differences between nucleotides could be improved further. Accordingly, there is a need to identify novel ways to improve nanopore sensing features.

SUMMARY

The disclosure relates to modified CsgF peptides, in particular truncated CsgF fragments, binding the CsgG pore and thereby introducing another additional channel or pore constriction within the CsgG pore. Other examples of the disclosure also relate to an isolated transmembrane pore complex, and the use of said CsgG:CsgF complexes and modified CsgF peptides or fragments in a nanopore sensing platform with two consecutive reader heads.

In one aspect, the present disclosure relates to a pore comprising a CsgG pore and a modified CsgF peptide, wherein the modified CsgF peptide is bound to CsgG and forms a constriction in the pore. In another aspect, the present disclosure relates to a pore as described herein, which is comprised in a membrane.

In another aspect, the present disclosure relates to a method for producing a pore as described herein, the method comprising: a. co-expressing one or more CsgG monomers and a CsgF peptide in a host cell, thereby allowing transmembrane pore complex formation in the cell; or b. contacting one or more purified CsgG monomers with a modified CsgF peptide, thereby allowing *in vitro* formation of the pore. In yet another aspect, the present disclosure relates to a method for producing a pore as described herein, wherein: a. the modified CsgF peptide is truncated; or b. the method comprises expressing a modified CsgF peptide comprising an enzyme cleavage site positioned such that cleavage of the CsgF peptide produces a truncated CsgF peptide comprising a CsgG-binding region and a region that forms a constriction in the pore, and wherein the method comprises cleaving the CsgF peptide. In yet another aspect, the present disclosure relates to a method for determining the presence, absence or one or more characteristics of a target analyte, comprising the steps of: (i) contacting the target analyte with a pore as described herein, such that the target analyte moves into the pore complex; and (ii) taking one or more measurements as the analyte moves through the pore complex and thereby determining the presence, absence or one or more characteristics of the analyte. In yet another aspect, the present disclosure relates to a method as described herein, wherein: a.the analyte is a (poly)peptide, a polysaccharide, a small organic or inorganic compound, such as pharmacologically active compounds, toxic compounds and pollutants; or b. wherein the analyte is a polynucleotide. In yet another aspect, the present disclosure relates to a method as described herein, wherein: a. the polynucleotide comprises at least one homopolymeric region; and/or b. the method comprises determining one or more characteristics selected from (i) the length of the polynucleotide, (ii) the identity of the polynucleotide, (iii) the sequence of the polynucleotide, (iv) the secondary structure of the polynucleotide and (v) whether or not the polynucleotide is modified. In yet another aspect, the present disclosure relates to a method of characterising a polynucleotide or a (poly)peptide using a pore as described herein.

In yet another aspect, the present disclosure relates to the use of the pore as described herein to determine the presence, absence or one or more characteristics of a target analyte. In yet another aspect, the present disclosure relates to a kit for characterising a target analyte comprising (a) pore as described herein and (b) the components of a membrane. In yet another aspect, the present disclosure

relates to a CsgF peptide that is a modified CsgF peptide comprising a CsgG-binding region, and a region that forms a constriction in the pore.

