-Gly-CAD-biotin overnight at 37°C, and their masses were analyzed by LC-MS following reduction. The L201K LCs were 100% conjugated in all samples (Table 5). The S202K LC in the single T135KHC mutant had more than double the efficiency of the double T135K-L448HC mutant (69.8% versus 31.8%). T135K was 100% conjugated when combined with just the L201KLC mutation, resulting in a DAR of 4.0. In combination with the S202KLC mutation, conjugation was reduced to 88.7% with an average DAR of 3.17. Combining T135K with the L448HC mutation resulted in 100% conjugation to the mAbs. However, the DAR was heterogeneous with 77.1% containing 2 biotins per HC and 22.9% containing only 1 biotin yielding an average DAR of 3.54. When

the double-HC mutations were combined with a LC mutation, conjugation efficiency to the HC dropped. The amount of DAR 1 species more than doubled and the DAR 2 species dropped 2.3- to 6.5-fold. Despite these two mAb having a potential DAR of 6, the average DAR was less than 4.

[Table 5]

Table 5 – Combining lysine mutations resulted in multiple acyl acceptor sites on a single mAb

Z-Gln-Gly-CAD-biotin	% HC conjugation DAR 1	% HC conjugation DAR 2	% LC conjugation DAR 1	Ave DAR
T135KHC/L201KLC	100.0%	0.0%	100.0%	4.00
T135KHC/S202KLC	88.7%	0.0%	69.8%	3.17
T135K-L448HC	22.9%	77.1%	0.0%	3.54
T135K-L448HC/L201KLC	56.0%	11.9%	100.0%	3.59
T135K-L448HC/S202KLC	52.8%	33.2%	31.8%	3.02

	% CH1 conjugation DAR 1	% LC conjugation DAR 1	Ave DAR
S136KHC/S202KLC	85.5%	72.5%	3.16
S136K-N297KHC/S202KLC	88.1%	59.7%	2.95
S136K-N297K-P445KHC/S202KLC	84.5%	56.0%	2.80

MAbs were incubated with Z-Gln-Gly-CAD-biotin and mTGase at 37°C overnight. The masses of the reduced T135K mutants were analyzed by LC-MS, and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined as above. The S136K mutant mAbs were digested with IdeS, reduced, and analyzed by ESI-MS. The signal from the Fc fragments was too low to analyze.

[0228] Samples containing the S136KHC mutation were incubated with mTGase and Z-Gln-Gly-CAD-biotin overnight at 37°C, and their masses were analyzed by ESI-MS following IdeS digestion and reduction. The CH1 and light chain of all samples were transamidated (Table 5). S136K was 84.5% to 88.1% conjugated for all samples. S202K transamidation was higher in the single S136K mutant versus the double and triple HC mutants (72.5% versus 56% or 59.7%). The DAR for S136KHC/S202KLC was 3.16 out of 4 potential sites. The ESI-MS signal for the Fc fragments containing the N297K mutation was very low, and the conjugation efficiency could not be determined. Therefore the DARs for the double- and triple-HC mutants were at least 2.95 and 2.8.

[0229] Transamidation of multiple sites by other acyl donor substrates was also examined. The T135K-based mutants were incubated with Z-Gln-Gly-N_3, Z-Gln-Gly-PEG_2-BCN, or Z-Gln-Gly-PEG_2-AuF and mTGase overnight at 37°C. Conjugation to the LC L201K and S202K sites by each of the substrates was similar between these multiple-mutation mAbs and the single L201K and S202K mutations shown in Table 4, except no conjugation of Z-Gln-Gly-PEG_2-BCN to the LC mutations was observed (FIG. 11). The two mutants containing a single T135K mutation plus a single LC mutation demonstrated conjugation efficiency to T135K similar to the single T135K in Table 4 for all acyl donor substrates. Adding the second mutation L448 mutation resulted in

mixtures of samples with DAR 1 and DAR 2 on the HC. The samples transamidated most efficiently were T135KHC/L201KLC+Z-Gln-Gly-N_3 (DAR 3.57 out of 4), T135K-L448HC+Z-Gln-Gly-N_3 (DAR 3.1 out of 4), and T135K-L448HC/L201KLC+Z-Gln-Gly-N_3 (DAR 5.04 out of 6).

Example 8

[0230]

Analysis of the Lysine Substitutions in Other IgG Isotypes

[0231] The Fc of IgG1, IgG2, IgG3, and IgG4 are 89.2% identical (FIG. 10, alignment sequence), therefore it was possible that lysine insertions or substitutions at positions in IgG2, IgG3, and IgG4 analogous to IgG1 could be engineered acyl acceptor sites for mTGase. First, wild-type IgG2, IgG3, and IgG4 were analyzed to determine whether there are any native acyl acceptor sites specific to these isotypes. MAbs were incubated with mTGase and Z-Gln-Gly-CAD-biotin overnight at 37°C. The samples were digested with IdeS and the masses of the Fcs were analyzed by ESI-MS. As with IgG1, there was no transamidation of wild-type IgG2, IgG3, or IgG4 (Table 6).

[Table 6]

Table 6 – IgG2, IgG3, and IgG4 have no acyl acceptor sites.

Z-Gln-Gly-CAD-biotin: +631Da

	Glycan	Calculated	Observed	ΔMass	% conjugated
IgG2	G0F	25362	25232	-130	0.0%
	G1F	25525	25394	-131	
IgG3	G0F	25396	25266	-130	0.0%
	G1F	25559	25428	-131	
IgG4	G0F	25344	25214	-130	0.0%
	G1F	25507	25376	-131	

MAbs were incubated with Z-Gln-Gly-CAD-biotin and mTGase at 37°C overnight, followed by digestion with IdeS to generate $F(ab')_2$ and Fc fragments. The masses of the IdeS-generated Fc fragments were analyzed by ESI-MS as above, and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined as disclosed herein.

[0232] Lysine substitutions were made at positions analogous to IgG1 (M252, N297, and P445). Except for IgG4 that encodes for a leucine at position 445, there are no differences at these residues between the isotypes (FIG. 10). Mutant mAbs were incubated with mTGase and Z-Gln-Gly-CAD-biotin overnight at 37°C. The samples were digested with IdeS and the masses of the Fcs were analyzed by ESI-MS. The mutants N297K and M252K were efficiently transamidated with the N297K mutants yielding more than one conjugation site per HC as with IgG1 (Table 7). IgG2-N297K and IgG4-N297K contained 2 acyl acceptor sites while IgG3 contained 3. The mutant P445K was only efficiently transamidated for the IgG2 isotype. P445K transamidation was only 62.6% and 50.6 for IgG3 and IgG4, respectively.

[Table 7]

Table 7 – Engineered acyl acceptor sites in IgG1 Fc were also acceptor sites in IgG2, IgG3, and IgG4.

ZQG-CAD-biotin = 631 Da

	Calculated	Glycan	Observed	ΔMass	% of total	% conjugation
IgG2 N297K	23803	-	24434	631	78.6%	100.0%
			25059	1256	21.4%	
IgG3 N297K	23837	-	24468	631	28.2%	100.0%
			25099	1262	40.8%	
			25730	1893	31.0%	
IgG4 N297K	23785	-	24416	631	81.6%	100.0%
			25047	1262	18.4%	
IgG2 M252K	25231	G0F	25231	0	14.2%	85.8%
	25231	G0F	25862	631	69.5%	
	25394	G1F	26024	630	16.3%	
IgG3 M252K	25265	G0F	25265	0	7.4%	92.6%
	25265	G0F	25896	631	50.7%	
	25428	G1F	26058	630	41.9%	
IgG4 M252K	25213	G0F	25844	631	57.4%	100.0%
	25376	G1F	26006	630	42.6%	
IgG2 P445K	25265	G0F	25895	630	64.7%	100.0%
	25428	G1F	26058	630	35.3%	
IgG3 P445K	25299	G0F	25299	0	21.1%	61.6%
	25462	G1F	25461	-1	17.3%	
	25299	G0F	25930	631	34.3%	
	25462	G1F	26092	630	27.4%	
IgG4 L445K	25231	G0F	25231	0	25.7%	50.6%
	25394	G1F	25393	-1	23.7%	
	25231	G0F	25862	631	27.6%	
	25394	G1F	26024	630	23.0%	

MAbs were incubated with Z-Gln-Gly-CAD-biotin and mTGase at 37°C overnight, followed by digestion with IdeS to generate $F(ab')_2$ and Fc fragments. The masses of the IdeS-generated Fc fragments were analyzed by ESI-MS as above, and the percent conjugation to Z-Gln-Gly-CAD-biotin (Δmass=631 Da) was determined as disclosed herein.

Example 9

[0233]

Transamidation Sites in the Fab' Region

[0234] The hinge of IgG is a flexible linker between CH1 and CH2, and it is therefore possible that this flexibility would allow transamidation of acyl acceptors in this region. Zhang, et al., 3D Structural Fluctuation of IgG1 Antibody Revealed by Individual Particle Electron Tomography, Sci Rep, (2015) 5:9803. Indeed, upper hinge mutants D221K, T223K, H224K, and T225K are efficiently transamidated; however, there was no transamidation of middle or lower hinge mutants. This lack of transamidation may be due to structural constraints of the interchain disulfide bonds and proximity to CH2, respectively. To determine whether these residues can be transamidated when relieved of any structural constraints, lysine mutations were made in the context of a Fab' containing the entire hinge region with Cys226 and Cys229 mutated to alanine (DTHTAPPAPAPELL).

[0235] The transamination of mutant Fab's was determined by ESI-MS as above using Z-

Gln-Gly-CAD-biotin as the acyl donor. The masses of the HC portion of the Fab's were as expected except for the mutant L235K. This mutation results in a C-terminal lysine residue that was cleaved, likely due to carboxypeptidase B as with full length IgG. Harris et al., Structural characterization of a recombinant CD4-IgG hybrid molecule, Eur J Biochem, (1990) 194:611-620; Harris, Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture, J Chromatogr.A, (1995) 705:129-134; Dick, Jr. et al., C-terminal lysine variants in fully human monoclonal antibodies: investigation of test methods and possible causes, Biotechnol. Bioeng., (2008) 100:1132-1143. While no transamidation of full-length IgG was seen, a minor amount of transamidation (4.7%) was seen in the wild-type Fab', possibly at Lys222 (FIG. 9). Scanning lysine mutagenesis of the Fab' revealed several acyl acceptor sites at positions D221, T223, H224, T225, P230, and E233. These Fab's were transamidated with 1 or 2 biotins, with one on the engineered lysine and the second on a native lysine.

[0236] As secondary structure plays a large part in determining whether a lysine is transamidated, and the secondary structure of the hinge between the Fab' mutants is unlikely to change significantly, it was unexpected that the native Lys222 and mutants C226K, P227K, C229K, A231K, P232K, L234K, and L235K were not transamidated. The primary sequences surrounding the lysine mutations were analyzed. It was previously demonstrated that a residue C-terminal (+1) to an acyl acceptor affect transamidation; specifically a +1 acidic or proline residue results in little to no transamidation see, e.g., U.S. Provisional No. 62/269,138 filed on December 18, 2015, and PCT/US2016/067165 filed on December 16, 2016, the entire contents of each of which are expressly incorporated herein by reference. The mutants C226K, P227K, C229K, and A231K all have a +1 proline and P232K has a +1 glutamate. In addition, Lys222 and L234K have a -1 acidic residue. It is possible that any acidic residue flanking an acyl acceptor inhibits transamidation. Therefore, alanine mutations were made to mutate either the -1 or +1 acidic residue or +1 proline. Indeed, mutating the acidic and proline residues resulted in efficient transamidation of the lysine substitutions (Table 8). The D221A mutation increased transamidation from 5% to 66%. Similarly, mutating the -1 or +1 acid residues in the P232K,E233A and E233A,L234K Fab's resulted in increased transamidation from 46% to 93% and 62% to 86%, respectively. Mutation of a +1 proline had similar results for lysine mutants C226K (52% to 71%), P227K (57% to 81%), C229K (33% to 91%), and A231K (22% to 93%).

