**CLAIMS:**

1. A method of screening a polypeptide for a desired activity against a target molecule, the method comprising:

a) culturing a Gram-negative bacterial cell comprising an exogenous polynucleotide encoding the polypeptide such that the polypeptide is produced in the cell,

b) allowing a lysis-defective phage to package the polynucleotide encoding the polypeptide, wherein the lysis-defective phage is retained within the bacterial cell,

c) permeabilising:

i) the outer membrane of the bacterial cell, or

ii) the inner and outer membranes of the bacterial cell,

d) contacting the bacterial cell with the target molecule, and

e) screening the polypeptide for the desired activity,

wherein the polypeptide is retained within the bacterial cell by the bacterial cell wall or inner membrane and/or the polypeptide is attached to the bacterial cell wall or inner membrane.

2. The method of claim 1, comprising permeabilising the inner and outer membranes of the bacterial cell.

3. The method of claim 1 or claim 2, wherein the polypeptide is associated with at least a second polypeptide to form a protein complex that is retained within the permeabilised bacterial cell and/or is attached to the bacterial cell wall.

4. The method of claim 3, wherein the polypeptide is fused to the second polypeptide or a subunit thereof.

5. The method of claim 3 or claim 4, wherein the second polypeptide is selected from:

i) a polypeptide having a molecular size such that the protein complex is retained inside the permeabilised bacterial cell wall;

ii) a DNA-binding protein;

iii) a bacterial cell wall-binding protein; and/or

iv) a phage coat protein of the lysis-defective phage.

6. The method of any one of claims 2 to 5, wherein the inner and outer bacterial membranes are permeabilised with one or more detergents or an organic solvent.

7. The method of claim 6, wherein the detergent is a non-ionic detergent.

8. The method of claim 7, wherein the non-ionic detergent is selected from Decanoyl-N-methylglucamide (Mega10), demithyloctylphosphine oxide (Apo8), n-octyl-β-D-thioglucopyranoside (8TGP), and a mixture of Decanoyl-N-methylglucamide (Mega10) and demithyloctylphosphine oxide (Apo8).

9. The method of claim 6, wherein the organic solvent is chloroform.

10. The method of claim 9, wherein the inner and outer membranes of the bacterial cell are permeabilised by incubating the bacterial cell in an aqueous solution saturated with chloroform.

11. The method of claim 10, wherein the bacterial cell is incubated in the aqueous solution saturated with chloroform for about 10 minutes at about 25°C.

12. The method of claim 1, wherein:
i) the bacterial outer membrane is permeabilised;
ii) the bacterial cell wall is at least partially hydrolysed; and
iii) the polypeptide is attached to the inner membrane.

13. The method of claim 12, wherein the bacterial cell wall is at least partially hydrolysed with lysozyme.

14. The method of claim 12 or claim 13, wherein the polypeptide is fused to a protein that attaches to the inner membrane.

15. The method of any one of claims 12 to 14, wherein the polypeptide is attached to the outer face of the inner membrane.

16. The method of any one of claims 1 to 15, wherein the polynucleotide encoding the polypeptide is a plasmid, cosmid, phagemid or phage DNA.

17. The method of any one of claims 1 to 16, wherein the lysis-defective phage is a temperate phage selected from lambda phage, 186, P2, a hybrid of 186 and P2, and P4.

18. The method of any one of claims 1 to 17, wherein the lysis-defective phage is a prophage.

19. The method of claim 18, wherein the bacterial cell comprises lysis-defective lambda, 186, P2, a hybrid of 182 and P2, and/or P4 prophage.

20. The method of claim 19, wherein the bacterial cell comprises P2 and P4 prophage.

21. The method of any one of claims 18 to 20, wherein allowing the lysis-defective phage to package the polynucleotide comprises inducing activation of the prophage in the bacterial cell to produce phage, wherein the phage package the polynucleotide.

22. The method of claim 21, wherein inducing activation of the prophage comprises producing one or more phage activator proteins in the bacterial cell.

23. The method of claim 22, wherein the bacterial cell comprises P2 and P4 prophages and the method comprises producing P2 and/or P4 activator proteins in the bacterial cell.

24. The method of claim 23, wherein the P2 and/or P4 activator proteins are selected from one or more of P2 *cox*, P2 *ogr*, P4 $\delta$ and/or P4 $\varepsilon$.

25. The method of claim 21, wherein inducing activation of the prophage comprises inactivating one or more phage repressor proteins in the bacterial cell.

