whereby the cell secretes the sortase A(-mediated/-catalyzed) conjugate of the first polypeptide domain and the second polypeptide domain,

thereby producing a polypeptide comprising at least two polypeptide domains.

~~In one aspect, there is provided a method for producing a polypeptide comprising at least two polypeptide domains comprising the step of~~

~~cultivating a cell comprising~~

~~a) a nucleic acid encoding a soluble S. aureus sortase A with a C-terminal endoplasmic reticulum retention signal,~~

~~b) a nucleic acid encoding a first polypeptide domain comprising at its C-terminus a sortase motif followed by an endoplasmic reticulum retention signal, and~~

~~c) a nucleic acid encoding a second polypeptide domain comprising at its N-terminus at least a diglycine,~~

~~whereby the cell secretes the sortase A conjugate of the first polypeptide domain and the second polypeptide domain,~~

~~thereby producing a polypeptide comprising at least two polypeptide domains.~~

There is also provided a method for producing a multispecific binder comprising at least two binding entities comprising the step of

- cultivating a cell comprising

a) a nucleic acid encoding a soluble sortase A with a C-terminal endoplasmic reticulum retention signal,

b) a nucleic acid encoding a first binding entity comprising at its C-terminus or in its C-terminal region a sortase motif followed by an endoplasmic reticulum retention signal, and

c) a nucleic acid encoding a second binding entity comprising at its N-terminus at least a diglycine,

whereby the cell secretes the sortase A(-mediated/catalyzed) conjugate of the first binding entity and the second binding entity,

whereby the first binding entity specifically binds to a first antigen or target and the second binding entity specifically binds to a second antigen or target,

thereby producing a multispecific binder comprising at least two binding entities.

In ~~another~~ one aspect, there is provided a method for producing a multispecific binder comprising at least two binding entities comprising the step of

- cultivating a HEK cell comprising

  a) a nucleic acid encoding a soluble S. aureus sortase A with a C-terminal endoplasmic reticulum retention signal,

  b) a nucleic acid encoding a first binding entity comprising at its C-terminus a sortase motif followed by an endoplasmic reticulum retention signal, and

  c) a nucleic acid encoding a second binding entity comprising at its N-terminus at least a diglycine,

whereby the HEK cell secretes the sortase A conjugate of the first binding entity and the second binding entity,

whereby the first binding entity specifically binds to a first antigen or target and the second binding entity specifically binds to a second antigen or target,

thereby producing a multispecific binder comprising at least two binding entities.

The sortase A may be the sortase A of Staphylococcus aureus (S. aureus). In one embodiment the nucleic acid encoding a (soluble) sortase A with a C-terminal endoplasmic reticulum retention signal encodes an amino acid sequence of SEQ ID NO: 51 or SEQ ID NO: 52.

Herein is reported a method for providing tailor-made, highly specific therapeutic molecules for the treatment of a disease, such as cancer or a viral infection, in a patient in need of a treatment, whereby the therapeutic molecule is adapted to the characteristics of the disease of the patient and/or to the genotype/phenotype of the patient.

the antibody heavy chain Fc-region polypeptide has an oligoglycine G_m (m = 2, or 3, or 4, or 5) amino acid sequence at its N-terminus, covalently by a sortase A catalyzed enzymatic reaction,

and

(ii) choosing the bispecific antibody with suitable binding specificity and/or selectivity and/or affinity and/or effector function and/or in vivo half-life and thereby determining a combination of antigen binding sites.

In another aspect, there is provided a method for determining a combination of antigen binding sites comprising the following steps

(i) determining a characteristic selected from the group consisting of ~~the~~ binding specificity, ~~and/or~~ selectivity, ~~and/or~~ affinity, ~~and/or~~ effector function, ~~and/or~~ in vivo half-life and combinations thereof of a multitude of bispecific antibodies prepared by combining (a) each member of a first multitude of antibody Fab fragments or scFv antibody fragments whereby each member comprises within the 20 C-terminal amino acid residues the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue) followed by an endoplasmic reticulum retention signal KDEL (SEQ ID NO: 02), whereby the Fab fragment or scFv antibody specifically binds to a first epitope or antigen, with (b) each member of a multitude of one-armed antibody fragments comprising a full length antibody heavy chain, a full length antibody light chain, and an antibody heavy chain Fc-region polypeptide, whereby the full length antibody heavy chain and the full length antibody light chain are cognate antibody chains complementary to each other and the pair of variable domains (VH and VL) thereof forms an antigen binding site that specifically binds to a second epitope or antigen, whereby the full length antibody heavy chain and the antibody heavy chain Fc-region polypeptide are covalently linked to each other via one or more disulfide bonds forming an antibody hinge region, and whereby the antibody heavy chain Fc-region polypeptide has an oligoglycine G_m (m = 2, or 3, or 4, or 5) amino acid sequence at its N-terminus, via a S.aureus sortase A-mediated intracellular enzymatic coupling reaction,

and

(ii) choosing the bispecific antibody with the suitable characteristic selected from the group consisting of binding specificity, ~~and/or~~ selectivity, ~~and/or~~ affinity, ~~and/or~~ effector function, ~~and/or~~ in vivo half-life and combinations thereof, and thereby determining a combination of antigen binding sites.