[0237] The effect of the hinge length on transamidation of mutants T223K, H224K, T225K, P228K, P230K, and E233K was analyzed by deleting all but the adjacent +1 residue. Removing the C-terminal hinge residues had no negative effect on transamidation of the engineered acyl acceptor site for mutants T223K, H224K, and T225K, and

increased transamidation of mutants P228K, P230K, and E233K (Table 8).

[Table 8]

Table 8 – Hinge lysine mutations in a Fab', but not a mAb, were acyl acceptors.

		% conjugation		
		+1 biotin	+2 biotins	Total
M9-Fab	DKTHTAPPAPAPELL	4.7%		4.7%
D221A	**A**KTHTAPPAPAPELL	65.9%		65.9%
D221K	**K**KTHTAPPAPAPELL	89.6%		89.6%
T223K	DK**K**HTAPPAPAPELL	51.1%	34.2%	85.3%
T223K-H	DK**K**H	56.6%	25.9%	82.5%
H224K	DKT**K**TAPPAPAPELL	87.7%	2.6%	90.2%
H224K-T	DKT**K**T	83.6%	5.4%	89.0%
T225K	DKTH**K**APPAPAPELL	69.6%	12.2%	81.8%
T225K-A	DKTH**K**A	62.6%	14.2%	76.8%
C226K	DKTHT**K**PPAPAPELL	52.2%		52.2%
C226K,P228A	DKTHT**KA**PAPAPELL	60.1%	11.2%	71.3%
P227K	DKTHTA**K**PAPAPELL	56.8%		56.8%
P227K,P228A	DKTHTA**KA**APAPELL	62.6%	17.9%	80.5%
P228K	DKTHTAP**K**APAPELL	16.5%		16.5%
P228K-A	DKTHTAP**K**A	65.8%	28.7%	94.4%
C229K	DKTHTAPP**K**PAPELL	32.5%		32.5%
C229K,P230A	DKTHTAPP**KA**APELL	77.3%	13.3%	90.6%
P230K	DKTHTAPPA**K**APELL	63.9%	14.8%	78.7%
P230K-A	DKTHTAPPA**K**A	75.7%	17.1%	92.8%
A231K	DKTHTAPPAP**K**PELL	22.0%		22.0%
A231K,P232A	DKTHTAPPAP**KA**ELL	79.3%	13.6%	92.9%
P232K	DKTHTAPPAPA**K**ELL	40.6%	5.5%	46.2%
P232K,E233A	DKTHTAPPAPA**KA**LL	85.0%	7.7%	92.6%
E233K	DKTHTAPPAPAP**K**LL	51.5%		51.5%
E233K-L	DKTHTAPPAPAP**K**L	82.4%	9.0%	91.4%
L234K	DKTHTAPPAPAPE**K**L	53.2%	8.5%	61.7%
E233A,L234K	DKTHTAPPAPAP**AK**L	75.4%	10.5%	86.3%
L235K	DKTHTAPPAPAPEL**K**	7.8%		7.8%

Purified mutant hinge mutant Fab's were screened for transamidation by incubating mTGase with Z-Gln-Gly-CAD-biotin overnight at 37°C. The masses of the HC were analyzed by ESI-MS and the percentage of conjugation was determined.

Example 10

[0238]

Generation of Dimeric Antibody Molecules

[0239] In addition to mTGase mediating transamidation between a small molecule acyl donor and a large molecule acyl acceptor, the transamidation of mutant Fabs with a mAb containing an acyl donor was analyzed. The mAb mutant N297Q contains two acyl donor sites at positions 295 and 297. Jeger et al., Site-specific and stoichiometric modification of antibodies by bacterial transglutaminase, Angew. Chem Int Ed Engl, (2010) 49:9995-9997. Fab' mutants D221K, H224K, T225K, P228K, P230K, and E233K were incubated with N297Q and mTGase overnight at 37°C. The samples were

reduced and analyzed on by SDS-PAGE. Unconjugated mAb and Fab' will yield bands at 23 kDa (LC), 25 kDa (Fab-Vh-CH1), 38 kDa (mTGase), and 49 kDa (HC). A single mAb-Fab' conjugation will result in a band at 73 kDa and conjugation of two Fab's to a mAb will result in a band at 98 kDa. Indeed, all samples contain bands at both ~75 and ~100 kDa (FIG. 11).

Conclusion to Examples

[0240] Given the number of native lysines in exposed loops and turns within an antibody, it was unexpected to find no suitable acyl acceptors on any antibody tested. These results are in direct contrast to the prior thinking in the field, including those reported by Mindt et al., 2008), who allegedly demonstrated conjugation of an average of 0.3 molecules of a fluorescent dye to a natural lysine residue on the antibody chCE7. Mindt also allegedly reported a low level (0.1 molecules/antibody) of transamidation of an acyl acceptor substrate to the same antibody, chCE7. However, numerous later publications could not replicate the findings of Mindt (see Strop et al., 2013; Jeger et al., 2010; and Siegmund et al., 2015). Specifically, Jeger noted that "no modification of native chCE7 or RTX was observed with any of the substrates" (see Jeger et al., 2010). Therefore, the findings disclosed in Mindt were likely either due to background fluorescence of the assay or specific to the acyl acceptor or donor being located in the variable regions of chCE7.

[0241] While no acyl acceptors were found on wild-type monoclonal antibodies, single amino acid lysine substitutions surprisingly revealed several positions throughout an antibody that can be engineered to be acyl acceptors. At least one site was identified in each of the constant region domains of the HC and LCs (FIG. 12). Beyond their position in solvent-exposed loops or turns, there was no clear consensus sequence surrounding the acyl acceptor sites. Examples of sequences of the sites that were transamidated greater than 70% were $KSK_{135}SG$, $STK_{136}GG$, $SSK_{193}GT$, $TLK_{252}IS$, $QYK_{297}ST$, $LSK_{445}G^*$, $QGK_{201}SS$, $GLK_{202}SP$, $PTK_{213}CS$. Similar sequences are found surrounding lysines that were not transamidated, such as in CH1 ($SSK_{133}ST$), CH3 ($LTK_{360}NQ$), and kappa ($QLK_{126}SG$, $LSK_{183}AD$).

[0242] An acidic or proline residue flanking a lysine did hinder the efficiency of transamidation, as demonstrated by mutations in the hinge of Fabs. In addition to mutating a residue to a lysine, acyl acceptor site can be engineered by mutating residues flanking an existing lysine. Based on the transamidation of Lys222 in the Fab mutant D221A, mutation of other proline or acidic residues flanking native lysine throughout the mAb is possible. For instance, IgG1 lysines $PK_{246}P$, $PK_{248}D$, $TK_{290}P$, $GK_{317}E$, and $DK_{414}S$, kappa lysines $SK_{169}D$ and $EK_{188}H$, and lambda lysine $EK_{207}T$ are flanked by acidic and proline residues, and mutation to something other than an acidic or proline residue may create an acyl acceptor site at that particular lysine. Further, it

may be possible that some sites were missed in the lysine scanning mutagenesis due to the mutant lysine being adjacent to an acidic or proline residue, as was the case for several Fab hinge mutants.

[0243] The structure surrounding the residue was also shown to influence transamidation. By relieving the core and lower hinge region of any structure contributed by CH2 and/or the interchain disulfide bonds through the generation of Fab fragments lysines that were not transamidated in the context of a mAb were transamidated in a Fab. In the context of a full-length mAb with interchain disulfide bonds at cysteines 226 and 229, P230K and E233K were not transamidated. However, in the context of a Fab fragment and no interchain disulfide bonds these residues were transamidated. A disulfide bond flanking an acyl acceptor site in amongst itself does not hinder transamidation, as the +1 position in the lambda mutant E213K is an interchain disulfide bond, and the mutant was transamidated. Therefore, either changing the three-dimensional structure of the hinge region by removing the interchain disulfide bonds and/or removing possible steric constraints of the nearby CH2 domain allowed transamidation of lysines at these sites.

[0244] MTGase has previously been investigated as a means to transamidate glutamines by engineering mAbs by one of two methods. Josten, et al., Use of microbial transglutaminase for the enzymatic biotinylation of antibodies, J Immunol.Methods, (2000) 240:47-54; Mindt, et al., Modification of different IgG1 antibodies via glutamine and lysine using bacterial and human tissue transglutaminase, Bioconjug.Chem, (2008) 19:271-278; Jeger, et al., Site-specific and stoichiometric modification of antibodies by bacterial transglutaminase, Angew.Chem Int Ed Engl, (2010) 49:9995-9997; Strop, et al., Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates, Chem Biol, (2013) 20:161-167. While effective in conjugating amine-based substrates on mAbs, both methods have disadvantages. The first approach requires deglycosylation of the mAb either enzymatically or by mutagenesis. Mindt, et al., Modification of different IgG1 antibodies via glutamine and lysine using bacterial and human tissue transglutaminase, Bioconjug.Chem, (2008) 19:271-278; Jeger, et al., Site-specific and stoichiometric modification of antibodies by bacterial transglutaminase, Angew.Chem Int Ed Engl, (2010) 49:9995-9997. Aglycosylated mAbs undergo a conformational change that decreases the thermal stability, increases protease susceptibility, and increases aggregation rates. Mimura, et al., The influence of glycosylation on the thermal stability and effector function expression of human IgG1-Fc: properties of a series of truncated glycoforms, Mol Immunol., (2000) 37:697-706; Kwon, et al., Effect of glycosylation on the stability of alpha1-antitrypsin toward urea denaturation and thermal deactivation, Biochim.Biophys.Acta, (1997) 1335:265-272; Wang, et al., pH dependent effect of glycosylation on protein stability,

Eur J Pharm Sci, (2008) 33:120-127; Yamaguchi, et al., Glycoform-dependent conformational alteration of the Fc region of human immunoglobulin G1 as revealed by NMR spectroscopy, Biochim.Biophys.Acta, (2006) 1760:693-700; Arnold, et al., The impact of glycosylation on the biological function and structure of human immunoglobulins, Annu Rev Immunol., (2007) 25:21-50; Zheng, et al., The impact of glycosylation on monoclonal antibody conformation and stability, MAbs., (2011) 3:568-576. The substitution N297K resulted in a site of transamidation, and utilizing this site also has the same disadvantage as the N297Q mutation.

[0245] The second approach involves engineering a 4-amino acid LLQG glutamine tag at either termini or within a solvent exposed region of the mAb. Strop, et al., Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates, Chem Biol, (2013) 20:161-167. The addition of 4 amino acids increases the likelihood of an immunogenic response in a patient which would reduce the efficacy of the ADC. In contrast, the modifications described herein utilize single amino acid substitutions or single amino acid, e.g., lysine, insertions as conjugation sites, thereby reducing the chance of eliciting an immune response in patients. Furthermore, multiple sites throughout the IgG, kappa, and lambda constant domains can be used if it is found that one site confers an undesirable property on the mAb such as increased aggregation or immunogenicity.

[0246] In summary, acyl acceptor sites can be engineeried in an antibody, or antigen binding fragment, by either insertion of a lysine between two native residues or by a lysine substitution at various positions throughout IgG1-4, kappa, or lambda. The optimal context for an acyl acceptor site is not only position-dependent, but also requires no acidic residue at the -1 or +1 position or no proline at the +1 position. Microbial transglutaminase conjugation technology can utilize these engineered sites to conjugate a variety of acyl donor-containing functional agents for making ADCs, bispecific antibodies, immunotoxins, or other mAb-protein complexes.