In yet another aspect, the present disclosure relates to a CsgF peptide as described herein which comprises: a. the amino acid sequence from residue 1 of SEQ ID NO: 6 up to any one of residues 28 to 45 of SEQ ID NO: 6, or a homologue or variant thereof; and/or b. SEQ ID NO: 39 (residues 1 to 29 of SEQ ID NO: 6), or a homologue or variant thereof; or c.which comprises SEQ ID NO: 15 (residues 1 to 34 of SEQ ID NO: 6), SEQ ID NO: 54 (residues 1 to 30 of SEQ ID NO: 6), SEQ ID NO: 40 (residues 1 to 45 of SEQ ID NO: 6), or SEQ ID NO: 55 (residues 1 to 35 of SEQ ID NO: 6), or a homologue or variant thereof. In yet another aspect, the present disclosure relates to a CsgF peptide as described herein, wherein: a. one or more residues in the region of between residues 1 and 28 to 45 of SEQ ID NO: 6, SEQ ID NO: 15, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 54, or SEQ ID NO: 55(residues 1 to 35) are modified, and optionally the peptide comprises a modification at one or more of the following positions: G1, T4, F5, R8, N9, N11, F12, A26 and Q29; or b. the modification is the introduction of a cysteine, a hydrophobic amino acid, a charged amino acid, a non-native reactive amino acid, or photoreactive amino acid; and/or c. the peptide comprises a modification at one or more of the following positions: N15, N17, A20, N24, A28 and D34, and optionally the peptide comprises one or more of the substitutions: N15S/A/T/Q/G/L/V/I/F/Y/W/R/K/D/C, N17S/A/T/Q/G/L/V/I/F/Y/W/R/K/D/C, A20S/T/Q/N/G/L/V/I/F/Y/W/R/K/D/C, N24S/T/Q/A/G/L/V/I/F/Y/W/R/K/D/C, A28S/T/Q/N/G/L/V/I/F/Y/W/R/K/D/C, and D34F/Y/W/R/K/N/Q/C; and/or d. the peptide comprises one or more of the following substitutions: G1C, T4C, N17S, and D34Y or D34N.

In yet another aspect, the present disclosure relates to a CsgF peptide as described herein, which further comprises: a. all or part of an enzyme cleavage site at the C-terminal end; and/or b. a signal peptide. In yet another aspect, the present disclosure relates to polynucleotide which encodes a CsgF peptide as described herein. In yet another aspect, the present disclosure relates to a pore complex comprising: (i) a CsgG pore comprising a first opening, a mid-section comprising a beta barrel, a second opening, and a lumen extending from the first opening through the mid-section to the second opening, wherein a luminal surface of the mid-section defines a CsgG constriction; and (ii) a plurality of modified CsgF peptides, each having a CsgF constriction region and a CsgG binding region, wherein the modified CsgF peptides form a CsgF constriction within the beta barrel of the CsgG pore and wherein the CsgG constriction and the CsgF constriction are co-axially spaced apart within the beta barrel of the CsgG pore.

The first example of the disclosure relates to a pore comprising a CsgG pore and a CsgF peptide. In one example, the CsgF peptide comprises a CsgG-binding region, and a region that forms a constriction in the pore. In one example the CsgF peptide is a truncated CsgF peptide lacking the C-terminal head domain of CsgF. In another example the CsgF peptide is a truncated CsgF peptide lacking the C-terminal head and a portion of the neck domain of CsgF. In another example the CsgF peptide is a truncated CsgF peptide lacking the C-terminal head and neck domains of CsgF. The pore is also referred to herein as a pore complex and as an isolated pore complex. The isolated pore complex comprising a CsgG pore, or a homologue or mutant thereof, and a modified CsgF peptide, or a homologue or mutant thereof, in particular truncated CsgF fragments, or homologues or mutants thereof. In one example, said modified CsgF peptide, or homologues or mutants, is located in the lumen

of the CsgG pore, or homologues or mutants thereof. In another example, said isolated pore complex has two or more channel constrictions, one located or provided by the CsgG pore, formed by its constriction loop, and another additional channel constriction or reader head, introduced by the modified CsgF peptide or its homologues or mutants. In one example, said CsgG-pore or CsgG-like pore, is not a wild-type pore, it is a mutant CsgG pore, with in particular examples mutations being present, for example, in said channel constriction loop. In another example, the isolated pore complex, comprising the modified CsgF peptide, or a homologue or mutant thereof, has a CsgF channel constriction with a diameter in the range from 0.5 nm to 2.0 nm. In one example, the pore complex comprises: (i) a CsgG pore comprising a first opening, a mid-section comprising a beta barrel, a second opening, and a lumen extending from the first opening through the mid-section to the second opening, wherein a luminal surface of the mid-section defines a CsgG constriction; and (ii) a plurality of modified CsgF peptides, each having a CsgF constriction region and a CsgF binding region (also referred to herein as a CsgG-binding domain or region of CsgF), wherein the modified CsgF peptides form a CsgF constriction within the beta barrel of the CsgG pore and wherein the CsgG constriction and the CsgF constriction are co-axially spaced apart within the beta barrel of the CsgG pore. The luminal surface of the CsgG pore may comprise one or more loop regions of CsgG monomers that define the CsgG constriction. The CsgF constriction region and the CsgF binding region typically correspond to a N-terminal portion of a CsgF mature peptide. In one example, the pore complex excludes CsgA, CsgB and CsgE.

