CLAIMS

What is claimed is:

1. A water-soluble G-protein coupled receptor (GPCR) polypeptide variant comprising a modified α- helical domain,

    a. wherein the modified α-helical domain comprises an amino acid sequence in which a plurality of hydrophobic amino acid residues within the α-helical domain of a G-protein coupled receptor (GPCR) selected from the group consisting of phenylalanine, isoleucine, valine and leucine are replaced with hydrophilic, non-ionic amino acid residues,

    b. wherein the pI of the polypeptide variant is substantially the same as the pI of the corresponding native GPCR polypeptide.

2. The variant of claim 1, wherein the variant has a biological activity of the native GPCR polypeptide.

3. The variant of claim 2 wherein the biological activity is ligand binding.

4. The variant of claim 3 wherein the biological activity is at least substantially similar binding affinity for a native ligand of the native GPCR.

5. The variant of claim 1 or 4, wherein the hydrophobic amino acid residues are located at α-helical positions b, c and f.

6. The variant of claim 1 or 4, wherein the hydrophobic amino acid residues at α-helical positions a, d, e and g are not replaced.

7. The variant of claim 1 or 4 wherein a plurality of leucines are replaced with glutamine (Q) and/or wherein a plurality of isoleucines are replaced with threonine (T) and/or wherein a plurality of valines are replaced with threonine (T) and/or wherein a plurality of phenylalanines are replaced with tyrosine (Y).

8. The variant of claim 7 wherein all or substantially all of the leucines in the α-helical positions b, c and f in the transmembrane domains are replaced and/or wherein wherein all or substantially all of the isoleucines in the α-helical positions b, c and f in

the transmembrane domains are replaced and/or wherein all or substantially all of the valines in the α-helical positions b, c and f in the transmembrane domains are replaced and/or wherein a plurality of phenylalanines are replaced with tyrosine (Y).

9. The variant of claim 1 or 4 wherein at least one or more leucine residues of the α-helical domain of the native membrane protein are replaced with glutamine (Q), one or more isoleucine and/or valine residues of the α-helical domain of the protein are replaced with threonine (T), one or more phenylalanine residues of the α-helical domain of the native membrane protein are replaced with tyrosine (Y).

10. The variant of claim 1, wherein about 10% to about 100% of the hydrophobic amino acid residues present in the hydrophilic face of α-helical domain is replaced.

11. The variant of claim 10, wherein at least about 15%, or about 25%, or about 50, or about 70% of the hydrophobic amino acid residues of the α-helical domain is replaced.

12. The variant of claim 1, wherein the GPCR is selected from the group comprising purinergic receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$), M_1 and M_3 muscarinic acetylcholine receptors, receptors for thrombin [protease-activated receptor (PAR)-1, PAR-2], thromboxane (TXA_2), sphingosine 1-phosphate ($S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$), lysophosphatidic acid (LPA_1, LPA_2, LPA_3), angiotensin II (AT_1), serotonin ($5\text{-}HT_{2c}$ and $5\text{-}HT_4$), somatostatin (sst_5), endothelin (ET_A and ET_B), cholecystokinin (CCK_1), V_{1a} vasopressin receptors, D_5 dopamine receptors, fMLP formyl peptide receptors, GAL_2 galanin receptors, EP_3 prostanoid receptors, A_1 adenosine receptors, α_1 adrenergic receptors, BB_2 bombesin receptors, B_2 bradykinin receptors, calcium-sensing receptors, chemokine receptors, KSHV-ORF74 chemokine receptors, NK_1 tachykinin receptors, thyroid-stimulating hormone (TSH) receptors, protease-activated receptors, neuropeptide receptors, adenosine A2B receptors, P2Y purinoceptors, metabolic glutamate receptors, GRK5, GPCR-30, and CXCR4.

13. The variant of claim 1 wherein the GPCR is a CXCR4.

14. A method for treating a mammal suffering from a disorder or disease that is mediated by the activity of a GPCR polypeptide, comprising administering to said mammal an effective amount of the water-soluble polypeptide variant of claim 1.

15. A pharmaceutical composition comprising an effective amount of a variant of claim 1, and a pharmaceutically acceptable diluent or carrier.

16. A water soluble GPCR polypeptide variant wherein at least one transmembrane domain has been substituted with a peptide selected from the group consisting of SEQ ID NOs. 3, 4, 5, 6, 7, 8 or 9.

17. A water-soluble G-protein coupled receptor (GPCR) polypeptide variant comprising a modified α- helical domain,

    a. wherein at least one or more leucine residues of the α-helical domain of the native membrane protein are replaced with glutamine (Q), one or more isoleucine and/or valine residues of the α-helical domain of the protein are replaced with threonine (T), one or more phenylalanine residues of the α-helical domain of the native membrane protein are replaced with tyrosine (Y);

    b. wherein the hydrophobic amino acid residues are located at α-helical positions b, c and f; and

    c. wherein the hydrophobic amino acid residues at α-helical positions a, d, e and g are not replaced.

