

[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 ~~aspect~~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 antigen binding polynucleotide encoding a first part of the antigen binding molecule; a cleavage 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); an anchor polynucleotide encoding a membrane anchor polypeptide;

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

thereby secreted by said target cell, and wherein said cleavage sites ~~are~~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

- 5 - 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.

[0012] In yet another ~~aspect~~example, the present disclosure refers to a method for detecting the presence of one or more secreted antibodies and/or one or more surface-bound antibodies
10 in a sample, the method comprising:

- providing an expression system as disclosed herein;
- delivering said expression system to one or more target cells, wherein said target cell transcribes said expression system, wherein once transcribed, said first cleavage site is cleaved in a first plurality of first part of the antigen binding molecule, so that said first plurality of first
15 part of the antigen binding molecule do not comprise said membrane anchor polypeptide, and are thereby secreted by said target cell, and wherein said first and second cleavage sites are 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

- 20 - 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.

[0013] In yet another aspect, the present disclosure refers to the expression system, the vector, the host cell or the kit as disclosed herein for use in screening antibody libraries or
25 antibody production

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying
30 drawings, in which:

[0015] **Fig. 1** shows the schematic of the exemplary expression systems and uses thereof. **Fig. 1A** is a schematic illustrating the exemplary expression systems as disclosed herein. **Fig.**

[0039] In one example, the first cleavage site is a minimal Furin cleavage consensus sequence. The term “Furin” used herein refers to a ubiquitous subtilisin-like proprotein convertase which cleaves proteins containing its recognition site. The terms not limiting to “Furin consensus sequence”, “minimal cleavage site”, “minimal Furin cleavage consensus
 5 sequence” or “Furin recognition site” as used herein refer to an amino acid sequence of RXKR (SEQ ID NO: 1) or RXRR (SEQ ID NO: 2), wherein X can be any amino acids. Furin recognises a protein that comprises a Furin consensus sequence, which leads to the cleavage of the protein that comprises the Furin consensus sequence. The cleavage can take place in, for example, the Golgi.

[0040] In one example, the Furin consensus sequence is selected from a group consisting of RRKR (SEQ ID NO: 3), RRRR (SEQ ID NO: 4), RSKR (SEQ ID NO: 5), RSRR (SEQ ID NO: 6), RKKR (SEQ ID NO: 7), RKRR (SEQ ID NO: 8), RQKR (SEQ ID NO: 9), RQRR (SEQ ID NO: 10), RTKR (SEQ ID NO: 11), RTRR (SEQ ID NO: 12), REKR (SEQ ID NO: 13), RERR (SEQ ID NO: 14), RDKR (SEQ ID NO: 15), RDRR (SEQ ID NO: 16), RHKR
 15 (SEQ ID NO: 17), RHRR (SEQ ID NO: 18), RFKR (SEQ ID NO: 19), RFRR (SEQ ID NO: 20), RAKR (SEQ ID NO: 21), RARR (SEQ ID NO: 22), RNKR (SEQ ID NO: 23), RNRR (SEQ ID NO: 24), RCKR (SEQ ID NO: 25), RCRR (SEQ ID NO: 26), RGKR (SEQ ID NO: 27), RGRR (SEQ ID NO: 28), RIKR (SEQ ID NO: 29), RIRR (SEQ ID NO: 30), RLKR (SEQ ID NO: 31), RLRR (SEQ ID NO: 32), RMKR (SEQ ID NO: 33), RMRR (SEQ ID NO: 34),
 20 RPKR (SEQ ID NO: 35), RPRR (SEQ ID NO: 36), RWKR (SEQ ID NO: 37), RWRR (SEQ ID NO: 38), RYKR (SEQ ID NO: 39), RYRR (SEQ ID NO: 40), RVKR (SEQ ID NO: 41) and RVRR (SEQ ID NO: 42). In a preferred example, the Furin consensus sequence is RRKR (SEQ ID NO: 3).