In a second example, the disclosure relates to a modified CsgF peptide, or a homologue or mutant thereof, wherein the protein or peptide is modified via a truncation or deletion of part of the protein, resulting in a CsgF fragment of SEQ ID NO:6 or of a homologue or mutant thereof. One example relates to a modified or truncated CsgF peptide, or a modified peptide of a CsgF homologue or mutant, said modified peptide comprising SEQ ID NO:39, or SEQ ID NO:40, or a homologue or mutant thereof, alternatively, said modified peptide comprises SEQ ID NO:15, or a homologue or mutant thereof, alternatively SEQ ID NO: 54, or SEQ ID NO: 55 or a homologue or mutant thereof . Another example discloses a modified CsgF peptide wherein one or more positions in the region comprising SEQ ID NO:15 are modified, and wherein said mutation(s) required to retain a minimal of 35 % amino acid identity to SEQ ID NO:15, in the peptide fragment corresponding to the region comprising SEQ ID NO:15.

One example relates to a pore comprising a CsgG pore and a modified CsgF peptide, wherein the modified CsgF peptide is bound to CsgG and forms a constriction in the pore

One example relates to a polynucleotide encoding said modified CsgF peptide, or homologue or mutant thereof, according to the second example of the disclosure.In another example, the isolated pore complex comprising a CsgG pore and a modified CsgF peptide, or homologues or mutants thereof, are characterized in that said modified CsgF peptide is a peptide provided by the peptides disclosed in the second example of the disclosure.

Another example relates to the isolated pore complex wherein the modified CsgF peptide and the CsgG pore or a monomer of said pore, or homologues or mutants thereof, are covalently coupled. And even more particularly, said coupling is made via a cysteine residue or via a non-native reactive or photo-reactive amino acid in a CsgG monomer at a position corresponding to 132, 133, 136, 138, 140,

142, 144, 145, 147, 149, 151, 153, 155, 183, 185, 187, 189, 191, 201, 203, 205, 207 or 209 of SEQ ID NO: 3, or of a homologue thereof.

A preferred example relates to an isolated transmembrane pore complex, or a membranous composition, which comprises the isolated pore complex of the disclosure, and the components of a membrane. Particularly, said transmembrane pore complex or membranous composition consists of the isolated pore complex of the disclosure, and the components of a membrane or an insulating layer.

One example relates to a method for producing a pore as disclosed herein, the method comprising co-expressing one or more CsgG monomers as disclosed herein and a CsgF peptide as disclosed herein in a host cell, thereby allowing transmembrane pore complex formation in the cell. The CsgF peptide may be produced in the cell by cleaving a modified CsgF peptide or protein comprising an enzyme cleavage site at a suitable position in the amino acid sequence.

One example relates to a method for producing a pore as disclosed herein, the method comprising contacting one or more purified CsgG monomers with one or more purified modified CsgF peptides, thereby allowing *in vitro* formation of the pore. The modified CsgF peptide may be a peptide comprising an enzyme cleavage site at a suitable position in the amino acid sequence, that is cleaved before or after formation of the pore.