[0247] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

INFORMAL SEQUENCE LISTING

[0248]

[Table 9-1]

SEQ ID NO	Antibody/Domain	Sequence
1	4F3F-VH	QVQLQQSGPELEKPGASVKISCKASGYSFTGYTMNWVK QSHGKSLEWIGLITPYNGASSYNQKFRGKATLTVDKSSS TAYMDLLSLTSEDSAVYFCARGGYDGRGFDYWGSGTPV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
2	4F3F-VK	DIELTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKS GTSPKRWIYDTSKLASGVPGRFSGSGSGNSYSLTISSVEA EDDATYYCQQWSKHPLTFGSGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC
3	4HK0-VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV RQAPGQGLEWMGWINPNSGGTNYAQKFQGWVTMTRD TSISTAYMELSRLRSDDTAVYYCARGGLEPRSVDYYYY GMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKHHHHHH
4	4HK0-VK	QSVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKP GQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEA GDEADYYCQVWDSSSDHVVFGGGTKLTVLGQPKAAPS VTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSS PVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYS CQVTHEGSTVEKTVAPTECS
5	1FC1	THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPQVKFNWYVDGVQVHNAKTKPREQQYNST YRVVSVLTVLHQNWLDGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
6	Antibody 02HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
7	Antibody 02LC	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[Table 9-2]

SEQ ID NO	Antibody/Domain	Sequence
8	Antibody 03HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGFFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
9	Antibody 03LC	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
10	Antibody 01HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
11	Antibody 01LC	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
12	Antibody 04HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
13	Antibody 04LC	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
14	Antibody 05HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-3]

SEQ ID NO	Antibody/Domain	Sequence
15	Antibody 05LC	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
16	Antibody 06HC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
17	Antibody 06LC	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
18	human gamma 1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
19	human kappa	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
20	human lambda	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHKSYSCQVTHEGSTVEKTVAPTECS
21	Antibody 01HC-A118K	KSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-4]

SEQ ID NO	Antibody/Domain	Sequence
22	Antibody 01HC-S119K	AKTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
23	Antibody 01HC-T120K	ASKKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
24	Antibody 01HC-G122K	ASTKKPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
25	Antibody 01HC-S131K	ASTKGPSVFPLAPKSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
26	Antibody 01HC-S132K	ASTKGPSVFPLAPSKKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK

[Table 9-5]

SEQ ID NO	Antibody/Domain	Sequence
27	Antibody 01HC-S134K	ASTKGPSVFPLAPSSKKTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
28	Antibody 01HC-T135K	ASTKGPSVFPLAPSSKSKSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
29	Antibody 01HC-S136K	ASTKGPSVFPLAPSSKSTKGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
30	Antibody 01HC-G137K	ASTKGPSVFPLAPSSKSTSKGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
31	Antibody 01HC-G138K	ASTKGPSVFPLAPSSKSTSGKTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-6]

SEQ ID NO	Antibody/Domain	Sequence
32	Antibody 01HC-T139K	ASTKGPSVFPLAPSSKSTSGGKAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
33	Antibody 01HC-E152K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPKPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
34	Antibody 01HC-P153K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEKVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
35	Antibody 01HC-S160K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNKGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
36	Antibody 01HC-A162K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGKLTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-7]

SEQ ID NO	Antibody/Domain	Sequence
37	Antibody 01HC-L163K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGAKTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
38	Antibody 01HC-T164K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALKSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
39	Antibody 01HC-S165K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTKGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
40	Antibody 01HC-G166K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSKVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
41	Antibody 01HC-V167K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGKHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-8]

SEQ ID NO	Antibody/Domain	Sequence
42	Antibody 01HC-S176K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQKSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
43	Antibody 01HC-S177K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSKGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
44	Antibody 01HC-G178K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSKLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
45	Antibody 01HC-L179K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGKYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
46	Antibody 01HC-P189K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVKSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-9]

SEQ ID NO	Antibody/Domain	Sequence
47	Antibody 01HC-S190K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPKSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
48	Antibody 01HC-S191K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSKSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
49	Antibody 01HC-S192K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
50	Antibody 01HC-L193K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSKGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
51	Antibody 01HC-G194K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLKTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-10]

SEQ ID NO	Antibody/Domain	Sequence
52	Antibody 01HC-T195K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGKQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
53	Antibody 01HC-Q196K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
54	Antibody 01HC-T197K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ KYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
55	Antibody 01HC-P206K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKKSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
56	Antibody 01HC-S207K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPKNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-11]

SEQ ID NO	Antibody/Domain	Sequence
57	Antibody 01HC-E216K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVKPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
58	Antibody 01HC-P217K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEKKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
59	Antibody 01HC-S219K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKKCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
60	Antibody 01HC-D221K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCKKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
61	Antibody 01HC-T223K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKKHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-12]

SEQ ID NO	Antibody/Domain	Sequence
62	Antibody 01HC-H224K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTKTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
63	Antibody 01HC-T225K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHKCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
64	Antibody 01HC-C226K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTKPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
65	Antibody 01HC-P227K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCKPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
66	Antibody 01HC-P228K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPKCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-13]

SEQ ID NO	Antibody/Domain	Sequence
67	Antibody 01HC-C229K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPKPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
68	Antibody 01HC-P230K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCKAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
69	Antibody 01HC-A231K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPKPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
70	Antibody 01HC-P232K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAKELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
71	Antibody 01HC-E233K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPKLL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-14]

SEQ ID NO	Antibody/Domain	Sequence
72	Antibody 01HC-L234K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEKL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
73	Antibody 01HC-L235K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELK GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
74	Antibody 01HC-G236K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL KGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
75	Antibody 01HC-G237K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GKPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
76	Antibody 01HC-P247K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKKKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK

[Table 9-15]

SEQ ID NO	Antibody/Domain	Sequence
77	Antibody 01HC-M252K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLKISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
78	Antibody 01HC-I253K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMKSRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
79	Antibody 01HC-S254K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMIKRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
80	Antibody 01HC-R255K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISKTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
81	Antibody 01HC-T256K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRKPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK

[Table 9-16]

SEQ ID NO	Antibody/Domain	Sequence
82	Antibody 01HC-D265K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVKVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
83	Antibody 01HC-S267K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
84	Antibody 01HC-H268K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSKEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
85	Antibody 01HC-E269K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHKDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
86	Antibody 01HC-D270K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEKPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-17]

SEQ ID NO	Antibody/Domain	Sequence
87	Antibody 01HC-P271K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDKEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
88	Antibody 01HC-E272K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPKVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
89	Antibody 01HC-D280K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVKGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
90	Antibody 01HC-G281K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDKVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
91	Antibody 01HC-V282K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGKEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-18]

SEQ ID NO	Antibody/Domain	Sequence
92	Antibody 01HC-E283K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVKVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
93	Antibody 01HC-V284K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEKHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
94	Antibody 01HC-H285K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVKNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
95	Antibody 01HC-N286K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHKAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
96	Antibody 01HC-A287K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNKKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-19]

SEQ ID NO	Antibody/Domain	Sequence
97	Antibody 01HC-T289K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
98	Antibody 01HC-Q295K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEKYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
99	Antibody 01HC-Y296K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQKNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
100	Antibody 01HC-N297K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYKSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
101	Antibody 01HC-S298K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNKTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-20]

SEQ ID NO	Antibody/Domain	Sequence
102	Antibody 01HC-L309K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVKHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
103	Antibody 01HC-H310K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLKQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
104	Antibody 01HC-Q311K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHKD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
105	Antibody 01HC-D312K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQK WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
106	Antibody 01HC-L314K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WKNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-21]

SEQ ID NO	Antibody/Domain	Sequence
107	Antibody 01HC-N315K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLKGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
108	Antibody 01HC-G316K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNKKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
109	Antibody 01HC-E318K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKKYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
110	Antibody 01HC-A327K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKKLPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
111	Antibody 01HC-P329K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALKAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK

[Table 9-22]

SEQ ID NO	Antibody/Domain	Sequence
112	Antibody 01HC-A330K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPKPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
113	Antibody 01HC-P331K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAKIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
114	Antibody 01HC-S337K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTIKKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
115	Antibody 01HC-A339K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKKKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
116	Antibody 01HC-G341K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKKQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-23]

SEQ ID NO	Antibody/Domain	Sequence
117	Antibody 01HC-Q342K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGKPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
118	Antibody 01HC-P343K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQKREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
119	Antibody 01HC-R344K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPKEPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
120	Antibody 01HC-E345K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPRKPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
121	Antibody 01HC-R355K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSKDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-24]

SEQ ID NO	Antibody/Domain	Sequence
122	Antibody 01HC-D356K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRKELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
123	Antibody 01HC-L358K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDEKTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
124	Antibody 01HC-T359K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELKKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
125	Antibody 01HC-N361K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKKQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
126	Antibody 01HC-Q362K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNKVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-25]

SEQ ID NO	Antibody/Domain	Sequence
127	Antibody 01HC-S375K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPKDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
128	Antibody 01HC-D376K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSKIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
129	Antibody 01HC-E382K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWKSNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
130	Antibody 01HC-N384K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESKGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
131	Antibody 01HC-G385K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNKQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-26]

SEQ ID NO	Antibody/Domain	Sequence
132	Antibody 01HC-Q386K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGKPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
133	Antibody 01HC-P387K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQKENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
134	Antibody 01HC-N389K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEKN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
135	Antibody 01HC-N390K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENK YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
136	Antibody 01HC-L398K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVKDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-27]

SEQ ID NO	Antibody/Domain	Sequence
137	Antibody 01HC-S400K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDKDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
138	Antibody 01HC-D401K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSKGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
139	Antibody 01HC-G402K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDKSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
140	Antibody 01HC-D413K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVKKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
141	Antibody 01HC-S415K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKKRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-28]

SEQ ID NO	Antibody/Domain	Sequence
142	Antibody 01HC-R416K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSKWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
143	Antibody 01HC-Q418K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWKQGNVFSCSVM HEALHNHYTQKSLSLSPGK
144	Antibody 01HC-Q419K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQKGNVFSCSVM HEALHNHYTQKSLSLSPGK
145	Antibody 01HC-G420K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQKNVFSCSVM HEALHNHYTQKSLSLSPGK
146	Antibody 01HC-N421K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGKVFSCSVM HEALHNHYTQKSLSLSPGK

[Table 9-29]

SEQ ID NO	Antibody/Domain	Sequence
147	Antibody 01HC-V422K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNKFSCSVM HEALHNHYTQKSLSLSPGK
148	Antibody 01HC-A431K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEKLHNHYTQKSLSLSPGK
149	Antibody 01HC-H433K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALKNHYTQKSLSLSPGK
150	Antibody 01HC-N434K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHKHYTQKSLSLSPGK
151	Antibody 01HC-H435K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNKYTQKSLSLSPGK

[Table 9-30]

SEQ ID NO	Antibody/Domain	Sequence
152	Antibody 01HC-Y436K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHKTQKSLSLSPGK
153	Antibody 01HC-S442K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLKLSPGK
154	Antibody 01HC-L443K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSKSPGK
155	Antibody 01HC-S444K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLKPGK
156	Antibody 01HC-P445K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSKGK

[Table 9-31]

SEQ ID NO	Antibody/Domain	Sequence
157	Antibody 01HC-G446K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPKK
158	Antibody 01LC-R108K	KTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
159	Antibody 01LC-T109K	RKVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
160	Antibody 01LC-V110K	RTKAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
161	Antibody 01LC-A112K	RTVAKPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
162	Antibody 01LC-D122K	RTVAAPSVFIFPPSKEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
163	Antibody 01LC-E123K	RTVAAPSVFIFPPSDKQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
164	Antibody 01LC-S127K	RTVAAPSVFIFPPSDEQLKKGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
165	Antibody 01LC-G128K	RTVAAPSVFIFPPSDEQLKSKTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
166	Antibody 01LC-T129K	RTVAAPSVFIFPPSDEQLKSGKASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
167	Antibody 01LC-R142K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPKEAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
168	Antibody 01LC-E143K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRKAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
169	Antibody 01LC-D151K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVKNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[Table 9-32]