26. The method of claim 25, wherein the bacterial cell comprises P2 and/or P4 prophage and inducing activation of P2 prophage comprises inactivating a temperature-sensitive repressor allele of P2 protein C in the bacterial cell.

27. The method of any one of claims 23 to 26, wherein the phage is lysis-defective due to deletion or mutation to an inactive form of the lysozyme gene, or deletion or mutation to an inactive form of the holin and lysozyme genes.

28. The method of any one of claims 23 to 27, wherein the P2 prophage lysozyme gene comprises a sequence of nucleotides comprising SEQ ID NO:17 and the P2 holin gene comprises a sequence of nucleotide comprising SEQ ID NO:18.

30. The method of claim 25, wherein the bacterial cell comprises lambda prophage and inducing activation of the prophage comprises inactivating a temperature-sensitive repressor allele of protein cI in the bacterial cell.

31. The method of claim 25, wherein the bacterial cell comprises lambda prophage and inducing activation of the lambda prophage comprises inactivating a temperature-sensitive repressor allele of lambda phage repressor protein, cI, in the bacterial cell.

32. The method of claim 25, wherein the bacterial cell comprises 186 prophage and inducing activation of the 186 prophage comprises inactivating a temperature-sensitive repressor allele of 186 protein cI in the bacterial cell.

33. The method of any one of claims 1 to 32, wherein the method further comprises an additional screening of the polypeptide for a desired activity against a target molecule in a Gram-negative bacterial cell, wherein

i) the polynucleotide encoding the polypeptide is not packaged into a lysis-defective phage, and/or

ii) the polypeptide is not retained within the bacterial cell by the bacterial cell wall and/or attached to the bacterial cell wall.

34. The method of claim 33, wherein the additional screening is performed prior to the method of any one of claims 1 to 32.

35. The method of claim 33 or claim 34, wherein the additional screening is performed using a lytic or temperate phage to package the polynucleotide encoding the polypeptide.

36. The method of claim 35, wherein the bacterial cell in the additional screening is lysed to release the phage.

37. The method of claim 35 or claim 36, wherein the phage in the additional screening is a lytic phage which lyses the bacterial cell.

38. The method of claim 37, wherein

i) the lytic phage comprises a first binding partner on the phage coat, and

ii) the polypeptide being screened for a desired activity is a fusion protein comprising a second binding partner,

wherein the fusion protein comprising the second binding partner binds to the first binding partner on the lytic phage coat.

39. The method of claim 37 or claim 38, wherein the lytic phage is lambda phage.

40. The method of claim 37 or claim 38, wherein the lytic phage is 186, P2, a hybrid of 186 and P2, and/or P4.

41. The method of any one of claims 38 to 40, wherein the first binding partner is calmodulin and the second binding partner is calmodulin binding peptide.

42. A Gram-negative bacterium comprising a lysis-defective phage with a temperature-sensitive phage repressor protein.

43. A Gram-negative bacterium comprising a lysis-defective phage and a polynucleotide encoding one or more phage activator proteins.

44. The Gram-negative bacterium of claim 42 or claim 43, wherein the lysis-defective phage is selected from lambda, 186, P2, a hybrid of 186 and P2, and/or P4.

45. The Gram-negative bacterium of claim 44, wherein the phage activator proteins are selected from one or more of P2 *cox*, P2 *ogr*, P4 $\delta$ and/or P4$\varepsilon$.

46. A kit comprising the Gram-negative bacterium of any one of claims 42 to 45.

47. The kit of claim 46, further comprising an agent capable of permeabilising the Gram-negative bacterial cell.

48. The kit of claim 47, wherein the agent capable of permeabilising the Gram-negative bacterial cell is selected from one or more detergents or an organic solvent.

49. The kit of claim 48, wherein the detergent is a non-ionic detergent selected from Decanoyl-N-methylglucamide (Mega10), demithyloctylphosphine oxide (Apo8), n-octyl-β-D-thioglucopyranoside (8TGP), polysorbate 20 (Tween20), and a mixture of Decanoyl-N-methylglucamide (Mega10) and demithyloctylphosphine oxide (Apo8).