A third example of the disclosure relates to a method for producing said transmembrane pore complex, wherein the pore is an isolated complex formed by a CsgG pore, or a homologue or mutant thereof, and a modified CsgF peptide, or a homologue or mutant thereof, the method comprising the steps of co-expressing CsgG SEQ ID NO:2, or a homologue or mutant thereof, and modified or truncated CsgF, comprising a fragment of SEQ ID NO:5, or a homologue or mutant thereof, in a suitable host cell, thereby allowing *in vivo* pore complex formation. In specific examples, said modified CsgF peptide, or homologue or mutant thereof, comprises SEQ ID NO: 12 or SEQ ID NO:14, or a homologue or mutant thereof. Alternatively, a method for producing an isolated pore complex comprises the steps of contacting CsgG monomers of SEQ ID NO:3, or a homologue or mutant thereof, with modified CsgF peptide(s), or a homologue or mutant thereof, for *in vitro* reconstitution of the pore complex. In particular examples, modified CsgF peptides of said method comprise SEQ ID NO:15 or SEQ ID NO:16, or homologues or mutants thereof.

Another example of the disclosure relates to a method for determining the presence, absence or one or more characteristics of a target analyte, comprising the steps of:

(i) contacting the target analyte with said isolated pore complex or transmembrane pore complex, such that the target analyte moves into the pore channel; and

(ii) taking one or more measurements as the analyte moves through the pore channel and thereby determining the presence, absence or one or more characteristics of the analyte.

In one example, said analyte is a polynucleotide. In particular, said method using a polynucleotide as an analyte alternatively comprises determining one or more characteristics selected from (i) the length of the polynucleotide, (ii) the identity of the polynucleotide, (iii) the sequence of the polynucleotide, (iv) the secondary structure of the polynucleotide and (v) whether or not the polynucleotide is modified.

In another example, the analyte is a protein, or peptide, and in further examples, said analyte is a polysaccharide, or a small organic or inorganic compound, such as for instance but not limited to pharmacologically active compounds, toxic compounds and pollutants.

In another example, a method for characterising a polynucleotide or a (poly)peptide using an isolated transmembrane pore complex is described, wherein the pore complex is an isolated complex comprising a CsgG pore, or a homologue or mutant thereof, and a modified CsgF peptide, or a homologue or mutant thereof. In particular, said CsgG pore, or homologue or mutant thereof, comprising six to ten CsgG monomers forming the CsgG pore channel.

A further example of the disclosure discloses the use of said isolated pore complex or transmembrane pore complex according to the previous examples of the disclosure to determine the presence, absence or one or more characteristics of a target analyte. Furthermore, the disclosure also relates to a kit for characterising a target analyte comprising (a) said isolated pore complex and (b) the components of a membrane.

DESCRIPTION OF THE FIGURES

The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes.

Figure 1. Structure of CsgG pore and the interface for complex formation with CsgF. Cross-sectional **(A)**, side **(B)** and top **(C)** views of CsgG oligomers (e.g., nonamers) (gold) in surface (A) and ribbon (B, C) representation, with a single CsgG protomer colored light blue **(D)** (based on the CsgG X-

CLAIMS

1. A pore comprising a CsgG pore and a modified CsgF peptide, wherein the modified CsgF peptide is bound to CsgG and forms a constriction in the pore.

2. The pore according to claim 1, wherein the CsgF peptide is a truncated CsgF peptide lacking the C-terminal head domain of CsgF and at least part of the neck domain of CsgF.

3. The pore according to claim 1 or 2, wherein the CsgF peptide has a length of from 25 to 50 amino acids.

4. The pore according to any one of claims 1 to 3, wherein the CsgF peptide comprises the amino acid sequence of SEQ ID NO: 6 from residue 1 up to any one of residues 28 to 45 of SEQ ID NO: 6, or the corresponding residues from a homologue of SEQ ID NO: 6, or variant of either thereof.

5. The pore according to any one of the preceding claims, wherein the CsgF peptide comprises SEQ ID NO: 39 (residues 1 to 29 of SEQ ID NO: 6), or a homologue or variant thereof, and optionally wherein the CsgF peptide comprises SEQ ID NO: 15 (residues 1 to 34 of SEQ ID NO: 6), SEQ ID NO: 40 (residues 1 to 45 of SEQ ID NO: 6), SEQ ID NO: 54 (residues 1 to 30 of SEQ ID NO: 6), or SEQ ID NO: 55 (residues 1 to 35 of SEQ ID NO: 6), or a homologue or variant thereof.

