[0005] In view of the above, there is a need to provide an expression system that permits screening of proteins, to enable the production of antigen binding molecules such as antibodies, and to keep the production of incorrectly processed antibody species to the minimum.

SUMMARY

[0006] In one example, the present disclosure refers to an expression system for an antigen binding molecule, wherein the antigen binding molecule is either secretable or membrane-bound, comprising:

- a first antigen binding polynucleotide encoding a first part of the antigen binding molecule;
- a cleavage polynucleotide encoding a cleavage site comprising a Furin consensus sequence RXKR (SEQ ID NO: 1) or RXRR (SEQ ID NO: 2), and a 2A polypeptide fragment thereof;
- an anchor polynucleotide encoding a membrane anchor polypeptide;

wherein the 2A polypeptide fragment thereof comprises one or more mutations in any one of the amino acid residue to control the cleavage efficiencies of the cleavage site to modulate ratio of production of the secretable antigen binding molecule versus the membrane-bound antigen binding molecule;
wherein the cleavage polynucleotide is in between the first antigen binding polynucleotide and the anchor polynucleotide;
wherein when the cleavage site is cleaved, the secretable antigen binding molecule comprising the first part of the antigen binding molecule is released;
wherein when the cleavage site is not cleaved, the membrane-bound antigen binding molecule comprising the first part of the antigen binding molecule, the Furin consensus sequence RXKR (SEQ ID NO: 1) or RXRR (SEQ ID NO: 2), the 2A polypeptide fragment thereof, and the membrane anchor polypeptide, is released.

[0006a] In one aspect, the present disclosure refers to an expression system for an antigen binding molecule, wherein the antigen binding molecule is either secretable or membrane-bound, comprising:

a first polynucleotide encoding a first part of the antigen binding molecule, wherein the first polynucleotide encoding the first part of the antigen binding molecule is an antibody heavy chain; a polynucleotide encoding a cleavage site wherein the cleavage site comprises a sequence selected from the group consisting of: RXKRGTNFS (SEQ ID NO: 82), RXRRGTNFS (SEQ ID NO: 92), RXKRAPNFS (SEQ ID NO: 88) RXRRAPNFS (SEQ ID NO: 98), RXKRATNFP (SEQ ID NO: 87) and RXRRATNFP (SEQ ID NO: 97); a polynucleotide encoding a membrane anchor polypeptide;

wherein the polynucleotide encoding the cleavage site is in between the first polynucleotide encoding the first part of the antigen binding molecule and the polynucleotide encoding the membrane anchor polypeptide; wherein when the cleavage site is cleaved, the secretable antigen binding molecule comprising the first part of the antigen binding molecule is released; wherein when the cleavage site is not cleaved, the membrane-bound antigen binding molecule comprising the first part of the antigen binding molecule, the sequence RXKRGTNFS (SEQ ID NO: 82), RXRRGTNFS (SEQ ID NO: 92), RXKRAPNFS (SEQ ID NO: 88), RXRRAPNFS (SEQ ID NO: 98), RXKRATNFP (SEQ ID NO: 87) or RXRRATNFP (SEQ ID NO: 97), and the membrane anchor polypeptide, is released.

[0007] In another example, the present disclosure refers to an expression system for an antigen binding molecule, wherein the antigen binding molecule is either secretable or membrane-bound, comprising:

- a first antigen binding polynucleotide encoding a first part of the antigen binding molecule;

Claims

1. An expression system for an antigen binding molecule, wherein the antigen binding molecule is either secretable or membrane-bound, comprising:
   - a first polynucleotide encoding a first part of the antigen binding molecule, wherein the first polynucleotide encoding the first part of the antigen binding molecule is an antibody heavy chain;
   - a polynucleotide encoding a cleavage site wherein the cleavage site comprises a sequence selected from the group consisting of: RXKRGTNFS (SEQ ID NO: 82), RXRRGTNFS (SEQ ID NO: 92), RXKRAPNFS (SEQ ID NO: 88) RXRRAPNFS (SEQ ID NO: 98), RXKRATNFP (SEQ ID NO: 87) and RXRRATNFP (SEQ ID NO: 97);
   - a polynucleotide encoding a membrane anchor polypeptide;

   wherein the polynucleotide encoding the cleavage site is in between the first polynucleotide encoding the first part of the antigen binding molecule and the polynucleotide encoding the membrane anchor polypeptide;