The sortase A may be the sortase A of Staphylococcus aureus (S. aureus). In one embodiment the nucleic acid encoding a (soluble) sortase A with a C-terminal endoplasmic reticulum retention signal encodes an amino acid sequence of SEQ ID NO: 51 or SEQ ID NO: 52.

In another aspect, there is provided a bispecific antibody obtained by a method as reported herein.

In another aspect, there is provided a bispecific antibody comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue) in one of its heavy chains.

The following embodiments as reported herein are given.

In one embodiment the members of the multitude of multispecific binders are each obtained by a method as reported herein.

In one embodiment a multispecific binder is selected based on its binding specificity and/or selectivity and/or affinity and/or effector function and/or in vivo half-life.

In one embodiment the binding entity is a cognate pair of an antibody heavy chain variable domain and an antibody light chain variable domain.

In one embodiment the multispecific binder is a bispecific antibody comprising two or four binding entities.

In one embodiment the first polypeptide domain and the second polypeptide domain are selected independently of each other from full length antibody, scFv, scFab, antibody heavy chain, antibody light chain, antibody heavy chain Fc-region fragment, pair of antibody light chain variable domain and antibody heavy chain

Patent Claims

~~1. A method for producing a polypeptide comprising at least two polypeptide domains comprising the step of~~

~~cultivating a cell comprising~~

~~a) a nucleic acid encoding a soluble S. aureus sortase A with a C-terminal endoplasmic reticulum retention signal,~~

~~b) a nucleic acid encoding a first polypeptide domain comprising at its C-terminus a sortase motif followed by an endoplasmic reticulum retention signal, and~~

~~c) a nucleic acid encoding a second polypeptide domain comprising at its N-terminus at least a diglycine,~~

~~whereby the cell secretes the sortase A conjugate of the first polypeptide domain and the second polypeptide domain,~~

~~thereby producing a polypeptide comprising at least two polypeptide domains.~~

~~2~~1. A method for producing a multispecific binder comprising at least two binding entities comprising the step of

- cultivating a HEK cell comprising

  a) a nucleic acid encoding a soluble S. aureus sortase A with a C-terminal endoplasmic reticulum retention signal,

  b) a nucleic acid encoding a first binding entity comprising at its C-terminus a sortase motif followed by an endoplasmic reticulum retention signal, and

  c) a nucleic acid encoding a second binding entity comprising at its N-terminus at least a diglycine,

whereby the HEK cell secretes the sortase A conjugate of the first binding entity and the second binding entity,

whereby the first binding entity specifically binds to a first antigen or target and the second binding entity specifically binds to a second antigen or target,

thereby producing a multispecific binder comprising at least two binding entities.

~~3~~2. A method for selecting a multispecific binder that specifically binds to two different epitopes or antigens comprising the step of

- selecting from a multitude of multispecific binders comprising different combinations of a first binding entity and a second binding entity a multispecific binder that specifically binds to two different epitopes or antigens,

wherein the members of the multitude of multispecific binders are each obtained by a method according to claim ~~2~~1.

~~4~~3. The method according to claim ~~3~~2, characterized in that a multispecific binder is selected based on a characteristic selected from the group consisting of its binding specificity, ~~and/or~~ selectivity, ~~and/or~~ affinity, ~~and/or~~ effector function, ~~and/or~~ in vivo half-life and combinations thereof.

~~5~~4. The method according to any one of claims ~~2~~1 to ~~4~~3, characterized in that the binding entity is a cognate pair of an antibody heavy chain variable domain and an antibody light chain variable domain.

~~6~~5. The method according to any one of claims ~~2~~1 to ~~5~~4, characterized in that the multispecific binder is a bispecific antibody comprising two or four binding entities.

~~7. The method according to claim 1, characterized in that the first polypeptide domain and the second polypeptide domain are selected independently of each other from full-length antibody, scFv, scFab, antibody heavy chain, antibody light chain, antibody heavy chain Fc region fragment, pair of antibody light chain variable domain and antibody heavy chain variable domain, VH, VL, CH1, CH2, CH3, CH4, CL, antibody hinge region, cytokine, receptor, receptor ligand, detectable label, tag, and partner of a binding pair.~~

86. The method according to any one of claims 1 to ~~75~~, characterized in that the endoplasmic reticulum retention signal is selected from SEQ ID NO: 02 (KDEL), SEQ ID NO: 03 (HDEL), or SEQ ID NO: 04 (SFIXXXXMP).

