antibodies might be linked to the conjugate at one location while some antibodies are linked to the same conjugate at another location, and some antibodies might not be linked at all.

[0005] There is a need for antibodies modified at site-specific positions optimized for uniformity, yield and/or activity to further the promising use of antibodies in, for example, therapy and diagnostics.

SUMMARY

[0006] Provided herein are antibodies modified at one or more site-specific positions with one or more non-natural amino acid residues. These site-specific positions are optimal for substitution of a natural amino acid residue with a non-natural amino acid residue. In certain embodiments, substitution at these site-specific positions yields antibodies that are uniform in substitution, *i.e.* that are substantially modified in the selected position. In certain embodiments, an antibody substituted at one or more of these site-specific positions has advantageous production yield, advantageous solubility, advantageous binding and/or advantageous activity. The properties of these antibodies are described in detail in the sections below.

[0006a] In one aspect, there is provided an antibody comprising a polypeptide chain having one or more non-natural amino acid residues at specific sites selected from the group consisting of heavy chain or light chain residues L22, L7, H25, H40, H19, and H70 according to the Kabat or Chothia numbering scheme, heavy chain residues H404, H121, H180, H136, and H190 according to the EU numbering scheme, or a post-translationally modified variant thereof.

[0007] In one example~~aspect~~, provided herein are antibodies comprising a polypeptide chain having at least one non-natural amino acid residue at a position in the polypeptide chain that is optimally substitutable. The antibody can be any polypeptide or multimeric polypeptide recognized as an antibody to those of skill in the art. The polypeptide chain can be any polypeptide chain of the antibody, including any heavy chain and any light chain. The position in the polypeptide chain that is optimally substitutable is any position in the polypeptide chain that can provide a substitution with optimal yield, uniformity, solubility, binding and/or activity. The sections below describe in detail the optimally substitutable positions of such polypeptide chains. Also described below are useful antibodies useful non-natural amino acids.

[0007a] In another aspect, there is provided a composition comprising the antibody as described herein, wherein said composition is substantially pure.

[0007b] In yet another aspect, there is provided a composition comprising the antibody as described herein wherein said antibody is at least 95% by mass of the total antibody mass of said composition.

[0008] In another ~~aspect~~example, provided herein are compositions comprising said antibodies. Advantageously, such compositions can have high uniformity because of the uniformity of the substitution of the antibodies provided herein. In certain embodiments, the compositions comprise a substantial amount of the antibody when measured by total weight of protein or when measured by total weight of antibody. In certain embodiments, the compositions comprise at least 80 % of the antibody, at least 85 % of the antibody, at least 90 % of the antibody or at least 95 % of the antibody by weight.

[0009] In another exampleaspect, provided herein are methods of making the antibodies. The antibodies can be made by any technique apparent to those of skill in the art for incorporating non-natural amino acids into site-specific positions of antibody chains. In certain embodiments, the antibodies are made by solid phase synthesis, semi-synthesis, *in vivo* translation, *in vitro* translation or cell-free translation.

[0010] In another exampleaspect, provided herein are methods of using the antibodies for therapy. Antibodies directed to a therapeutic target can incorporate one or more site-specific non-natural amino acids according to the description herein. These antibodies can be used for treating or preventing a disease or condition associated with the therapeutic target. Advantageously, a site-specific non-natural amino acid can be used to link the antibody to a therapeutic payload to facilitate efficacy. Exemplary antibodies, therapeutic targets and diseases or conditions are described herein.

[0011] In another exampleaspect, provided herein are methods of using the antibodies for detection. Antibodies directed to a detection target can incorporate one or more site-specific non-natural amino acids according to the description herein. These antibodies can be used with a label to signal binding to the detection target. Advantageously, a site-specific non-natural amino acid can be used to link the antibody to a label to facilitate detection. Exemplary antibodies, detection targets and labels are described herein.

[0011a] In another aspect, there is provided an antibody conjugate comprising the antibody as described herein linked to one or more therapeutic moieties or labeling moieties.

[0012] In another exampleaspect, provided herein are methods of modifying the stability of antibodies. Antibodies can be modified with a non-natural amino acid as described herein to facilitate linking to a molecular entity that can modify the stability of the antibody. For instance, a site-specific non-natural amino acid can facilitate linking to a molecular shield, for example polyethylene glycol, to increase the stability of an antibody. Exemplary non-natural amino acids and linking moieties are described herein.

BRIEF DESCRIPTION OF THE FIGURES

[0013] FIG. 1 provides the structure of exemplary cytotoxic reagent DBCO-MMAF.

[0014] FIG. 2 provides a single peak hydrophobic interaction chromatography (HIC) assay profile.

[0015] FIG. 3 provides a not-well-resolved (NWR) HIC assay profile.

[0016] FIG. 4 provides a well-resolved (WR) HIC assay profile.