SEQ ID NO	Antibody/Domain	Sequence
170	Antibody 01LC-N152K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDKALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
171	Antibody 01LC-A153K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNKLQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
172	Antibody 01LC-L154K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNAKQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
173	Antibody 01LC-Q155K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALKSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
174	Antibody 01LC-S156K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQKGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
175	Antibody 01LC-G157K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSKNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
176	Antibody 01LC-E165K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTKQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
177	Antibody 01LC-D167K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQKSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
178	Antibody 01LC-S168K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDKKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
179	Antibody 01LC-D170K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKKSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
180	Antibody 01LC-S182K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLKKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
181	Antibody 01LC-A184K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKK DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
182	Antibody 01LC-E187K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYKKHKVYACEVTHQGLSSPVTKSFNRGEC
183	Antibody 01LC-H189K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKKKVYACEVTHQGLSSPVTKSFNRGEC
184	Antibody 01LC-V191K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKKYACEVTHQGLSSPVTKSFNRGEC

[Table 9-33]

SEQ ID NO	Antibody/Domain	Sequence
185	Antibody 01LC-Q199K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHKGLSSPVTKSFNRGEC
186	Antibody 01LC-G200K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQKLSSPVTKSFNRGEC
187	Antibody 01LC-L201K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGKSSPVTKSFNRGEC
188	Antibody 01LC-S202K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLKSPVTKSFNRGEC
189	Antibody 01LC-S203K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSKPVTKSFNRGEC
190	Antibody 01LC-P204K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSKVTKSFNRGEC
191	Antibody 01LC-N210K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFKRGEC
192	Antibody 01LC-R211K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNKGEC
193	Antibody 01LC-G212K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRKEC
194	Antibody 01LC-E213K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGKC
195	Antibody 05LC-G110K	KQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
196	Antibody 05LC-Q111K	GKPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
197	Antibody 05LC-P112K	GQKKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVT VAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS
198	Antibody 05LC-A115K	GQPKAKPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
199	Antibody 05LC-S125K	GQPKAAPSVTLFPPSKEELQANKATLVCLISDFYPGAVT VAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS

[Table 9-34]

SEQ ID NO	Antibody/Domain	Sequence
200	Antibody 05LC-E126K	GQPKAAPSVTLFPPSSKELQANKATLVCLISDFYPGAVT VAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS
201	Antibody 05LC-L128K	GQPKAAPSVTLFPPSSEEKQANKATLVCLISDFYPGAVT VAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS
202	Antibody 05LC-Q129K	GQPKAAPSVTLFPPSSEELKANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
203	Antibody 05LC-A130K	GQPKAAPSVTLFPPSSEELQKNKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
204	Antibody 05LC-N131K	GQPKAAPSVTLFPPSSEELQAKKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
205	Antibody 05LC-G145K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPKAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
206	Antibody 05LC-A146K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGKVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
207	Antibody 05LC-V147K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAKTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
208	Antibody 05LC-S155K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADKSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
209	Antibody 05LC-S156K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSKPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
210	Antibody 05LC-P157K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSKVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
211	Antibody 05LC-A160K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKKGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
212	Antibody 05LC-G161K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAKVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
213	Antibody 05LC-S171K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQKNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
214	Antibody 05LC-N172K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSKNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS

[Table 9-35]

SEQ ID NO	Antibody/Domain	Sequence
215	Antibody 05LC-N173K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNKKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
216	Antibody 05LC-T184K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLKPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
217	Antibody 05LC-E186K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPKQ WKSHRSYSCQVTHEGSTVEKTVAPTECS
218	Antibody 05LC-Q187K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEK WKSHRSYSCQVTHEGSTVEKTVAPTECS
219	Antibody 05LC-S190K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKKHRSYSCQVTHEGSTVEKTVAPTECS
220	Antibody 05LC-H191K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSKRSYSCQVTHEGSTVEKTVAPTECS
221	Antibody 05LC-E201K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHKGSTVEKTVAPTECS
222	Antibody 05LC-G202K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEKSTVEKTVAPTECS
223	Antibody 05LC-S203K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGKTVEKTVAPTECS
224	Antibody 05LC-P211K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAKTECS
225	Antibody 05LC-T212K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPKECS
226	Antibody 05LC-E213K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTKCS
227	Antibody 05LC-S215K	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECK

[Table 9-36]

SEQ ID NO	Antibody/Domain	Sequence
228	Antibody 01HC-S191.K.S192	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
229	Antibody 01HC-S192.K.L193	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSKLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
230	Antibody 01HC-L193.K.G194	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLKGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
231	Antibody 01-LC-K	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGECK
232	Antibody 01-LC-KL	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGECKL
233	Antibody 01-LC-LK	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGECLK
234	Antibody 01-LC-LKL	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGECLKL
235	Antibody 01-LC-GGSGK	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSGK
236	Antibody 01-LC-GGSGKL	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGECGGSGKL

[Table 9-37]

SEQ ID NO	Antibody/Domain	Sequence
237	Antibody 01HC-T135K-L448HC	ASTKGPSVFPLAPSSKSKSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGKL
238	Antibody 01HC-S136K-N297K	ASTKGPSVFPLAPSSKSTKGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYKSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
239	Antibody 01HC-S136K-N297K-P445K	ASTKGPSVFPLAPSSKSTKGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYKSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSKGK
240	hu IgG2	AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD WLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
241	hu IgG3	GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQF KWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENN YNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVM HEALHNRFTQKSLSLSPGK
242	hu IgG4	GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGK

[Table 9-38]

SEQ ID NO	Antibody/Domain	Sequence
243	Antibody 01IgG2HC	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
244	Antibody 01IgG2HC-M252K	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLKISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
245	Antibody 01IgG2HC-N297K	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFKSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
246	Antibody 01IgG2HC-P445K	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQ TYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLN GKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSKGK
247	Antibody 01IgG3HC	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPK SCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSG QPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFS CSVMHEALHNRFTQKSLSLSPGK

[Table 9-39]

SEQ ID NO	Antibody/Domain	Sequence
248	Antibody 01IgG3HC-M252K	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPK SCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRC PAPELLGGPSVFLFPPKPKDTLKISRTPEVTCVVVDVSHE DPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSG QPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFS CSVMHEALHNRFTQKSLSLSPGK
249	Antibody 01IgG3HC-N297K	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPK SCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFKWYVDGVEVHNAKTKPREEQYKSTFRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSG QPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFS CSVMHEALHNRFTQKSLSLSPGK
250	Antibody 01IgG3HC-P445K	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPK SCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSG QPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFS CSVMHEALHNRFTQKSLSLSKGK
251	Antibody 01IgG4HC	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGK

[Table 9-40]

SEQ ID NO	Antibody/Domain	Sequence
252	Antibody 01IgG4HC-M252K	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGP SVFLFPPKPKDTLKISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGK
253	Antibody 01IgG4HC-N297K	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFKSTYRVVSVLTVLHQDWL NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGK
254	Antibody 01IgG4HC-L445K	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSKGK
255	Antibody 01FabHC	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAPEL L
256	Antibody 01FabHC-D221A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCAKTHTAPPAPAPEL L
257	Antibody 01FabHC-D221K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCKKTHTAPPAPAPEL L
258	Antibody 01FabHC-T223K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKKHTAPPAPAPEL L
259	Antibody 01FabHC-T223K-H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKKH

[Table 9-41]

SEQ ID NO	Antibody/Domain	Sequence
260	Antibody 01FabHC-H224K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTKTAPPAPAPEL L
261	Antibody 01FabHC-H224K-T	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTKT
262	Antibody 01FabHC-T225K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHKAPPAPAPEL L
263	Antibody 01FabHC-T225K-A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHKA
264	Antibody 01FabHC-C226K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTKPPAPAPEL L
265	Antibody 01FabHC-C226K,P228A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTKAPAPAPEL L
266	Antibody 01FabHC-P227K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAKPAPAPEL L
267	Antibody 01FabHC-P227K,P228A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAKAAPAPE LL
268	Antibody 01FabHC-P228K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPKAPAPEL L
269	Antibody 01FabHC-P228K-A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPKA
270	Antibody 01FabHC-C229K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPKPAPEL L
271	Antibody 01FabHC-C229K,P230A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPKAAPEL L

[Table 9-42]

SEQ ID NO	Antibody/Domain	Sequence
272	Antibody 01FabHC-P230K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAKAPEL L
273	Antibody 01FabHC-P230K-A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAKA
274	Antibody 01FabHC-A231K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPKPEL L
275	Antibody 01FabHC-A231K,P232A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPKAEL L
276	Antibody 01FabHC-P232K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAKEL L
277	Antibody 01FabHC-P232K,E233A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAKA LL
278	Antibody 01FabHC-E233K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAPKL L
279	Antibody 01FabHC-E233K-L	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAPKL
280	Antibody 01FabHC-L234K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAPEK L
281	Antibody 01FabHC-E233A,L234K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAPA KL
282	Antibody 01FabHC-L235K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSKSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTAPPAPAPEL K

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SEQ ID NO	Antibody/Domain	Sequence
283	Antibody 01HC-N297Q	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
284	Signal sequence	MGWSCIILFLVATATGVHS

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

The term “comprise” and variants of the term such as “comprises” or “comprising” are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge.

Definitions of the specific embodiments of the invention as claimed herein follow.

According to a first embodiment of the invention, there is provided a conjugated immunoglobulin comprising an immunoglobulin or an antigen-binding portion thereof, and an acyl donor substrate, wherein

a) the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

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wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, or

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

b) the acyl donor substrate comprises a glutamine residue and is according to one of Formulae (I) or (II):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(Y) \qquad (I)$$

$$(Y)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad (II)$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

Y is a therapeutic agent or a diagnostic agent,

wherein the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, is conjugated to the glutamine residue of the acyl donor substrate.

According to a second embodiment of the invention, there is provided a conjugated immunoglobulin comprising an immunoglobulin, or antigen-binding portion thereof, and an acyl donor substrate, wherein

a) the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, or

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

b) the engineered lysine residue is conjugated to a glutamine residue on the acyl donor substrate, wherein the acyl donor substrate further comprises a reactive group and is according to one of Formulae (III) or (IV):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(X) \qquad \text{(III)}$$

$$(X)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad \text{(IV)}$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

X is a reactive group; and

c) the reactive group is conjugated to a therapeutic agent or a diagnostic agent.

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According to a third embodiment of the invention, there is provided a pharmaceutical composition comprising the conjugated immunoglobulin of the first embodiment, and a pharmaceutically acceptable carrier.

According to a fourth embodiment of the invention, there is provided a method for generating the conjugated immunoglobulin of the first embodiment, the method comprising:

contacting an immunoglobulin, or antigen-binding portion thereof, with a microbial transglutaminase and an acyl donor substrate,

a) wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

b) wherein the acyl donor substrate comprises a glutamine residue, and is according to one of Formulae (I) or (II):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(Y) \qquad (I)$$

$$(Y)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad (II)$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

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Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

Y is a therapeutic or a diagnostic agent, and

wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate, thereby generating the conjugated immunoglobulin.

