SUMMARY

[0005] In one example, the present disclosure refers to an expression system for an antigen binding molecule, comprising:

- a first polynucleotide encoding a first part of the antigen binding molecule;
- a second polynucleotide encoding a first cleavage site comprising an amino acid sequence Z-R-X1-X2-R-X3-X4-X5-X6 (SEQ ID NO: 256), wherein the second polynucleotide is located downstream of the first polynucleotide;
- a third polynucleotide encoding a second cleavage site comprising a 2A polypeptide or a fragment thereof, wherein the third polynucleotide is located downstream of the second polynucleotide; and
- a fourth polynucleotide encoding a second part of the antigen binding molecule, wherein the fourth polynucleotide is located downstream of the third polynucleotide,

wherein when the first and second cleavage sites are cleaved, an antigen binding molecule comprising the first and second parts of the antigen binding molecule is released,

wherein Z is R, P, G, A, K, N, Q, H, I, L, M, F, S, T, W, Y, or V, and

wherein X1, X2, X3, X4, X5 and X6 is R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V.

[0005a] In one aspect, the present disclosure refers to an expression system for an antigen binding molecule, comprising:

- a first polynucleotide encoding a first part of the antigen binding molecule;
- a second polynucleotide encoding a first cleavage site comprising an amino acid sequence Z-R-X1-X2-R-X3-X4-X5-X6 (SEQ ID NO: 256), wherein the second polynucleotide is located downstream of the first polynucleotide;
- a third polynucleotide encoding a second cleavage site comprising a 2A polypeptide or a fragment thereof that comprises at least the last 10 amino acids of the 2A polypeptide, wherein the third polynucleotide is located downstream of the second polynucleotide; and
- a fourth polynucleotide encoding a second part of the antigen binding molecule, wherein the fourth polynucleotide is located downstream of the third polynucleotide,

wherein when the first and second cleavage sites are cleaved, an antigen binding molecule comprising the first and second parts of the antigen binding molecule is released,

wherein Z is R, P, G, A, K, N, Q, H, I, L, M, F, S, T, W, Y, or V,

wherein X1, X3, X4, X5 and X6 is R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V, and wherein X2 is K or R.

[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 polynucleotide encoding a first part of the antigen binding molecule;
- a second polynucleotide encoding a first cleavage site comprising an amino acid sequence Z-R-X1-X2-R-X3-X4-X5-X6 (SEQ ID NO: 256), wherein the second polynucleotide is located downstream of the first polynucleotide;
- a third polynucleotide encoding a second cleavage site comprising a 2A polypeptide or a fragment thereof, wherein the third polynucleotide is located downstream of the second polynucleotide;
- a fourth polynucleotide encoding a second part of the antigen binding molecule, wherein the fourth polynucleotide is located downstream of the third polynucleotide;
- a fifth polynucleotide encoding a third cleavage site comprising a Furin cleavage sequence variant comprising:

(i) a Furin consensus sequence R-X-K-R (SEQ ID NO: 18); or

(ii) a Furin consensus sequence R-X-R-R (SEQ ID NO: 19),

wherein the fifth polynucleotide is located downstream of the fourth polynucleotide; and

- a sixth polynucleotide encoding a membrane anchor polypeptide, wherein the sixth polynucleotide is located downstream of the fifth polynucleotide;

wherein when the first, second and third cleavage sites are cleaved, a secretable antigen binding molecule comprising the first and second parts of the antigen binding molecule is released;

wherein when the first and second cleavage sites are cleaved and the third cleavage site is not cleaved, a membrane-bound antigen binding molecule comprising the first and second parts of the antigen binding molecule, the third cleavage site, and the membrane anchor polypeptide is released,

wherein Z is R, P, G, A, K, N, Q, H, I, L, M, F, S, T, W, Y, or V,

wherein X1, X2, X3, X4, X5 and X6 is R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V, and

wherein X is any amino acid.