50. The kit of claim 48, wherein the organic solvent is chloroform.

51. A method of screening a polypeptide for a desired activity against a target molecule, the method comprising:

a) culturing a Gram-negative bacterial cell comprising a polynucleotide encoding the polypeptide such that the polypeptide is produced,

b) permeabilising the inner and outer membranes of the bacterial cell with chloroform, wherein the polypeptide and polynucleotide encoding the polypeptide are retained inside the permeabilised bacterial cell,

c) contacting the permeabilised bacterial cell with the target molecule such that it diffuses into the permeabilised bacterial cell, and

d) screening the polypeptide for the desired activity.

52. A method of screening a polypeptide for a desired activity against a target molecule, the method comprising:

a) culturing a Gram-negative bacterial cell comprising a polynucleotide encoding the polypeptide such that the polypeptide is produced and attaches to the bacterial cell wall,

b) permeabilising the inner and outer membranes of the bacterial cell with chloroform, wherein the polynucleotide encoding the polypeptide is retained inside the permeabilised bacterial cell,

c) contacting the permeabilised bacterial cell with the target molecule, and

d) screening the polypeptide for the desired activity.

53. The method of claim 51 or claim 52, wherein step d) comprises:

i) determining if the polypeptide binds, and/or the extent of binding to, the target molecule, and/or

ii) determining if the polypeptide enzymatically modifies, and/or the rate of enzymatic modification of, the target molecule.

54. The method of any one of claims 51 to 53, wherein the polypeptide is associated with at least a second polypeptide to form a protein complex that is retained inside the permeabilised bacterial cell and/or attached to the bacterial cell wall.

55. The method of claim 54, wherein the polypeptide is fused to the second polypeptide, or a subunit thereof.

56. The method of claim 54 or claim 55, wherein the second polypeptide is selected from:

i) a polypeptide having a molecular size such that the protein complex is retained inside the permeabilised bacterial cell wall;

ii) a DNA-binding protein;

iii) a bacterial cell wall-binding protein; and/or

iv) a phage coat protein.

57. A method for identifying a polypeptide with a desired activity, the method comprising:

a) screening a library of polypeptides using a method of any one of claims 1 to 41 or 51 to 56; and

b) selecting one or more polypeptides with the desired activity.

58. A Gram-negative bacterial cell obtained by permeabilising the inner and outer membranes of the bacterial cell with chloroform, wherein the bacterial cell comprises an exogenous polypeptide associated with a second polypeptide to form a protein complex that is retained inside the permeabilised bacterial cell.

59. A Gram-negative bacterial cell obtained by permeabilising the inner and outer membranes of the bacterial cell with chloroform, wherein the bacterial cell comprises comprises an exogenous polypeptide attached to the bacterial cell wall.

60. A kit comprising:

a) a vector comprising

i) a site for inserting into the vector a polynucleotide encoding a first polypeptide, and

ii) an open reading frame encoding a second polypeptide which associates with the first polypeptide to form a protein complex that is retained inside a permeabilised Gram-negative bacterial cell, and

b) chloroform for permeabilising a bacterial cell.

61. A kit comprising:

a) a vector comprising

i) a site for inserting into the vector a polynucleotide encoding a first polypeptide, and

ii) an open reading frame encoding a second polypeptide which associates with the first polypeptide to form a protein complex that attaches to a Gram-negative bacterial cell wall, and

b) chloroform for permeabilising a bacterial cell.

**CLAIMS:**

1. A method of screening a polypeptide for a desired activity against a target molecule, the method comprising:

a) culturing a Gram-negative bacterial cell comprising an exogenous polynucleotide encoding the polypeptide such that the polypeptide is produced in the cell,

b) allowing a lysis-defective phage to package the polynucleotide encoding the polypeptide, wherein the lysis-defective phage is retained within the bacterial cell,

c) permeabilising:

i) the outer membrane of the bacterial cell, or

ii) the inner and outer membranes of the bacterial cell,

d) contacting the bacterial cell with the target molecule, and

e) screening the polypeptide for the desired activity,

wherein the polypeptide is retained within the bacterial cell by the bacterial cell wall or inner membrane and/or the polypeptide is attached to the bacterial cell wall or inner membrane.

2. The method of claim 1, wherein the polypeptide is associated with at least a second polypeptide to form a protein complex that is retained within the permeabilised bacterial cell and/or is attached to the bacterial cell wall.

3. The method of claim 1 or claim 2, wherein the inner and outer bacterial membranes are permeabilised with one or more detergents or an organic solvent.

4. The method of claim 1, wherein:

i) the bacterial outer membrane is permeabilised;

ii) the bacterial cell wall is at least partially hydrolysed; and

iii) the polypeptide is attached to the inner membrane.