According to a fifth embodiment of the invention, there is provided a method for generating the conjugated immunoglobulin of the second embodiment, the method comprising:

i) contacting an immunoglobulin, or antigen-binding portion thereof, with a microbial transglutaminase and an acyl donor substrate,

a) wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

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Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof; and

b) wherein the acyl donor substrate comprises a glutamine residue and a reactive group,

wherein the acyl donor substrate is according to one of Formulae (III) or (IV):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(X) \qquad \text{(III)}$$

$$(X)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad \text{(IV)}$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

X is a reactive group;

wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate, and

ii) conjugating a therapeutic or a diagnostic agent to the reactive group of the acyl donor substrate, thereby generating the conjugated immunoglobulin.

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Claims

1. A conjugated immunoglobulin comprising an immunoglobulin or an antigen-binding portion thereof, and an acyl donor substrate, wherein

a) the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, or

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

b) the acyl donor substrate comprises a glutamine residue and is according to one of Formulae (I) or (II):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(Y) \qquad (I)$$

$$(Y)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad (II)$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

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m is an integer from 0 to 5;

n is an integer from 0 to 5; and

Y is a therapeutic agent or a diagnostic agent,

wherein the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, is conjugated to the glutamine residue of the acyl donor substrate.

2. A conjugated immunoglobulin comprising an immunoglobulin, or antigen-binding portion thereof, and an acyl donor substrate, wherein

a) the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, or

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

b) the engineered lysine residue is conjugated to a glutamine residue on the acyl donor substrate, wherein the acyl donor substrate further comprises a reactive group and is according to one of Formulae (III) or (IV):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(X) \qquad \text{(III)}$$

$$(X)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad \text{(IV)}$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

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each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

X is a reactive group; and

c) the reactive group is conjugated to a therapeutic agent or a diagnostic agent.

3. The conjugated immunoglobulin of claim 1 or claim 2, wherein the heavy chain further comprises an amino acid residue which has been added to its C-terminus at position 448, and wherein said amino acid residue is not proline or an acidic amino acid residue.

4. The conjugated immunoglobulin of claim 3, wherein the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

5. The conjugated immunoglobulin of claim 1 or claim 2, wherein the immunoglobulin, or antigen-binding portion thereof, further comprises a second engineered lysine residue, wherein the second engineered lysine residue is a second lysine residue insertion or a second natural amino acid residue which has been mutated to a lysine residue, and wherein the second engineered lysine residue is conjugated to the glutamine residue of the acyl donor substrate.

6. The conjugated immunoglobulin of claim 5, wherein the heavy chain further comprises at least one amino acid residue which has been added to its C-terminus at position 448, and wherein the at least one amino acid residue is not proline or an acidic amino acid residue.

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7. The conjugated immunoglobulin of claim 6, wherein the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

8. The conjugated immunoglobulin of claim 1 or claim 2, wherein the amino acid residue after the engineered lysine residue:

i) is not a proline residue or an acidic amino acid residue; or

ii) is an amino acid residue insertion, wherein the amino acid residue insertion is a non-acidic amino acid residue or a non-proline residue insertion; or

(iii) is a natural acidic amino acid residue or a natural proline residue, which has been mutated to a non-acidic amino acid residue or a non-proline residue; and/or

wherein the amino acid residue before the enginnered lysine residue:

i) is not an acidic amino acid residue; or

i) is a non-acidic amino acid residue insertion; or

iii) is a natural acidic amino acid residue which has been mutated to a non-acidic amino acid residue.

9. The conjugated immunoglobulin of claim 1 or claim 2, wherein the immunoglobulin, or antigen-binding portion thereof, comprises a heavy chain which further comprises at least one amino acid residue which has been added to its C-terminus at position 448, and wherein the at least one amino acid residue is not proline or an acidic amino acid residue.

10. The conjugated immunoglobulin of claim 1, wherein:

i) the acyl donor substrate is according to formula (I), and wherein Z is a CBZ group; wherein each L is independently a polyethylene glycol moiety (PEG) (-O($(CH_2)_2$)-), ethyl amine (-NH($(CH_2)_2$)-) or propyl amine (-NH($(CH_2)_3$)-); and wherein n is 0, 1, 2, 3, 4 or 5; or

ii) the acyl donor substrate is according to formula (I), wherein Z is a CBZ group, and L is an amino acid; or

iii) the acyl donor substrate is according to formula (II), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is Gly.

11. The conjugated immunoglobulin of claim 1, wherein the therapeutic or diagnostic agent Y is auristatin F.

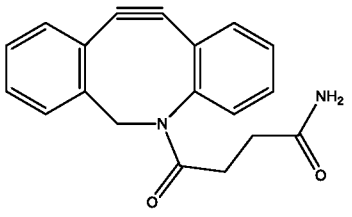
12. The conjugated immunoglobulin of claim 2, wherein:

i) the acyl donor substrate is according to formula (III), and wherein Z is a CBZ group; wherein each L is independently a polyethylene glycol moiety (PEG) (-O($(CH_2)_2$)-), ethyl amine (-NH($(CH_2)_2$)-) or propyl amine (-NH($(CH_2)_3$)-); and wherein n is 0, 1, 2, 3, 4, or 5; or

ii) the acyl donor substrate is according to formula (III), wherein Z is a CBZ group, and wherein one or more L is an amino acid; or

iii) the acyl donor substrate is according to formula (IV), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is Gly.

13. The conjugated immunoglobulin of claim 2, wherein X is a reactive group selected from the group consisting of (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol



(BCN), (dibenzocyclooctyne; DBCO), trans-cyclooctene (TCO), azido (N_3), alkyne, tetrazine methylcyclopropene, norbornene, hydrazide/hydrazine, and aldehyde.

14. The conjugated immunoglobulin of claim 1 or claim 2, wherein:

i) the therapeutic agent is an antibody or antigen-binding portion thereof, a chemotherapeutic agent, a drug agent, a radioactive agent, a cytotoxic agent, an antibiotic, a small molecule, a nucleic acid, or a polypeptide; or

ii) the diagnostic agent is a fluorophore, a fluorescent dye, a radionuclide, or an enzyme; or

iii) the immunoglobulin, or antigen-binding portion thereof, is an IgG_1 immunoglobulin, or antigen-binding portion thereof; or

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iv) the immunoglobulin, or antigen-binding portion thereof, is an IgG_2, IgG_3, or IgG_4 immunoglobulin, or antigen-binding portion thereof; or

v) the immunoglobulin, or antigen-binding portion thereof, is an IgA_1, an IgA_2, or an IgM immunoglobulin, or antigen-binding portion thereof; or

vi) the immunoglobulin, or antigen-binding portion thereof, is an IgD or IgE, immunoglobulin, or antigen-binding portion thereof; or

vii) the immunoglobulin, or antigen-binding portion thereof, is a fragment-antigen binding (Fab).

15. The conjugated immunoglobulin of any one of claims 1 to 14, wherein the immunoglobulin, or antigen-binding portion thereof:

i) is a human immunoglobulin, or antigen-binding portion thereof, or a humanized immunoglobulin, or antigen-binding portion thereof; or

ii) is a chimeric immunoglobulin, or antigen-binding portion thereof, or a non-human immunoglobulin, or antigen-binding portion thereof; or

iii) comprises two heavy chains and two light chains.

16. A pharmaceutical composition comprising the conjugated immunoglobulin of any one of claims 1 to 15, and a pharmaceutically acceptable carrier.

17. A method for generating the conjugated immunoglobulin of claim 1, the method comprising:

contacting an immunoglobulin, or antigen-binding portion thereof, with a microbial transglutaminase and an acyl donor substrate,

a) wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

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Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

b) wherein the acyl donor substrate comprises a glutamine residue, and is according to one of Formulae (I) or (II):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(Y) \qquad (I)$$

$$(Y)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad (II)$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

Y is a therapeutic or a diagnostic agent, and

wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate, thereby generating the conjugated immunoglobulin.

18. A method for generating the conjugated immunoglobulin of claim 2, the method comprising:

i) contacting an immunoglobulin, or antigen-binding portion thereof, with a microbial transglutaminase and an acyl donor substrate,

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a) wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a lysine residue insertion or a natural amino acid residue which has been mutated to a lysine residue,

wherein the lysine residue insertion is a lysine residue which has been inserted between Serine 191 and Serine 192 or between Serine 192 and Leucine 193 on a heavy chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), or Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

Leucine 201 (L201K) or Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof; and

b) wherein the acyl donor substrate comprises a glutamine residue and a reactive group,

wherein the acyl donor substrate is according to one of Formulae (III) or (IV):

$$(Z)_m\text{-Gln-}(L)_n\text{-}(X) \qquad \text{(III)}$$

$$(X)\text{-}(L)_n\text{-Gln-}(Z)_m \qquad \text{(IV)}$$

wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

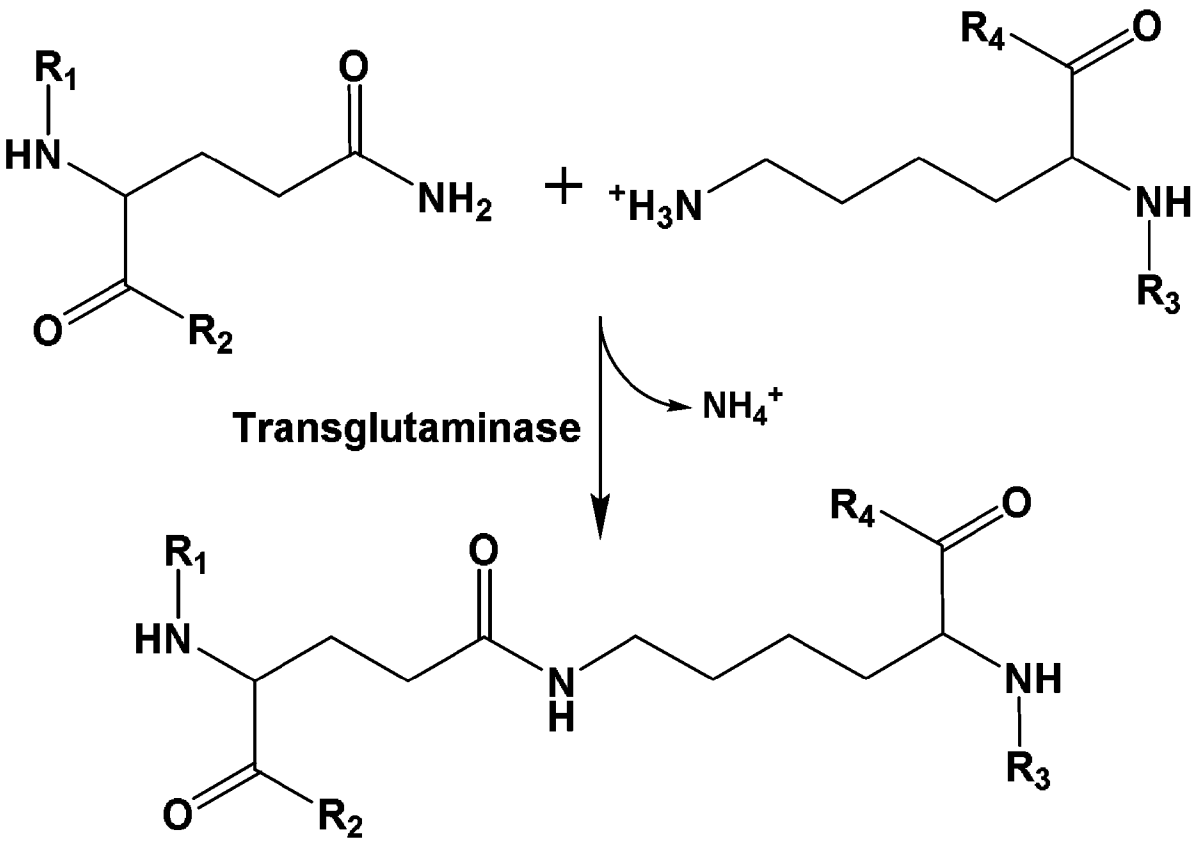
X is a reactive group;

wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate, and

ii) conjugating a therapeutic or a diagnostic agent to the reactive group of the acyl donor substrate, thereby generating the conjugated immunoglobulin.