6. The pore according to claim 5, wherein in the CsgF peptide one or more residues in SEQ ID NO: 15, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 54, or SEQ ID NO: 55 are modified, and optionally wherein the CsgF peptide comprises a modification at one or more of the following positions: G1, T4, F5, R8, N9, N11, F12, A26 and Q29.

7. The pore according to claim 6, wherein the modification is the introduction of a cysteine, a hydrophobic amino acid, a charged amino acid, a non-native reactive amino acid, or photoreactive amino acid.

8. The pore according to claim 6 or 7, wherein the CsgF peptide comprises a modification at one or more of the following positions: N15, N17, A20, N24, A28 and D34.

9. The pore according to claim 8, wherein the CsgF peptide comprises one or more of the substitutions: N15S/A/T/Q/G/L/V/I/F/Y/W/R/K/D/C, N17S/A/T/Q/G/L/V/I/F/Y/W/R/K/D/C, A20S/T/Q/N/G/L/V/I/F/Y/W/R/K/D/C, N24S/T/Q/A/G/L/V/I/F/Y/W/R/K/D/C, A28S/T/Q/N/G/L/V/I/F/Y/W/R/K/D/C, and D34F/Y/W/R/K/N/Q/C.

10. The pore according to any one of claims 6 to 9, wherein the CsgF peptide comprises one or more of the following substitutions: G1C, T4C, N17S, and D34Y or D34N.

11. The pore according to any one of claims 6 to 10, wherein the CsgF peptide further comprises all or part of an enzyme cleavage site at the C-terminal end.

12. The pore according to any one of the preceding claims, wherein:

a. the truncated CsgF peptide is inserted into the lumen of the CsgG pore; and/or

b. the CsgG pore comprises from 6 to 10 CsgG monomers; and/or

c. the ratio of CsgG monomers to truncated CsgF peptides in the pore is 1:1; and/or

d. the CsgF peptide and the CsgG pore are covalently coupled.

13. The pore according to claim 12, wherein:

I. the covalent coupling is via:

(i) a cysteine residue at a position corresponding to 132, 133, 136, 138, 140, 142, 144, 145, 147, 149, 151, 153, 155, 183, 185, 187, 189, 191, 201, 203, 205, 207 or 209 of SEQ ID NO: 3, or of a homologue thereof;

(ii) a non-native reactive or photoreactive amino acid at a position corresponding to 132, 133, 136, 138, 140, 142, 144, 145, 147, 149, 151, 153, 155, 183, 185, 187, 189, 191, 201, 203, 205, 207 or 209 of SEQ ID NO: 3, or of a homologue thereof; and/or

II. the CsgF peptide and the CsgG pore are covalently linked via residues at a position corresponding to one or more of the following pairs of positions of SEQ ID NO: 6 and SEQ ID NO: 3, respectively: 1 and 153, 4 and 133, 5 and 136, 8 and 187, 8 and 203, 9 and 203, 11 and 142, 11 and 201, 12 and 149, 12 and 203, 26 and 191, and 29 and 144; and/or

III. the covalent linkage is a disulphide bond, or click chemistry.

14. The pore according to any one of the preceding claims, wherein:

I. the interaction between the CsgF peptide and the CsgG pore is stabilised by hydrophobic interactions, or electrostatic or covalent interactions at a position corresponding to one or more of the following pairs of positions of SEQ ID NO: 6 and SEQ ID NO: 3, respectively: 1 and 153, 4 and 133, 5 and 136, 8 and 187, 8 and 203, 9 and 203, 11 and 142, 11 and 201, 12 and 149, 12 and 203, 26 and 191, and 29 and 144; and/or

II. the CsgG pore comprises at least one monomer comprising one or more modification corresponding to the following modifications in SEQ ID NO:3:

(i) a modification at one or more positions Y51, N55 and F56;

(ii) at least one substitution selected from R97W or R97Y and R93W or R93Y;

(iii) deletion of V105, A106 and I107 of SEQ ID NO:3;