CLAIMS

What is claimed is:

1. A water-soluble polypeptide comprising a modified α-helical domain, wherein the modified α-helical domain comprises an amino acid sequence in which one or more hydrophobic amino acid residues within a α-helical domain of a native membrane protein is replaced with one or more hydrophilic amino acid residues.

2. The water-soluble polypeptide of claim 1, wherein the α-helical domain is 7-transmembrane α-helical domain.

3. The water-soluble polypeptide of claim 1, wherein the native membrane protein is a G-protein coupled receptor (GPCR).

4. The water-soluble polypeptide of claim 1, wherein the native membrane protein is an integral membrane protein.

5. The water-soluble polypeptide of claim 1, wherein the hydrophilic residues are selected from the group consisting of glutamine (Q), threonine (T), tyrosine (Y) and any combination thereof.

6. The water-soluble polypeptide of claim 1, wherein one or more of the hydrophobic residues leucine (L), isoleucine (I), valine (V) and phenylalanine (F) are replaced.

7. The water-soluble polypeptide of claim 1, wherein one or more phenylalanine residues of the α-helical domain of the native membrane protein are replaced with tyrosine.

8. The water-soluble polypeptide of any one of claims 1 and 7, wherein one or more isoleucine and/or valine residues of the α-helical domain of the protein are replaced with threonine.

9. The water-soluble polypeptide of any one of claims 1, 7 and 8, wherein one or more leucine residues of the α-helical domain of the native membrane protein are replaced with glutamine.

10. The water-soluble polypeptide of claim 1, wherein the native membrane protein is an olfactory receptor or chemokine receptor CXCR4.

11. The water-soluble polypeptide of claim 1, wherein the native membrane protein is a mammalian protein

12. The water-soluble polypeptide of claim 10, wherein the native membrane protein is mOR103-15 or or chemokine receptor CXCR4.

13. The water-soluble polypeptide of claim 12, wherein the hydrophobic amino acid residues at α-helical positions b, c and f are replaced.

14. The water-soluble polypeptide of claim 12, wherein the hydrophobic amino acid residues at α-helical positions a, d, e and g are not replaced.

15. The water-soluble polypeptide of claim 1, comprising the amino acid sequence of MERRNHTGRV SEFVLLGFPA PAPQRALQFF QSLQAYVQTL TENIQTITAIRNHPTLHKPM YYFLANMSFYL ETWYTTVTTP KMQAGYIGSE ENHGQLISFE ACMTQLYFFQ GLGCTECTLL AVMAYDRYVA TCHPLHYPVI VSSRQCVQMA AGSWAGGFGT SMTVKVYQISR LSYCGPNTIN HFFCDVSPLL NLSCTDMSTA ELTDFILAIF ILLGPLSVTG ASYMAITGAV MRIPSAAGRH KAFSTCASHL TTVITYYAAS IYTYARPKAL SAFDTNKLVS VLYAVIVPLL NPIIYCLRNQ EVKKALRRTL HLAQGDANT KKSSRDGGSS GTETSQVAPA (SEQ ID NO: 2);

or

MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANYNKTFLPTIYSIIYQTGTV GNGLVILVMGYQKKLRSMTDKYRLHLSTADLQFVTTLPYWATDATANWYF GNFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVY VGVWTPAQLLTTPDYTFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLI LPGIVILSCYCIIISKLSHSKGHQKRKALKTTTTLIQAFFACWQPYYTGISIDSYI LLEIIKQGCEFENTVHKWISITTEAQAFYHCCTNPTQYAYLGAKFKTSAQHALT SVSRGSSLKILSKGKRGGHSSVSTESESSSSFHS (SEQ ID NO:10).

16. The water-soluble polypeptide of claim 2, wherein the modified 7-transmembrane α-helical domain comprises one or more of the following amino acid sequences:

    a. PQRALQFFQSLQAYVQTLTENIQTITAI R (SEQ ID NO: 3)
    b. M YYFLANMSFYLETWYTTVTTPKMQAGYI (SEQ ID NO: 4)
    c. CMTQLYFFQGLGCTECTLLAVMAYDRYVA TC (SEQ ID NO: 5)
    d. RQCVQMAAGSWAGGFGTSMTVKVYQ (SEQ ID NO: 6)
    e. LTDFILAIFILLGPLSVTGASYMAITGAV (SEQ ID NO: 7)
    f. HKAFSTCASHLTTVITYYAAS IYTY (SEQ ID NO: 8)
    g. TNKLVSVLYAVIVPLLNPIIYCLRN (SEQ ID NO: 9).

17. The water-soluble polypeptide of any one of claims 1 to 16, wherein the polypeptide retains the biological activity of the native membrane protein.

18. The water-soluble polypeptide of any one of claims 1 to 16, wherein one or more amino acids within potential odorant binding sites are not replaced.

19. A method of preparing a water-soluble polypeptide comprising replacing one or more hydrophobic amino acid residues within the α-helical domain of a native membrane protein with one or more hydrophilic amino acid residues.

20. The method of claim 19, wherein the α-helical domain is a 7-transmembrane α-helical domain.

21. The method of claim 19, wherein the native membrane protein is a G-protein coupled receptor (GPCR).

22. The method of claim 19, wherein the hydrophilic residues are selected from the group consisting of glutamine (Q), threonine (T), tyrosine (Y) and any combination thereof.