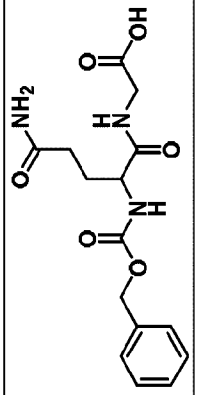
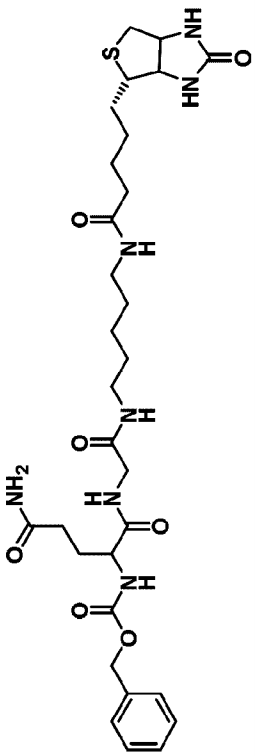
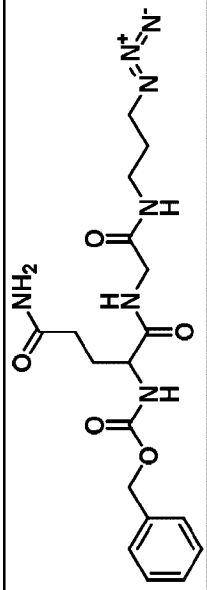
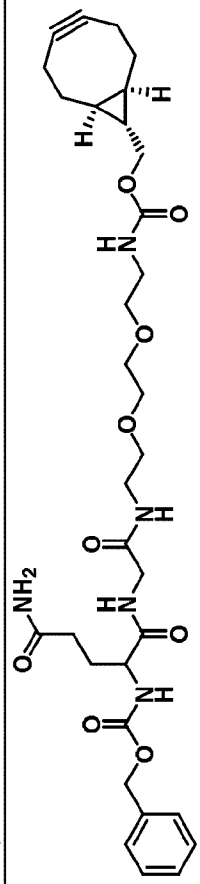
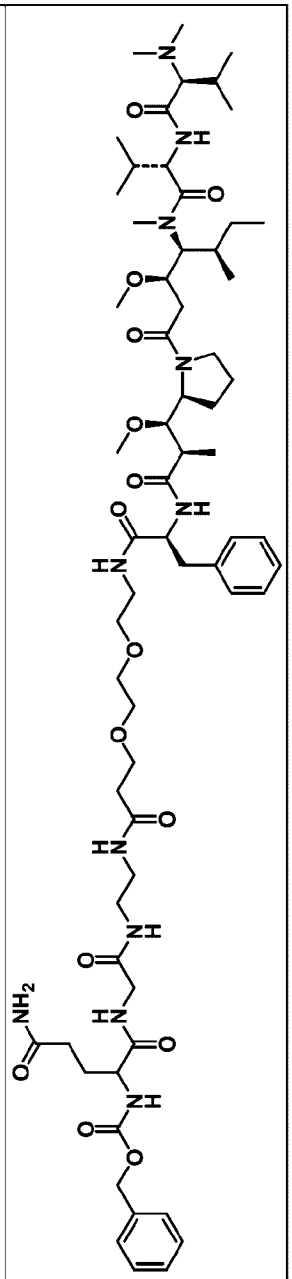
[Fig. 1]

FIGURE 1



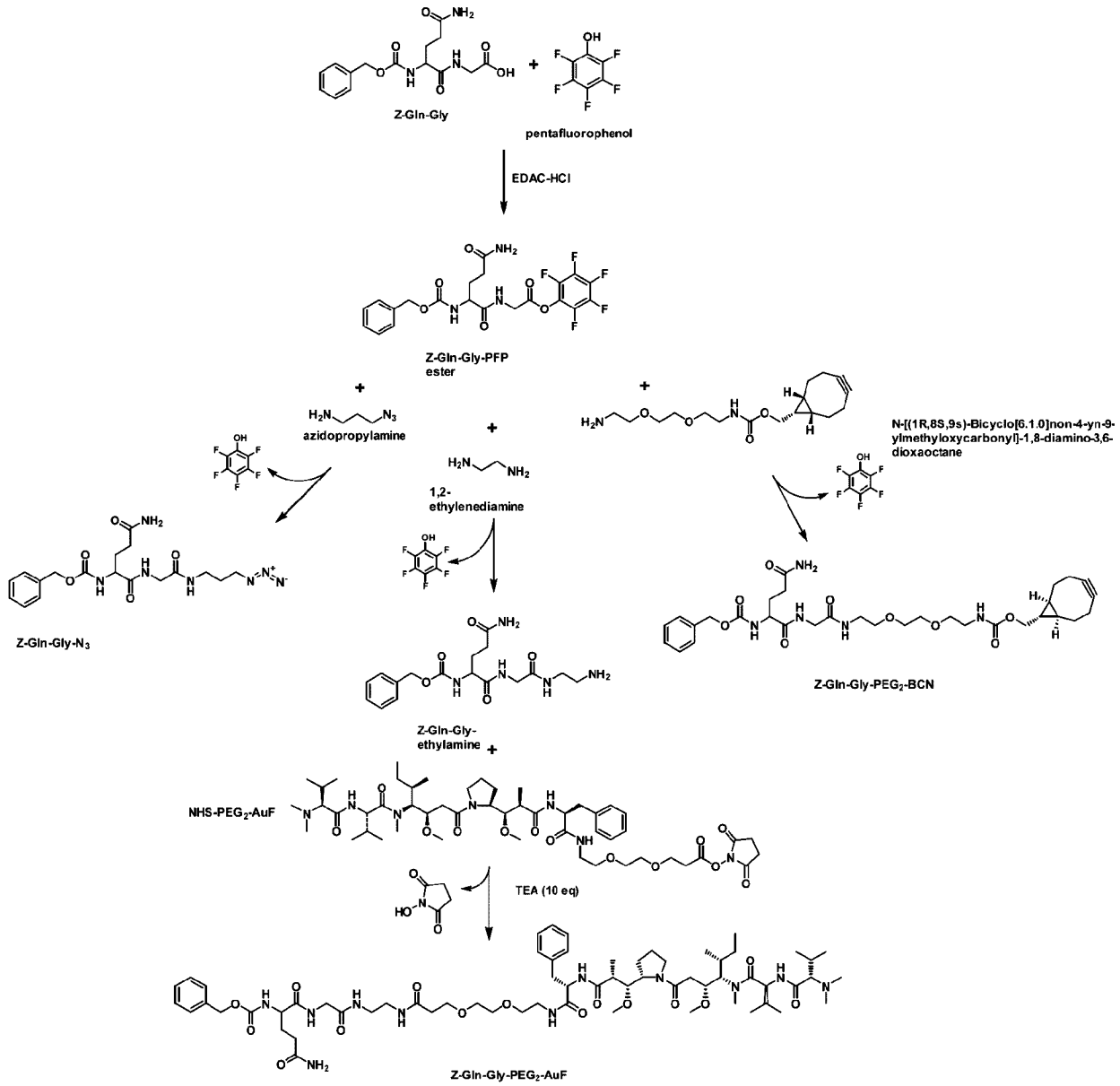
[Fig. 2]

FIGURE 2

Z-Gln-Gly	
Z-Gln-Gly-CAD-Biotin	
Z-Gln-Gly-N_3	
Z-Gln-Gly-PEG_2-BCN	
Z-Gln-Gly-PEG_2-Auristatin F	

[Fig. 3]

FIGURE 3



[Fig. 4]

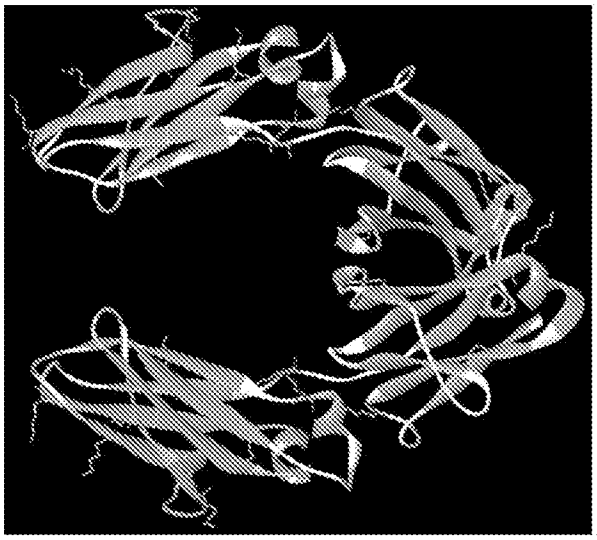


FIG. 4C. Fc

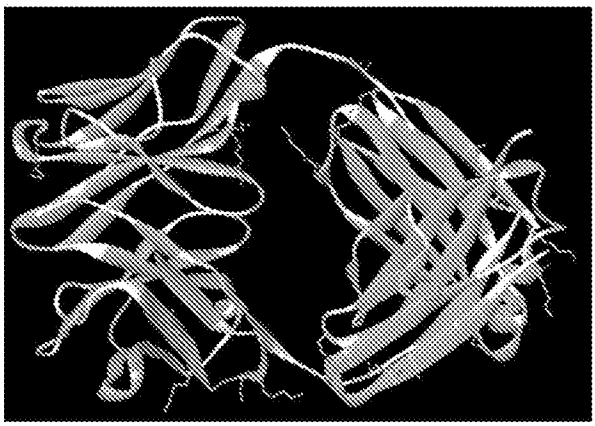


FIG. 4B. Fab-lambda

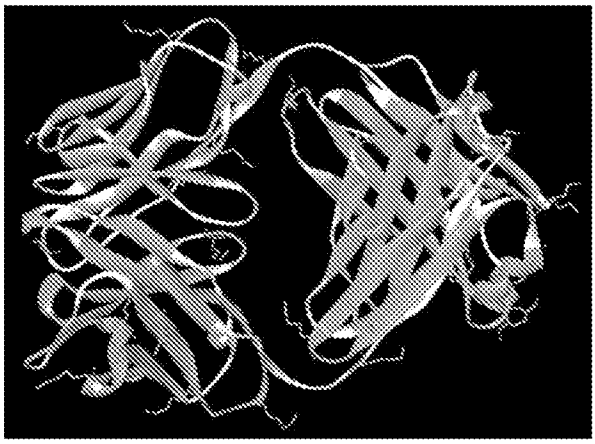


FIG. 4A. Fab-kappa

[Fig. 5]