(iv) deletion of one or more of positions R192, F193, I194, D105, Y196, Q197, R198, L199 and E201 of SEQ ID NO:3;

(v) at least one substitution selected from K94N/Q/R/F/Y/W/L/S, D43S, E44S, F48S/N/Q/Y/W/I/V/H/R/K, Q87N/R/K, N91K/R, R97F/Y/W/V/I/K/S/Q/H, E101I/L/A/H, N102K/Q/L/I/V/S/H, R110F/G/N, Q114R/K, R142Q/S, T150Y/A/V/L/S/Q/N; and/or

(vi) a modification at one or more of positions I41, R93, A98, Q100, G103, T104, A106, I107, N108, L113, S115, T117, Y130, K135, E170, S208, D233, D238, E244, Q42, E44, L90, N91, I95, A99, E101 and Q114.

15. The pore according to claim 14, wherein the CsgG pore comprises at least one monomer comprising one or more modification corresponding to the following modifications in SEQ ID NO:3:

(i) at least one substitution selected from Y51A/I/V/S/T, N55A/I/V/S/T and F56/A/I/V/S/T/Q;

(ii) the substitution R97W;

(iii) deletion of F193, I194, D195, Y196, Q197, R198 and L199 or deletion of D195, Y196, Q197, R198 and L199;

(iv) deletion of V105, A106 and I107;

(v) a substitution selected from K94Q and K94N;

(vi) at least one substitution selected from Q42K or Q42R; E44N or E44Q; L90R or L90K; N91R or N91K; I95R or I95K; A99R or A99K; E101H, E101K, E101N, E101Q or E101T; and/or Q114K; and/or

(vii) the substitution N55V.

16. The pore of any one of the preceding claims, wherein:

a. the CsgG pore comprises at least one monomer that comprises R or K at a position corresponding to R192 of SEQ ID NO:3; and/or

b. the pore is a double pore comprising two CsgG pores, wherein the CsgF peptide is inserted into the lumen of at least one of the CsgG pores.

17. A pore according to any one of the preceding claims, which is comprised in a membrane.

18. A method for producing a pore according to any one of claims 1 to 17, the method comprising:

a. co-expressing one or more CsgG monomers and a CsgF peptide in a host cell, thereby allowing transmembrane pore complex formation in the cell; or

b. contacting one or more purified CsgG monomers with a modified CsgF peptide, thereby allowing *in vitro* formation of the pore.

19. A method for producing a pore according to claim 18, wherein:

a. the modified CsgF peptide is truncated; or

b. the method comprises expressing a modified CsgF peptide comprising an enzyme cleavage site positioned such that cleavage of the CsgF peptide produces a truncated CsgF peptide comprising a CsgG-binding region and a region that forms a constriction in the pore, and wherein the method comprises cleaving the CsgF peptide.

20. The method according to claim 18 or 19, wherein the modified CsgF peptide comprises SEQ ID NO:15 or SEQ ID NO:16, or a homologue or mutant thereof.

21. A method for determining the presence, absence or one or more characteristics of a target analyte, comprising the steps of:

(i) contacting the target analyte with a pore according to any one of claims 1 to 17, such that the target analyte moves into the pore complex; and

(ii) taking one or more measurements as the analyte moves through the pore complex and thereby determining the presence, absence or one or more characteristics of the analyte.

22. A method according to claim 21, wherein:

a. the analyte is a (poly)peptide, a polysaccharide, a small organic or inorganic compound, such as pharmacologically active compounds, toxic compounds and pollutants; or

b. wherein the analyte is a polynucleotide.

23. A method according to claim 22(b), wherein:

a. the polynucleotide comprises at least one homopolymeric region; and/or

b. the method comprises determining one or more characteristics selected from (i) the length of the polynucleotide, (ii) the identity of the polynucleotide, (iii) the sequence of the polynucleotide, (iv) the secondary structure of the polynucleotide and (v) whether or not the polynucleotide is modified.

24. A method of characterising a polynucleotide or a (poly)peptide using a pore according to any one of claims 1 to 17.

25. Use of the pore according to any one of claims 1 to 17 to determine the presence, absence or one or more characteristics of a target analyte.