[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;
- a second polynucleotide encoding a first cleavage site comprising an amino acid sequence Z-R-X1-X2-R-X3-X4-X5-X6 (SEQ ID NO: 256), wherein the second polynucleotide is located downstream of the first polynucleotide;
- a third polynucleotide encoding a second cleavage site comprising a 2A polypeptide or a fragment thereof that comprises at least the last 10 amino acids of the 2A polypeptide, wherein the third polynucleotide is located downstream of the second polynucleotide;
- a fourth polynucleotide encoding a second part of the antigen binding molecule, wherein the fourth polynucleotide is located downstream of the third polynucleotide;
- a fifth polynucleotide encoding a third cleavage site comprising a Furin cleavage sequence variant comprising:

(i) a Furin consensus sequence R-X-K-R (SEQ ID NO: 18); or

(ii) a Furin consensus sequence R-X-R-R (SEQ ID NO: 19),

wherein the fifth polynucleotide is located downstream of the fourth polynucleotide; and

- a sixth polynucleotide encoding a membrane anchor polypeptide, wherein the sixth polynucleotide is located downstream of the fifth polynucleotide;

wherein when the first, second and third cleavage sites are cleaved, a secretable antigen binding molecule comprising the first and second parts of the antigen binding molecule is released;

wherein when the first and second cleavage sites are cleaved and the third cleavage site is not cleaved, a membrane-bound antigen binding molecule comprising the first and second parts of the antigen binding molecule, the third cleavage site, and the membrane anchor polypeptide is released,

wherein Z is R, P, G, A, K, N, Q, H, I, L, M, F, S, T, W, Y, or V,

wherein X1, X3, X4, X5 and X6 is R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V, wherein X2 is K or R, and

wherein X is any amino acid.

[0007] In one aspect, the present disclosure refers to a vector comprising the expression system disclosed herein.

[0008] In one aspect, the present disclosure refers to a host cell comprising the expression system disclosed herein or the vector disclosed herein.

[0009] In one aspect, the present disclosure refers to a kit comprising the expression system disclosed herein, the vector disclosed herein, or the host cell disclosed herein.

[0010] In one aspect, the present disclosure refers to the expression system disclosed herein, the vector disclosed herein, the host cell disclosed herein, or the kit disclosed herein for use in screening antibody libraries or antibody production.

[0011] In one aspect, the present disclosure refers to a method of producing one or more secretable antigen binding molecules and/or one or more membrane-bound antigen binding molecules, comprising culturing the host cell disclosed herein under suitable culture conditions such that one or more secretable antigen binding molecules and/or one or more membrane-bound antigen binding molecules are produced.

CLAIMS

1. An expression system for an antigen binding molecule, comprising:

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

- a second polynucleotide encoding a first cleavage site comprising an amino acid sequence Z-R-X1-X2-R-X3-X4-X5-X6 (SEQ ID NO: 256), wherein the second polynucleotide is located downstream of the first polynucleotide;

- a third polynucleotide encoding a second cleavage site comprising a 2A polypeptide or a fragment thereof that comprises at least the last 10 amino acids of the 2A polypeptide, wherein the third polynucleotide is located downstream of the second polynucleotide; and

- a fourth polynucleotide encoding a second part of the antigen binding molecule, wherein the fourth polynucleotide is located downstream of the third polynucleotide,

wherein when the first and second cleavage sites are cleaved, an antigen binding molecule comprising the first and second parts of the antigen binding molecule is released,

wherein Z is R, P, G, A, K, N, Q, H, I, L, M, F, S, T, W, Y, or V,

wherein X1, X3, X4, X5 and X6 is R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V, and wherein X2 is K or R.