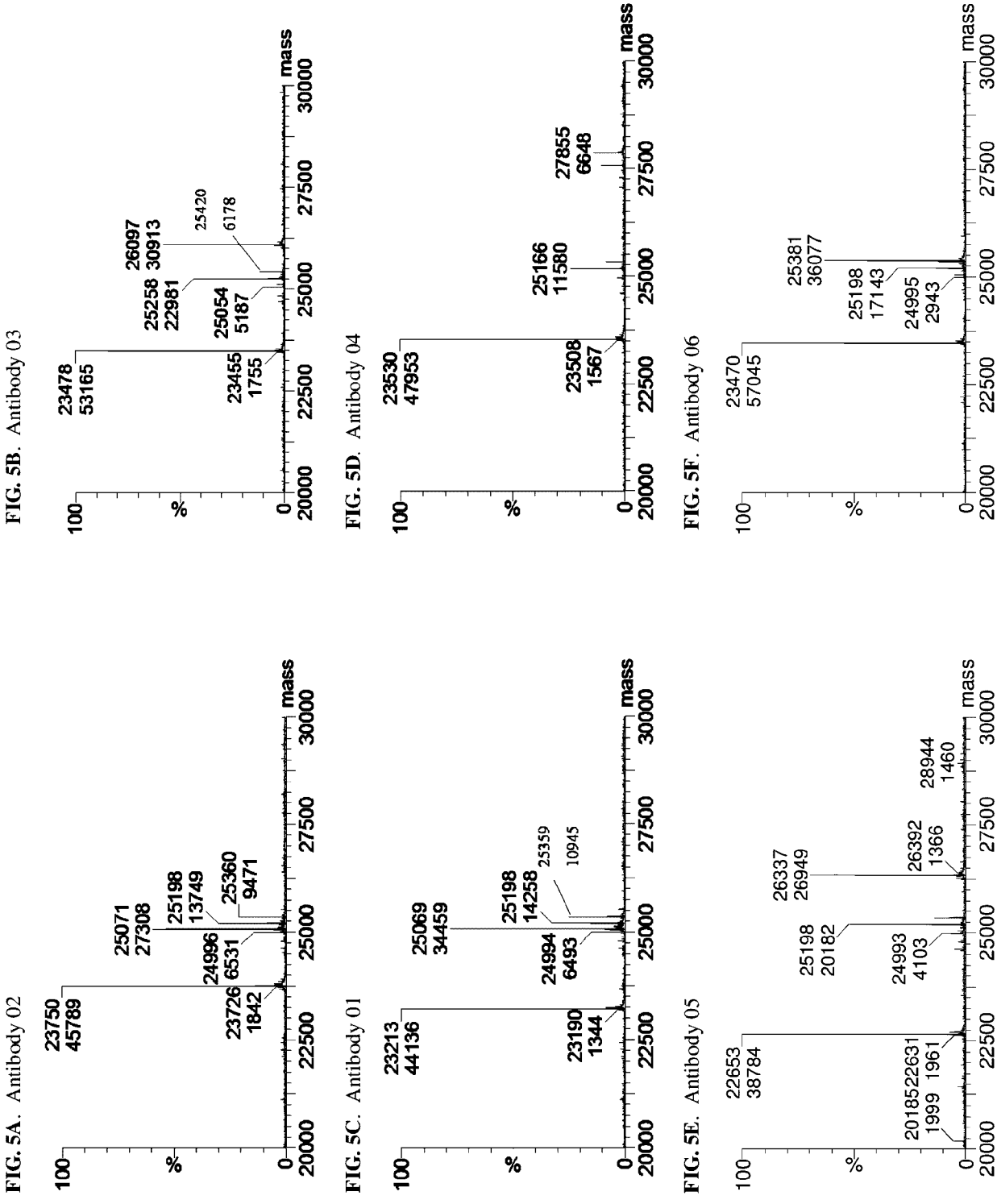


FIG. 5A. Antibody 02
FIG. 5B. Antibody 03
FIG. 5C. Antibody 01
FIG. 5D. Antibody 04
FIG. 5E. Antibody 05
FIG. 5F. Antibody 06
100
%
0
20000
22500
25000
27500
30000
mass
23750 45789
25071 27308
25198 13749
24996 6531
25360 9471
23726 1842
23478 53165
26097 30913
25258 22981
25420
6178
25054 5187
23455 1755
23213 44136
25069 34459
25198 14258
25359
10945
24994 6493
23190 1344
23530 47953
25166 11580
27855 6648
23508 1567
23470 57045
25381 36077
25198 17143
24995 2943
22653 38784
26337 26949
25198 20182
24993 4103
26392 1366
28944 1460
2018522631 1999 1961

[Fig. 6]