[0041] The second cleavage polynucleotide of the expression system can be at least 10
 25 nucleotides in length. In another example, the ~~first~~second cleavage polynucleotide can be, but is not limited to, a length of about 10 to 200 nucleotides, or about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, about 140, about 150, about 160, about 170, about 180, about 190 or about 200 nucleotides in length. In a preferred example, the second cleavage polynucleotide is 15 nucleotides in
 30 length. In another preferred example, the second cleavage polynucleotide is 57 nucleotides in length.

[0042] The second cleavage polynucleotide encodes for a second cleavage site. The second cleavage site is a self-processing cleavage site. The term “self-processing cleavage site” as used

herein refers to a peptide sequence that has self-cleaving or self-processing ability, wherein the peptide does not require an external molecule or enzyme such as a protease to cleave the peptide sequence. The second cleavage site can be at least 4 amino acids in length. In another example, the second ~~first~~ cleavage site can be, but is not limited to, a length of about 4 to 50 amino acids, about 10 to 20 amino acids, about 20 to 30 amino acids, about 30 to 40 amino acids, about 40 to 50 amino acids, or about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 amino acid residues in length. In one example, the second ~~first~~ cleavage site is 5 amino acids in length. In another example, the second ~~first~~ cleavage site is 19 amino acids in length.

[0043] In one example, the self-processing second cleavage site is a 2A polypeptide or a fragment thereof. The terms not limiting to “2A polypeptide”, “2A peptide”, “2A protein” used herein refer to a peptide that mediates “self-cleavage” or “self-processing” of proteins during translation in eukaryotic cells. The 2A polypeptide is, for example, usually 18–25 amino-acid (aa) in length, and originates from viruses. 2A polypeptide is capable of self-cleaving, which occurs co-translationally between, for example, between the last two amino acids, glycine and proline. In one example, the 2A polypeptide or a fragment thereof is selected from a group consisting of P2A, F2A, E2A and T2A, or a fragment thereof. F2A is a 2A peptide derived from foot-and-mouth disease virus; E2A is a 2A peptide derived from equine rhinitis A virus; T2A is a 2A peptide derived from Thosea asigna virus; P2A is a 2A peptide derived from porcine teschovirus-1. In another example, the 2A polypeptide or a fragment thereof is a P2A polypeptide or a fragment thereof. In one example, the P2A polypeptide is ATNFSLLKQAGDVEENPGP (SEQ ID NO: 43). In another example, the F2A polypeptide is APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 44). In another example, the E2A polypeptide is QCTNYALLKLAGDVESNPGP (SEQ ID NO: 45). In another example, the T2A polypeptide is EGRGSLLTCGDVEENPGP (SEQ ID NO: 46). The corresponding nucleotide sequences are shown in Table 1 below.

[0044] **Table 1.** Nucleotide and amino acid sequences of P2A, F2A, E2A and T2A.

Item	Nucleotide sequence	Amino acid sequence
P2A	GCT ACT AAC TTC AGC CTG CTG AAG CAG GCT GGA GAC GTG GAG GAG AAC CCT GGG CCC (SEQ ID NO: 47)	ATNFSLLKQAGDVEENPGP (SEQ ID NO: 43)

different cleavage efficiency. The cleavage efficiency of 2A and furin are also affected their flanking amino acids.