2. 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;

- a second polynucleotide encoding a first cleavage site comprising an amino acid sequence Z-R-X1-X2-R-X3-X4-X5-X6 (SEQ ID NO: 256), wherein the second polynucleotide is located downstream of the first polynucleotide;

- a third polynucleotide encoding a second cleavage site comprising a 2A polypeptide or a fragment thereof that comprises at least the last 10 amino acids of the 2A polypeptide, wherein the third polynucleotide is located downstream of the second polynucleotide;

- a fourth polynucleotide encoding a second part of the antigen binding molecule, wherein the fourth polynucleotide is located downstream of the third polynucleotide;

- a fifth polynucleotide encoding a third cleavage site comprising a Furin cleavage sequence variant comprising:

(i) a Furin consensus sequence R-X-K-R (SEQ ID NO: 18); or

(ii) a Furin consensus sequence R-X-R-R (SEQ ID NO: 19),

wherein the fifth polynucleotide is located downstream of the fourth polynucleotide; and

- a sixth polynucleotide encoding a membrane anchor polypeptide, wherein the sixth polynucleotide is located downstream of the fifth polynucleotide;

wherein when the first, second and third cleavage sites are cleaved, a secretable antigen binding molecule comprising the first and second parts of the antigen binding molecule is released;

wherein when the first and second cleavage sites are cleaved and the third cleavage site is not cleaved, a membrane-bound antigen binding molecule comprising the first and second parts of the antigen binding molecule, the third cleavage site, and the membrane anchor polypeptide is released,

wherein Z is R, P, G, A, K, N, Q, H, I, L, M, F, S, T, W, Y, or V,

wherein X1, X3, X4, X5 and X6 is R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V, wherein X2 is K or R, and

wherein X is any amino acid.

3. The expression system of claim 1 or 2, wherein Z is R and the amino acid sequence of the first cleavage site comprises an amino acid sequence R-R-X1-X2-R-X3-X4-X5-X6 (SEQ ID NO: 1).

4. The expression system of any one of claims 1-3, wherein X1 is R, and X3, X4, X5, and X6 is any one of P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V.

5. The expression system of any one of claims 1 or 2, wherein the first cleavage site comprises an amino acid sequence set forth in SEQ ID NOs: 2-17 and SEQ ID NOs: 66-160.

6. The expression system of any one of claims 1-3 and 5, wherein X3 is any one of P, G, A, D, E, H, I, L, M, F, or S; X4 is any one of P, A, I, L, T, or V; X5 is any one of R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V; and X6 is any one of R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V.

7. The expression system of any one of claims 1-2 and 5-6, wherein the first cleavage site comprises an amino acid sequence set forth in SEQ ID NOs: 2-17, 66-70, 74-84, 86, 87, 91, 93, 95-99, 104, 106, 115, 116, 120, 123-129, and 130-160.

8. The expression system of any one of claims 1, 2, 5-6 and 7, wherein Z is R, P, G, A, K, N, Q, H, I, L, M, F, S, T, W, Y, or V; X3 is any one of S, A, D, or E; X4 is any one of V, A, I, L or T; X5 is any one of R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V; and X6 is any one of R, P, G, A, D, C, E, K, N, Q, H, I, L, M, F, S, T, W, Y, or V.

9. The expression system of any one of claims 1-8, wherein the first cleavage site comprises an amino acid sequence set forth in SEQ ID NOs 2, 87, 91, 93, 106, 115, 116, and 120.

10. The expression system of any one of claims 1-9, further comprising a polynucleotide encoding a linker SGSG between the second and third polynucleotides.

11. The expression system of claim 2, wherein the fifth polynucleotide encodes a third cleavage site comprising a Furin cleavage sequence variant comprising:

(i) a Furin consensus sequence R-X-K-R (SEQ ID NO: 18) and at least the first five amino acids derived from a 2A polypeptide; or

(ii) a Furin consensus sequence R-X-R-R (SEQ ID NO: 19) and at least the first five amino acids derived from a 2A polypeptide.

12. The expression system of claim 11, wherein the fifth polynucleotide encodes a third cleavage site comprising a Furin cleavage sequence variant comprising:

(i) a Furin consensus sequence R-X-K-R (SEQ ID NO: 18) and the first five amino acids derived from a 2A polypeptide, wherein the first five amino acids derived from the 2A polypeptide comprise one or more point mutations; or

(ii) a Furin consensus sequence R-X-R-R (SEQ ID NO: 19) and the first five amino acids derived from a 2A polypeptide, wherein the first five amino acids derived from the 2A polypeptide comprise one or more point mutations.