FIGURE 6

```
hu Cγ
118
ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP
218
KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
318
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
418 447
QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

hu Cκ
108
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
208
SFNRGEC

hu Cλ
110
GQPKAAPSVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTTPSK QSNNKYAASS YLSLTPEQWK SHKSYSCQVT HEGSTVEKTV
210
APTECS
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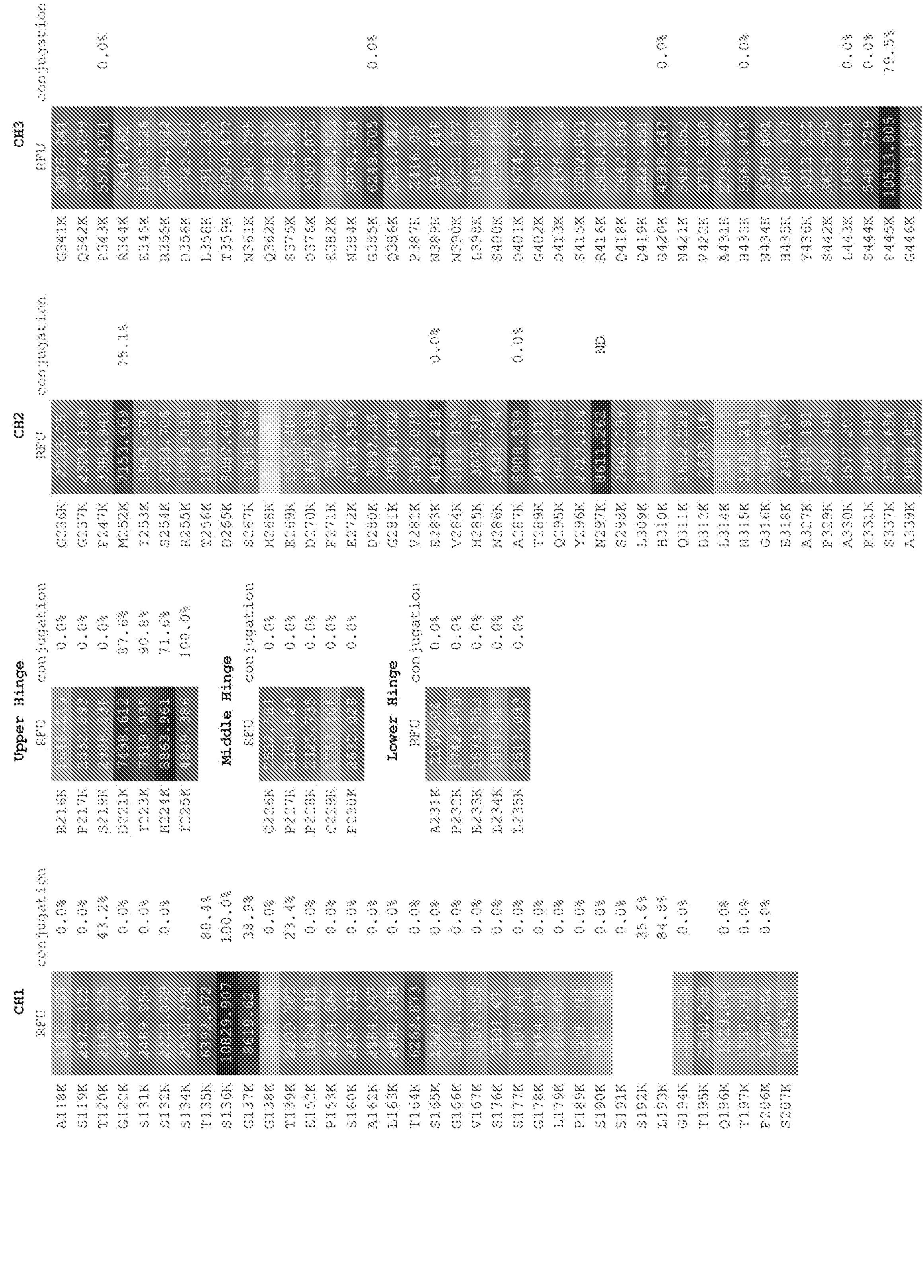
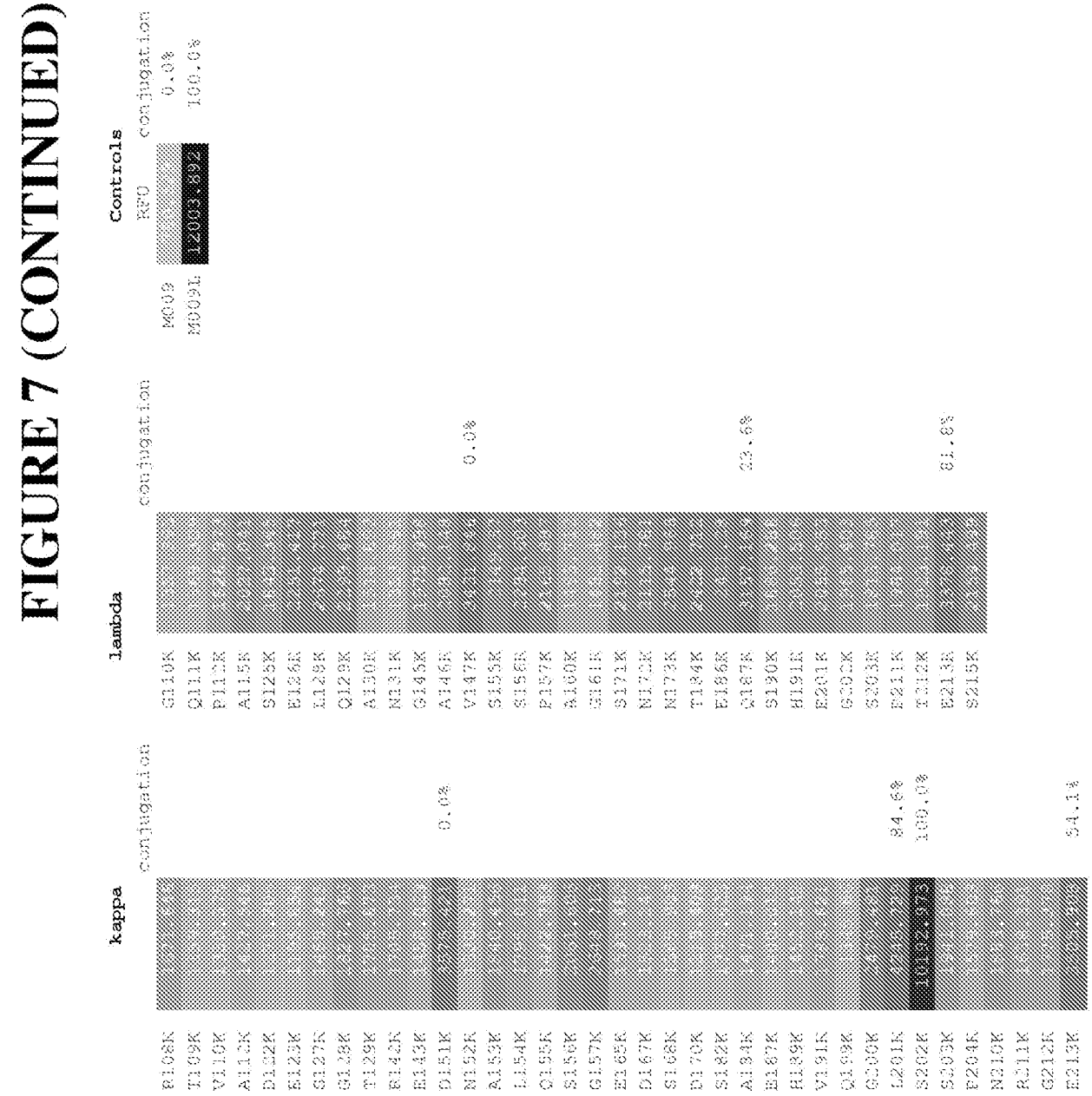
[Fig. 7-1]

FIGURE 7



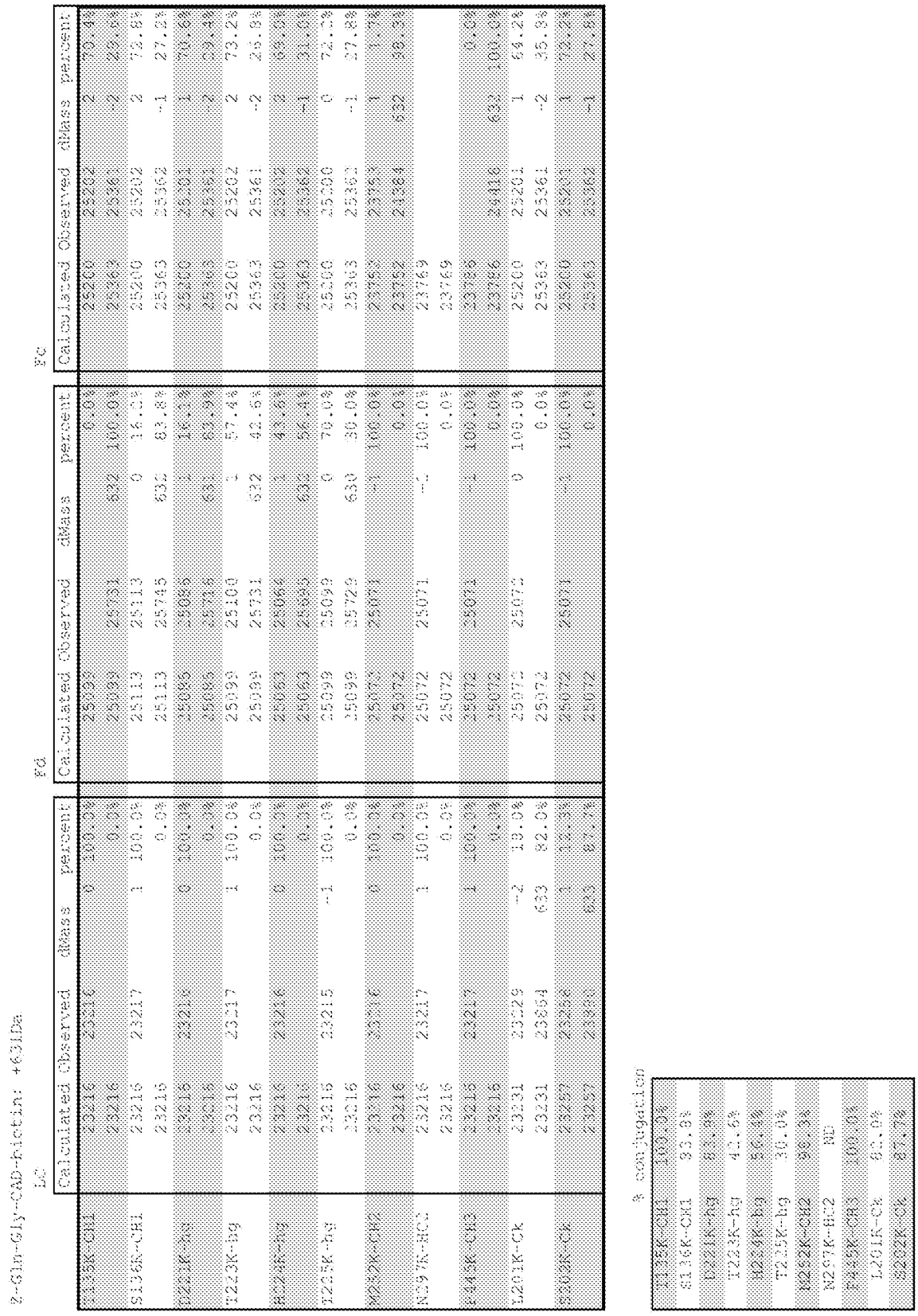
[Fig. 7-2]

FIGURE 7 (CONTINUED)



[Fig. 8]

FIGURE 8



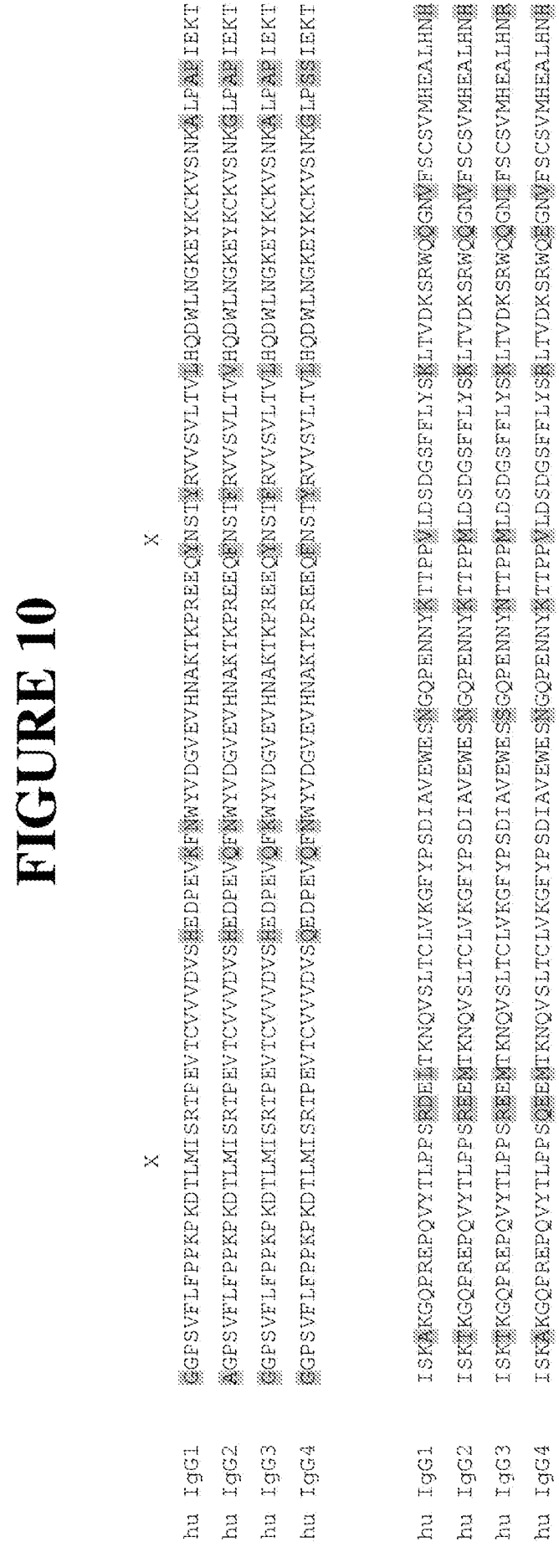
[Fig. 9]

FIGURE 9

	Z-Gln-Gly-Ms				Z-Gln-Gly-PEG8-BCN				Z-Gln-Gly-PEG8-Auf			
	% HC conjugation DAR 1	% HC conjugation DAR 2	% LC conjugation DAR 1	Ave DAR	% HC conjugation DAR 1	% HC conjugation DAR 2	% LC conjugation DAR 1	Ave DAR	% HC conjugation DAR 1	% HC conjugation DAR 2	% LC conjugation DAR 1	Ave DAR
T135AHC/L201KLC	78.6%	0.0%	100.0%	3.57	ND	ND	0.0%	0.21*	56.8%	0.0%	94.2%	2.06435
T135AHC/S202KLC	74.9%	0.0%	70.3%	2.30	41.9%	0.0%	0.0%	0.84	38.4%	0.0%	46.6%	1.65927
T135AHC/L443HC	41.8%	56.7%	0.0%	3.10	ND	ND	0.0%	ND	37.1%	24.7%	0.0%	2.12845
T135K/L443HC/L201KLC	32.2%	59.3%	100.0%	5.04	ND	ND	0.0%	0.80*	61.2%	12.4%	96.8%	3.57463
T135K/L443HC/S202KLC	38.2%	55.2%	78.1%	4.43	44.2%	19.2%	0.0%	1.64	0.5%	0.0%	38.1%	1.75388

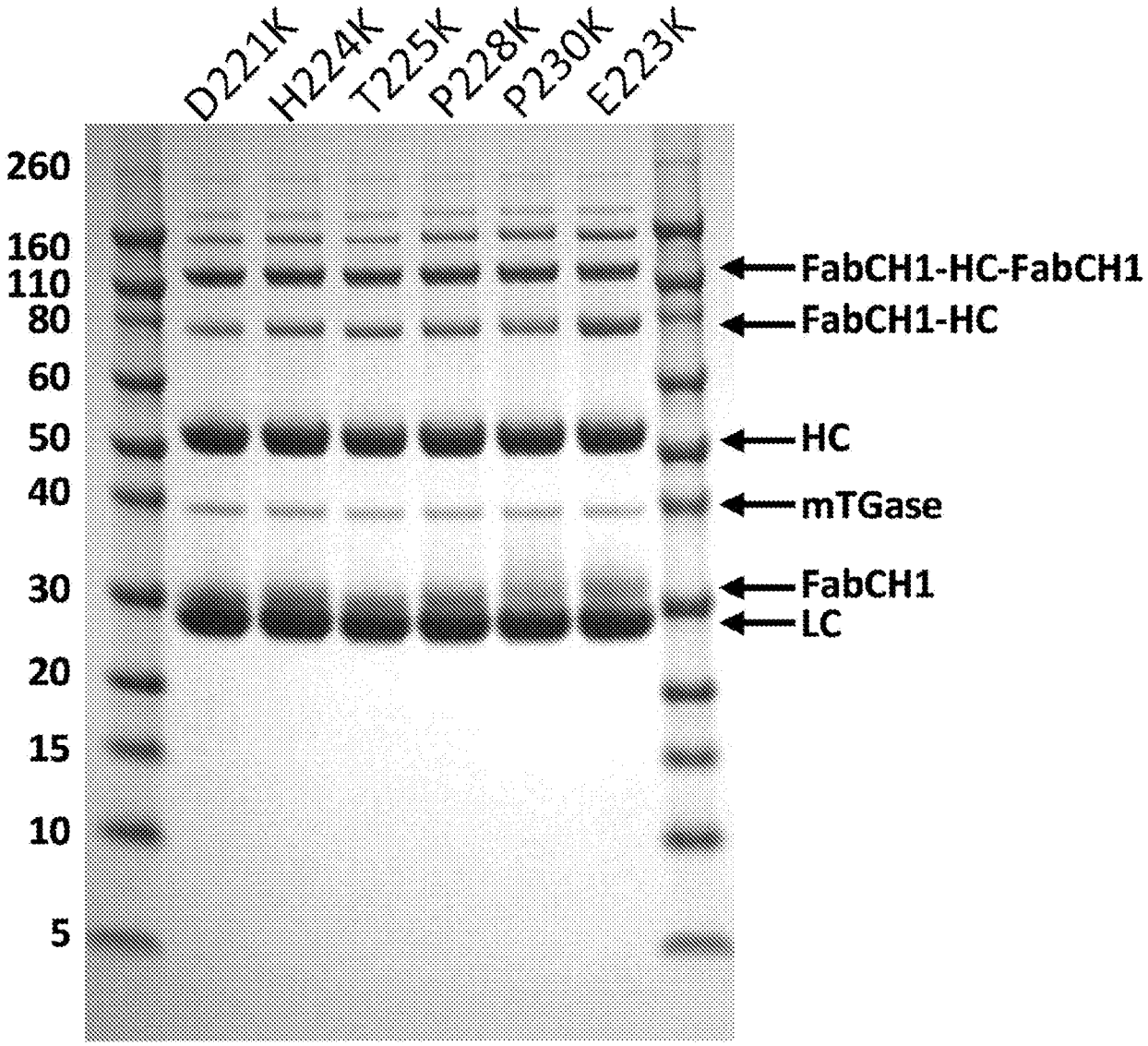
[Fig. 10]

FIGURE 10



[Fig. 11]

FIGURE 11



[Fig. 12]

FIGURE 12