Claims

1. A method of producing an immunoligand/payload conjugate, the method comprising
a) providing an immunoligand selected from the group consisting of:

(i) an antibody,
(ii) an antibody-based binding protein being a protein containing at least one antibody-derived VH, VL, or CH immunoglobulin domain,
(iii) an antibody fragment binding to a receptor, antigen, growth factor, cytokine and/or hormone, and/or
(iv) an antibody mimetic selected from the group consisting of DARPins, C-type lectins, A-domain proteins of S. aureus, transferrins, lipocalins, 10th type III domains of fibronectin, Kunitz domain protease inhibitors, affilins, gamma crystallin derived binders, cysteine knots or knottins, thioredoxin A scaffold based binders, nucleic acid aptamers, artificial antibodies produced by molecular imprinting of polymers, and stradobodies, and

b) enzymatically conjugating at least one payload to the immunoligand by a sequence-specific sortase or a catalytic domain thereof, wherein the payload is a toxin having a molecular weight not exceeding 2,500 Dalton,
wherein either

- the immunoligand comprises a sortase recognition motif and the toxin is modified with a Glyn-modification, wherein n>1, or
- the toxin comprises a sortase recognition motif and the immunoligand is modified with a Glyn-modification, wherein n>1,

thereby producing the immunoligand/payload conjugate.

2. The method of claim 1, wherein the immunoligand binds at least one entity selected from the group consisting of a receptor, an antigen, a growth factor, a cytokine, and a hormone.

3. The method of claim 1, wherein at least one catalytic domain of the sortase is fused to the C-terminus of either the immunoligand or the payload.

4. The method of claim 1, wherein comprising conjugating said immunoligand to at least two

different payloads, at least one of which is the toxin, and wherein said immunoligand has at least two subunits each being conjugated to a payload.

5. The method of claim 1, wherein said immunoligand comprises two subunits, at least one of which has a peptide spacer appended to the C-terminus thereof, said spacer comprises at least two amino acids.

6. The method of claim 5, wherein the spacer is 2-5 amino acids.

7. The method of claim 1, wherein the ratio of immunoligand and payload is stoichiometrically defined.

8. The method of claim 7, wherein said method comprises removal of partially reacted C-terminally modified immunoligand substrate.

9. The method of claim 1, wherein the method comprises an affinity purification step to separate incomplete conjugates from complete conjugates using an affinity purification tag which is attached to the immunoligand via a sortase recognition motif.

10. The method of claim 9, wherein the immunoligand contains at least two affinity purification tags.

11. The method of claim 1, wherein the conjugation of the payload to the immunoligand is site-specific.

12. The method of claim 1, wherein the conjugation comprises a transpeptidation of the sortase recognition motif.

13. The method of claim 1, wherein the sortase recognition motif is LPXTG (SEQ ID NO:27) or NPQTN (SEQ ID NO:28).

14. The method of claim 1, wherein the conjugation is performed in crude cell culture supernatant.