13. The expression system of claim 12, wherein the one or more point mutations is selected from the group consisting of A1P, A1G, T2G, T2P, N3P, N3A, F4P, F4A and S5P.

14. The expression system of any one of claims 1-13, wherein the 2A polypeptide is selected from a group consisting of P2A, F2A, E2A, and T2A, wherein optionally the 2A polypeptide is T2A.

15. The expression system of any one of claims 2-14, 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 domain or murin B7-1 antigen, wherein optionally the membrane anchor polypeptide is GPI.

16. The expression system of any one of claims 1-15, wherein the second polynucleotide encodes a first cleavage site having a higher cleavage efficiency of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% compared to an expression system comprising a second polynucleotide that encodes a first cleavage site comprising a Furin consensus sequence R-X-K-R (SEQ ID NO: 18) or R-X-R-R (SEQ ID NO: 19).

17. The expression system of any one of claims 1-16, wherein the expression system is capable of releasing an antigen binding molecule comprising a first part of the antigen binding molecule having the correct polypeptide sequence, wherein the first part of the antigen binding molecule does not contain one or more residual amino acids from the second cleavage site after the cleavage of the first and second cleavage sites.

18. The expression system of any one of claims 1-17, wherein the expression system is capable of improving the homogeneity of the antigen binding molecules produced, wherein homogeneity is achieved when a plurality of antigen binding molecules are produced, wherein each antigen binding molecule in the plurality of antigen binding molecules comprises a first part having the same amino acid sequence and/or molecular weight.

19. The expression system of any one of claims 1-18, wherein the expression system is delivered to a target cell prior to transcription, wherein optionally the target cell is an animal cell, and wherein optionally the animal cell is a mammalian cell.

20. The expression system of any one of claims 1, 2, 5, and 10-19, wherein the second polynucleotide comprises a nucleotide sequence set forth in SEQ ID NOs: 35-50 and SEQ ID NOs: 161-255.

21. A vector comprising the expression system of any one of claims 1-20.

22. A host cell comprising the expression system of any one of claims 1-20 or the vector of claim 21.

23. A kit comprising the expression system of any one of claims 1-20, the vector of claim 21, or the host cell of claim 22.

24. The expression system of any one of claims 1-20, the vector of claim 21, the host cell of claim 22, or the kit of claim 23 for use in screening antibody libraries or antibody production.

25. A method of producing one or more secretable antigen binding molecules and/or one or more membrane-bound antigen binding molecules, comprising culturing the host cell of claim 22 under suitable culture conditions such that one or more secretable antigen binding molecules and/or one or more membrane-bound antigen binding molecules are produced.

26. The method of claim 25, further comprising recovering the one or more secretable antigen binding molecules.

27. A method for detecting the presence of one or more secretable antigen binding molecules and/or one or more membrane-bound antigen binding molecules, the method comprising:

- providing an expression system of any one of claims 1-20;

- delivering said expression system to one or more target cells;

wherein said target cells transcribe said expression system into one or more amino acid sequences,

wherein when all of the cleavage sites in the one or more amino acid sequences are cleaved, one or more secretable antigen binding molecules comprising the first and second parts of the antigen binding molecules are secreted by the target cells,

wherein when the first and second cleavage sites of the one or more amino acid sequences are cleaved and the third cleavage site of the one or more amino acid sequences is not cleaved, one or more membrane-bound antigen binding molecules comprising the first and second parts of the antigen binding molecules are bound to the surface of the target cells; and

- detecting the presence or absence of the one or more secretable antigen binding molecules and/or one or more membrane-bound antigen binding molecules.

28. The method of claim 27, wherein:

(i) the presence or absence of the one or more secretable antigen binding molecules is detected using enzyme-linked immunosorbent assay (ELISA) or alternative binding assay; and/or

(ii) the presence or absence of the one or more membrane-bound antigen binding molecules is detected using flow cytometry analysis, wherein the first part of the antigen binding molecules, the second part of the antigen binding molecules and/or an antigen specific to the antigen binding molecules are stained prior to the flow cytometry analysis.