[00104] The control targeting vector without the membrane anchor had high level secretion of antibodies into the culture medium but very little display of antibodies on the cell surface (Fig. 5C). In contrast, GPI vector had high display levels but no secretion of antibodies. The RRKR-GPI vector showed higher display levels than the GPI vector but no secretion of antibodies. The flanking amino acids from GPI may inhibit the cleavage of furin at RRKR (SEQ ID NO: 3). Linking HC with GPI through all four 2A peptides resulted in both display and secretion at different ratios. The secreted HC polypeptides from these vectors had a bigger size compared to that expressed by the control vector (Fig. 5C). Peptide mapping analysis indicated they were attached with 2A or 2A-GPI residues. Higher secretion and lower display indicated higher cleavage efficiency of a 2A peptide. The results indicated that amongst the different 2A peptides, E2A showed the highest cleavage efficiency followed by T2A, F2A and P2A. Using RRKR-2A to link HC and membrane anchor resulted in higher secretion and lower display for P2A, ~~E2A-F2A~~ and T2A but not for E2A. The secretion level from RRKR-P2A-GPI, RRKR-F2A-GPI and RRKR-T2A-GPI had been increased to a level close to the control vector. The HC polypeptides secreted from RRKR-F2A-GPI, RRKR-~~ET~~2A-GPI and RRKR-T2A-GPI vectors still contained a significant proportion of species attached with 2A or 2A-GPI residues. Only RRKR-P2A-GPI vector produced antibodies without the incorrect species (Fig. 5C). However, the display level from this vector was too low to be separated the stained cells from the blank cells.

[00105] **Engineering P2A peptide and furin recognition sequence for mediating the ratios of membrane bound to secretory antibodies**

[00106] To increase the ratios of display to secretion of antibodies from the RRKR-P2A-GPI vector, point mutations of P2A were created, wherein the point mutations include the individual residue to glycine, proline or alanine (Fig. 6). Proline and glycine influence secondary structure, constraining or providing high flexibility to peptide chains, respectively. In contrast, alanine has little influence on secondary structure. Another set of targeting vector containing P2A with same mutations were tested to understand how the mutations affect the cleavage of efficiency of P2A. Nine point mutations, A1G, T2G, A1P, T2P, N3P, F4P, S5P, N3A and F4A increased the display level from the RRKR-P2A-GPI vectors compared the wild type P2A. The level of expression is provided in Table 2. The increased level varied depending on the

Claims

1. 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 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) 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 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) 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.

2. The expression system of claim 21, further comprising a second antigen binding polynucleotide encoding a second part of the antigen binding molecule.

3. The expression system of claim ~~2-1~~ or ~~32~~, wherein the cleavage site polynucleotide encodes the cleavage site sequence RRKRGTNFS (SEQ ID NO: 62) or RRRRGTNFS (SEQ ID NO: 72)~~2A polypeptide fragment thereof comprises one or more mutations in amino acid residue 1, 2, 3, 4 or 5.~~
4. The expression system of ~~any one of~~ claims ~~1-3~~ or ~~2~~, wherein the cleavage site polynucleotide encodes the cleavage site sequence RRKRAPNFS (SEQ ID NO: 68) or RRRRAPNFS (SEQ ID NO: 78)~~2A polypeptide fragment thereof is selected from a group consisting of a P2A, F2A, E2A and T2A fragment thereof.~~
5. The expression system of claim 1 or 2, wherein the cleavage site polynucleotide encodes the cleavage site sequence RRKRATNFP (SEQ ID NO: 67) or RRRRATNFP (SEQ ID NO: 77).
- ~~5.6.~~ The expression system of any one of claims ~~1-45~~, wherein the first part of the antigen binding molecule is an antibody heavy chain~~amino acid is mutated to glycine, proline or alanine.~~
- ~~6.7.~~ The expression system of any one of claims ~~42-56~~, wherein the second part of the antigen binding molecule is an antibody light chain~~one or more mutations is selected from the group consisting of A1P, A1G, T2G, T2P, N3P, F4P, S5P, N3A and F4A.~~
- ~~7.8.~~ The expression system of any one of claims ~~1 to 7~~, wherein the membrane anchor polypeptide is glycopospholipid 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.
9. The expression system of any one of claims 1 to 3 or 6 to 8, wherein the expression system comprises: a second antigen binding polynucleotide encoding a light chain antibody; a first antigen binding polynucleotide encoding a heavy chain antibody; a

cleavage polynucleotide encoding RRKRGTNFS (SEQ ID NO: 62); and an anchor polynucleotide encoding a membrane anchor polypeptide.