97. The method according to any one of claims 1 to ~~86~~, characterized in that the sortase motif is LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue).

~~108~~. The method according to any one of claims 1 to ~~97~~, characterized in that the first binding domain or the first binding entity has within the 20 C-terminal amino acid residues the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue).

~~11. The method according to any one of claims 1 to 10, characterized in that the cell is a mammalian cell or a yeast cell.~~

~~12. The method according to claim 11, characterized in that the mammalian cell is selected from a HEK cell, a CHO cell, or a BHK cell.~~

~~139~~. A method for selecting a bispecific antibody comprising the following steps

(i) determining the cell surface makers present in a cell containing sample and selecting thereof at least a first surface marker and a second surface marker,

(ii) transfecting a cell with (a) a nucleic acid encoding an antibody Fab fragment, or an antibody scFab, or a scFv antibody comprising within the 20 C-terminal amino acid residues the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue) followed by an endoplasmic reticulum retention signal KDEL (SEQ ID NO: 02), whereby the Fab fragment or scFv antibody specifically binds to the first surface marker or its ligand, (b) a nucleic acid encoding an one-armed antibody fragment comprising a full length antibody heavy chain, a full length antibody light chain, and an antibody heavy chain Fc-region polypeptide, whereby the full length antibody heavy chain and the full length antibody light chain are cognate antibody chains complementary to each other and the pair of variable domains (VH and VL) thereof forms an antigen binding site that specifically binds to the second surface marker or its ligand, whereby the full length antibody heavy chain and

the antibody heavy chain Fc-region polypeptide are covalently linked to each other via one or more disulfide bonds forming an antibody hinge region, and whereby the antibody heavy chain Fc-region polypeptide has an oligoglycine G_m (m = 2, or 3, or 4, or 5) amino acid sequence at its N-terminus, and (c) a nucleic acid encoding a soluble S. aureus sortase A with a C-terminal endoplasmic reticulum retention signal,

and thereby producing the bispecific antibody.

~~14~~10. A method for determining a combination of antigen binding sites comprising the following steps

(i) determining a characteristic selected from the group consisting of ~~the~~ binding specificity, ~~and/or~~ selectivity, ~~and/or~~ affinity, ~~and/or~~ effector function, ~~and/or~~ in vivo half-life and combinations thereof of a multitude of bispecific antibodies prepared by combining (a) each member of a first multitude of antibody Fab fragments or scFv antibody fragments whereby each member comprises within the 20 C-terminal amino acid residues the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue) followed by an endoplasmic reticulum retention signal KDEL (SEQ ID NO: 02), whereby the Fab fragment or scFv antibody specifically binds to a first epitope or antigen, with (b) each member of a multitude of one-armed antibody fragments comprising a full length antibody heavy chain, a full length antibody light chain, and an antibody heavy chain Fc-region polypeptide, whereby the full length antibody heavy chain and the full length antibody light chain are cognate antibody chains complementary to each other and the pair of variable domains (VH and VL) thereof forms an antigen binding site that specifically binds to a second epitope or antigen, whereby the full length antibody heavy chain and the antibody heavy chain Fc-region polypeptide are covalently linked to each other via one or more disulfide bonds forming an antibody hinge region, and whereby the antibody heavy chain Fc-region polypeptide has an oligoglycine G_m (m = 2, or 3, or 4, or 5) amino acid sequence at its N-terminus, via a S.aureus sortase A-mediated intracellular enzymatic coupling reaction,

and

(ii) choosing the bispecific antibody with the suitable characteristic selected from the group consisting of binding specificity, ~~and/or~~ selectivity, ~~and/or~~ affinity, ~~and/or~~ effector function, ~~and/or~~ in vivo half-life and combinations thereof, and thereby determining a combination of antigen binding sites.

~~15. A bispecific antibody obtained by a method according to any one of claim 6 to claim 13.~~

~~16~~11. A bispecific antibody comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue) in one of its heavy chains.

~~17~~12. The method according to any one of claims ~~6~~ 5 to ~~13~~ 9 or the antibody according to any one of claims 15 or 16, characterized in that Fc-region comprises a mutation of the naturally occurring amino acid residue at position 329 and at least one further mutation of at least one amino acid residue selected from the group comprising amino acid residues at position 228, 233, 234, 235, 236, 237, 297, 318, 320, 322 and 331 to a different residue, wherein the residues in the Fc-region are numbered according to the EU index of Kabat.

~~18. A pharmaceutical formulation comprising a bispecific antibody according to claim 15 or claim 16 or claim 17.~~

~~19. Use of a bispecific antibody according to claim 15 or claim 16 or claim 17 in the manufacture of a medicament.~~