   wherein when the cleavage site is cleaved, the secretable antigen binding molecule comprising the first part of the antigen binding molecule is released;

   wherein when the cleavage site is not cleaved, the membrane-bound antigen binding molecule comprising the first part of the antigen binding molecule, the sequence RXKRGTNFS (SEQ ID NO: 82), RXRRGTNFS (SEQ ID NO: 92), RXKRAPNFS (SEQ ID NO: 88), RXRRAPNFS (SEQ ID NO: 98), RXKRATNFP (SEQ ID NO: 87) or RXRRATNFP (SEQ ID NO: 97), and the membrane anchor polypeptide, is released.

2. The expression system of claim 1, further comprising a second polynucleotide encoding a second part of the antigen binding molecule.

3. The expression system of claim 1 or 2, wherein the polynucleotide encoding the cleavage site encodes the sequence RRKRGTNFS (SEQ ID NO: 62) or RRRRGTNFS (SEQ ID NO: 72).

4. The expression system of claim 1 or 2, wherein the polynucleotide encoding the cleavage site encodes the sequence RRKRAPNFS (SEQ ID NO: 68) or RRRRAPNFS (SEQ ID NO: 78).

5. The expression system of claim 1 or 2, wherein the polynucleotide encoding the cleavage site encodes the sequence RRKRATNFP (SEQ ID NO: 67) or RRRRATNFP (SEQ ID NO: 77).

6. The expression system of any one of claims 2-5, wherein the second part of the antigen binding molecule is an antibody light chain.

7. The expression system of any one of claims 1 to 6, wherein the membrane anchor polypeptide is glycophospholipid transmembrane domain (GPI), platelet-derived growth factor receptor (PDGFR) beta chain transmembrane domain (PTM), or immunoglobulin C2-type extracellular-transmembrane-cytosolic domains of murin B7-1 antigen.

8. The expression system of any one of claims 1 to 3, 6 or 7 wherein the expression system comprises: a second polynucleotide encoding a light chain antibody; a first polynucleotide encoding a heavy chain antibody; a polynucleotide encoding a cleavage site sequence RRKRGTNFS (SEQ ID NO: 62); and a polynucleotide encoding a membrane anchor polypeptide.

9. The expression system of any one of claims 1, 2, 4, 6 or 7, wherein the expression system comprises: a second polynucleotide encoding a light chain antibody; a first polynucleotide encoding a heavy chain antibody; a polynucleotide encoding a cleavage site sequence RRKRAPNFS (SEQ ID NO: 68); and a polynucleotide encoding a membrane anchor polypeptide.

10. The expression system of any one of claims 1, 2 or 5-7, wherein the expression system comprises: a second polynucleotide encoding a light chain antibody; a first

polynucleotide encoding a heavy chain antibody; a polynucleotide encoding a cleavage site sequence RRKRATNFP (SEQ ID NO: 67); and an anchor polynucleotide encoding a membrane anchor polypeptide.

11. A vector comprising the expression system of any one of claims 1-10.

12. A host cell comprising the expression system of any one of claims 1-10 or the vector of claim 11.

13. A kit comprising the expression system of any one of claims 1-10, the vector of claim 11, or the host cell of claim 12.

14. A method for detecting the presence of one or more secreted antibodies and/or one or more surface-bound antibodies in a sample, the method comprising:

- providing an expression system of any one of claims 1-10;
- delivering said expression system to one or more target cells, wherein said target cell transcribes said expression system, wherein once transcribed, said cleavage site is cleaved in a first plurality of first part of the antigen binding molecule, so that said first plurality of first part of the antigen binding molecule do not comprise said membrane anchor polypeptide, and are thereby secreted by said target cell, and wherein said cleavage site is not cleaved in a second plurality of first part of the antigen binding molecule, so that said second plurality of first part of the antigen binding molecule comprise said membrane anchor polypeptide, and are thereby bound to the surface of said target cell; and
- detecting the presence or absence of said first plurality of first part of the antigen binding molecule secreted by said target cell and/or detecting a quantity of said second plurality of first part of the antigen binding molecule on the surface of said target cell.

15. The expression system of any one of claims 1-10, the vector of claim 11, the host cell of claim 12 or the kit of claim 13 for use in screening antibody libraries or antibody production.