10. The expression system of any one of claims 1, 2, 4 or 6 to 8, wherein the expression system comprises: a second antigen binding polynucleotide encoding a light chain antibody; a first antigen binding polynucleotide encoding a heavy chain antibody; a cleavage polynucleotide encoding RRKRAPNFS (SEQ ID NO: 68); and an anchor polynucleotide encoding a membrane anchor polypeptide.

11. The expression system of any one of claims 1, 2 or 5 to 8, wherein the expression system comprises: a second antigen binding polynucleotide encoding a light chain antibody; a first antigen binding polynucleotide encoding a heavy chain antibody; a cleavage polynucleotide encoding RRKRATNFP (SEQ ID NO: 67); and an anchor polynucleotide encoding a membrane anchor polypeptide.

~~8. 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 first cleavage polynucleotide encoding a first cleavage site, wherein the first cleavage site is a minimal Furin cleavage consensus sequence RXKR (SEQ ID NO: 1) or RXRR (SEQ ID NO: 2);~~

~~— a second cleavage polynucleotide encoding a self-processing second cleavage site, wherein the self-processing second cleavage site is a 2A polypeptide or a fragment thereof;~~

~~— an anchor polynucleotide encoding a membrane anchor polypeptide;~~

~~wherein the 2A polypeptide or the fragment thereof comprises one or more mutations in any one of the amino acid residue to control the cleavage efficiencies of the first cleavage site and the second cleavage site to modulate ratio of production of the secretable antigen binding molecule versus the membrane-bound antigen binding molecule;~~

~~wherein the first and second cleavage polynucleotides are in between the first antigen binding polynucleotide and the anchor polynucleotide;~~

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

wherein when the first and second cleavage sites are not cleaved, the membrane-bound antigen binding molecule comprising the first part of the antigen binding molecule, the minimal Furin cleavage consensus sequence RXKR (SEQ ID NO: 1) or RXRR (SEQ ID NO: 2), the 2A polypeptide or a fragment thereof, and the membrane anchor polypeptide, is released.

9. ~~The expression system of claim 8, further comprising a second antigen binding polynucleotide encoding a second part of the antigen binding molecule.~~

10. ~~The expression system of claim 8 or 9, wherein the 2A polypeptide or a fragment thereof comprises one or more mutations in amino acid residue 1, 2, 3, 4 or 5.~~

11. ~~The expression system of any one of claims 8-10, wherein the 2A polypeptide or a fragment thereof is selected from a group consisting of P2A, F2A, E2A and T2A, or a fragment thereof.~~

12. ~~The expression system of any one of claims 8-11, wherein the amino acid is mutated to glycine, proline or alanine.~~

13. ~~The expression system of any one of claims 8-12, wherein the one or more mutations is selected from the group consisting of A1P, A1G, T2G, T2P, N3P, F4P, S5P, N3A and F4A.~~

14. ~~The expression system of claim 8, wherein the membrane anchor polypeptide is glycephospholipid 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.~~

15. 12. A vector comprising the expression system of any one of claims 1-~~14~~11.

~~16.13.~~ A host cell comprising the expression system of any one of claims 1-~~14~~11 or the vector of claim ~~15~~12.

~~17.14.~~ A kit comprising the expression system of any one of claims 1-~~14~~11, the vector of claim ~~15~~12, or the host cell of claim ~~16~~13.

~~18.15.~~ 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-~~7~~11;
- 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 sites ~~are~~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.

~~19. 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 8-14;~~
- ~~— delivering said expression system to one or more target cells, wherein said target cell transcribes said expression system, wherein once transcribed, said first 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 first and second cleavage sites are 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.

2016. The expression system of any one of claims 1-1411, the vector of claim 1512, the host cell of claim 1613 or the kit of claim 1714 for use in screening antibody libraries or antibody production.