26. A kit for characterising a target analyte comprising (a) pore according to any one of claims 1 to 17 and (b) the components of a membrane.

27. A CsgF peptide that is a modified CsgF peptide comprising a CsgG-binding region, and a region that forms a constriction in the pore.

28. The CsgF peptide according to claim 27, which is a truncated CsgF peptide lacking the C-terminal head domain of CsgF.

29. The CsgF peptide according to claim 28, which:

a. lacks at least part of the neck domain of CsgF, and optionally

which lacks the C-terminal head domain of CsgF and the neck domain of CsgF; and/or

b. has a length of from 25 to 50 amino acids.

30. A CsgF peptide according to any one of claims 27 to 29, which comprises:

a. the amino acid sequence from residue 1 of SEQ ID NO: 6 up to any one of residues 28 to 45 of SEQ ID NO: 6, or a homologue or variant thereof; and/or

b. SEQ ID NO: 39 (residues 1 to 29 of SEQ ID NO: 6), or a homologue or variant thereof; or

c. which comprises SEQ ID NO: 15 (residues 1 to 34 of SEQ ID NO: 6), SEQ ID NO: 54 (residues 1 to 30 of SEQ ID NO: 6), SEQ ID NO: 40 (residues 1 to 45 of SEQ ID NO: 6), or SEQ ID NO: 55 (residues 1 to 35 of SEQ ID NO: 6), or a homologue or variant thereof.

31. A CsgF peptide according to claim 30, wherein:

a. one or more residues in the region of between residues 1 and 28 to 45 of SEQ ID NO: 6, SEQ ID NO: 15, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 54, or SEQ ID NO: 55(residues 1 to 35) are modified, and optionally

the peptide comprises a modification at one or more of the following positions: G1, T4, F5, R8, N9, N11, F12, A26 and Q29; or

b. the modification is the introduction of a cysteine, a hydrophobic amino acid, a charged amino acid, a non-native reactive amino acid, or photoreactive amino acid; and/or

c. the peptide comprises a modification at one or more of the following positions: N15, N17, A20, N24, A28 and D34, and optionally

the peptide comprises one or more of the substitutions: N15S/A/T/Q/G/L/V/I/F/Y/W/R/K/D/C, N17S/A/T/Q/G/L/V/I/F/Y/W/R/K/D/C, A20S/T/Q/N/G/L/V/I/F/Y/W/R/K/D/C, N24S/T/Q/A/G/L/V/I/F/Y/W/R/K/D/C, A28S/T/Q/N/G/L/V/I/F/Y/W/R/K/D/C, and D34F/Y/W/R/K/N/Q/C; and/or

d. the peptide comprises one or more of the following substitutions: G1C, T4C, N17S, and D34Y or D34N.

32. A CsgF peptide according to any one of claims 27 to 31, which further comprises:

a. all or part of an enzyme cleavage site at the C-terminal end; and/or

b. a signal peptide.

33. A polynucleotide which encodes a CsgF peptide according to any one of claims 27 to 31.

34. A pore complex comprising:

(i) a CsgG pore comprising a first opening, a mid-section comprising a beta barrel, a second opening, and a lumen extending from the first opening through the mid-section to the second opening, wherein a luminal surface of the mid-section defines a CsgG constriction; and

(ii) a plurality of modified CsgF peptides, each having a CsgF constriction region and a CsgG binding region, wherein the modified CsgF peptides form a CsgF constriction within the beta barrel of the CsgG pore and wherein the CsgG constriction and the CsgF constriction are co-axially spaced apart within the beta barrel of the CsgG pore.

35. The pore complex of claim 34, wherein:

a. the luminal surface comprises one or more loop regions of CsgG monomers that define the CsgG constriction; or

b. the CsgF constriction region and the CsgG binding region correspond to a N-terminal portion of a CsgF mature peptide; or

c. the pore complex excludes CsgA, CsgB and CsgE; or

d. the CsgF peptides are covalently coupled to the CsgG pore.