5. The method of any one of claims 1 to 4, wherein the polynucleotide encoding the polypeptide is a plasmid, cosmid, phagemid or phage DNA.

6. The method of any one of claims 1 to 5, wherein the lysis-defective phage is a temperate phage selected from lambda phage, 186, P2, a hybrid of 186 and P2, and P4, and/or wherein the lysis-defective phage is a prophage.

7. The method of claim 6, wherein allowing the lysis-defective phage to package the polynucleotide comprises inducing activation of the prophage in the bacterial cell to produce phage, wherein the phage package the polynucleotide, and/or wherein inducing activation of the prophage comprises producing one or more phage activator proteins in the bacterial cell.

8. The method of claim 7, wherein inducing activation of the prophage comprises inactivating one or more phage repressor proteins in the bacterial cell.

9. The method of claim 8, wherein the phage is lysis-defective due to deletion or mutation to an inactive form of the lysozyme gene, or deletion or mutation to an inactive form of the holin and lysozyme genes.

10. The method of claim 8, wherein the bacterial cell comprises lambda prophage and inducing activation of the lambda prophage comprises inactivating a temperature-sensitive repressor allele of lambda phage repressor protein, cI, in the bacterial cell.

11. The method of any one of claims 1 to 10, wherein the method further comprises an additional screening of the polypeptide for a desired activity against a target molecule in a Gram-negative bacterial cell, wherein

i) the polynucleotide encoding the polypeptide is not packaged into a lysis-defective phage, and/or

ii) the polypeptide is not retained within the bacterial cell by the bacterial cell wall and/or attached to the bacterial cell wall.

12. The method of claim 11, wherein the additional screening is performed using a lytic or temperate phage to package the polynucleotide encoding the polypeptide.

13. The method of claim 12, wherein the bacterial cell in the additional screening is lysed to release the phage.

14. The method of claim 13, wherein

i) the lytic phage comprises a first binding partner on the phage coat, and

ii) the polypeptide being screened for a desired activity is a fusion protein comprising a second binding partner,

wherein the fusion protein comprising the second binding partner binds to the first binding partner on the lytic phage coat.

15. The method of claim 14, wherein the first binding partner is calmodulin and the second binding partner is calmodulin binding peptide.

16. A method of screening a polypeptide for a desired activity against a target molecule, the method comprising:

a) culturing a Gram-negative bacterial cell comprising a polynucleotide encoding the polypeptide such that the polypeptide is produced,

b) allowing a lysis-defective phage to package the polynucleotide encoding the polypedtide,

c) permeabilising the inner and outer membranes of the bacterial cell with chloroform, wherein the polypeptide and polynucleotide encoding the polypeptide are retained inside the permeabilised bacterial cell,

d) contacting the permeabilised bacterial cell with the target molecule such that it diffuses into the permeabilised bacterial cell, and

e) screening the polypeptide for the desired activity.

17. A method of screening a polypeptide for a desired activity against a target molecule, the method comprising:

a) culturing a Gram-negative bacterial cell comprising a polynucleotide encoding the polypeptide such that the polypeptide is produced and attaches to the bacterial cell wall,

b) permeabilising the inner and outer membranes of the bacterial cell with chloroform, wherein the polynucleotide encoding the polypeptide is retained inside the permeabilised bacterial cell,

c) contacting the permeabilised bacterial cell with the target molecule, and

d) screening the polypeptide for the desired activity.

18. The method of claim 16 or claim 17, wherein step d) comprises:

i) determining if the polypeptide binds, and/or the extent of binding to, the target molecule, and/or

ii) determining if the polypeptide enzymatically modifies, and/or the rate of enzymatic modification of, the target molecule.

19. The method of any one of claims 16 to 18, wherein the polypeptide is associated with at least a second polypeptide to form a protein complex that is retained inside the permeabilised bacterial cell and/or attached to the bacterial cell wall.

20. The method of claim 19, wherein the second polypeptide is selected from:

i) a polypeptide having a molecular size such that the protein complex is retained inside the permeabilised bacterial cell wall;

ii) a DNA-binding protein;

iii) a bacterial cell wall-binding protein; and/or

iv) a phage coat protein.

21. A method for identifying a polypeptide with a desired activity, the method comprising:

a) screening a library of polypeptides using a method of any one of claims 1 to 20; and

b) selecting one or more polypeptides with the desired activity.