23. The method of claim 19, wherein the hydrophobic residues leucine (L), isoleucine (I), valine (V) and phenylalanine (F) are replaced.

24. The method of claim 20, wherein one or more phenylalanine residues of the 7-transmembrane α-helical domain of the native membrane protein are replaced with tyrosine.

25. The method of any one of claims 19 and 24, wherein one or more isoleucine and/or valine residues of the α-helical domain of the native membrane protein are replaced with threonine.

26. The method of any one of claims 19, 24 and 25, wherein one or more leucine residues of the α-helical domain of the native membrane protein are replaced with glutamine.

27. The method of claim 19, wherein the native protein is an olfactory receptor.

28. The method of claim 19, wherein the native protein is a mammalian protein.

29. The method of claim 27, wherein the native protein is mOR103-15, or chemokine receptor CXCR4.

30. The method of claim 19, wherein the polypeptide comprises the amino acid sequence MERRNHTGRV SEFVLLGFPA PAPQRALQFF QSLQAYVQTL TENIQTITAI RNHPTLHKPM YYFLANMSFYL ETWYTTVTTP KMQAGYIGSE ENHGQLISFE ACMTQLYFFQ GLGCTECTLL AVMAYDRYVA TCHPLHYPVI VSSRQCVQMA AGSWAGGFGT SMTVKVYQISR LSYCGPNTIN HFFCDVSPLL NLSCTDMSTA ELTDFILAIF ILLGPLSVTG ASYMAITGAV MRIPSAAGRH KAFSTCASHL TTVITYYAAS IYTYARPKAL SAFDTNKLVS VLYAVIVPLL NPIIYCLRNQ EVKKALRRTL HLAQGDANT KKSSRDGGSS GTETSQVAPA (SEQ ID NO: 2);

or

MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANYNKTFLPTIYSIIYQTGTV GNGLVILVMGYQKKLRSMTDKYRLHLSTADLQFVTTLPYWATDATANWYF GNFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVY VGVWTPAQLLTTPDYTFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLI LPGIVILSCYCIIISKLSHSKGHQKRKALKTTTTLIQAFFACWQPYYTGISIDSYI LLEIIKQGCEFENTVHKWISTTEAQAFYHCCTNPTQYAYLGAKFKTSAQHALT SVSRGSSLKILSKGKRGGHSSVSTESESSSSFHS (SEQ ID NO:10).

31. The method of claim 24, wherein the modified 7-transmembrane α-helical domain comprises one or more of the following amino acid sequences:

    a. PQRALQFFQSLQAYVQTLTENIQTITAI R (SEQ ID NO: 3)
    b. M YYFLANMSFYLETWYTTVTTPKMQAGYI (SEQ ID NO: 4)
    c. CMTQLYFFQGLGCTECTLLAVMAYDRYVA TC (SEQ ID NO: 5)
    d. RQCVQMAAGSWAGGFGTSMTVKVYQ (SEQ ID NO: 6)
    e. LTDFILAIFILLGPLSVTGASYMAITGAV (SEQ ID NO: 7)
    f. HKAFSTCASHLTTVITYYAAS IYTY (SEQ ID NO: 8)
    g. TNKLVSVLYAVIVPLLNPIIYCLRN (SEQ ID NO: 9) SEQ ID NO: 2.

32. The method of any one of claim 19 to 31, wherein the water-soluble polypeptide retains the biological activity of the native membrane protein.

33. The method of any one of claim 19 to 31, further comprising determining the secondary structure of the polypeptide.

34. The method of claim 33, wherein the secondary structure is determined using circular dichroism.

35. The method of any one of claims 19 to 31, further comprising measuring ligand binding.

36. The method of any one of claims 19 to 31, wherein the polypeptide is prepared using a cell-free system.

37. The method of any one of claim 19 to 31, wherein the protein is an olfactory receptor and further comprising measuring odorant binding to the olfactory receptor.

38. The method of claim 35, wherein the ligand binding affinity of the water-soluble polypeptide is compared to the native membrane protein.

39. The method of claim 35, wherein ligand binding is measured using microscale thermophoresis.

40. The method of claim 35, wherein ligand binding is measured using a calcium influx assay.

41. A polypeptide produced according to the method of any one of claims 19 to 31.

42. A cell transfected with the polypeptide of any one of claims 1 to 18 and 41.

43. The method of claim 42, wherein the cell is a mammalian cell.

44. The cell of claim 43, wherein the cell is a HEK293 cell.

45. A method for treating a mammal suffering from a disorder or disease that is mediated by the activity of a native membrane protein, comprising administering to said mammal an effective amount of a water-soluble polypeptide comprising a modified α-helical domain, wherein the modified α-helical domain comprises an amino acid sequence in which one or more hydrophobic amino acid residues within a α-helical domain of the native membrane protein is replaced with one or more hydrophilic amino acid residues.

46. The method of claim 45, wherein the α-helical domain is 7-transmembrane α-helical domain.

47. The method of claim 45, wherein the native membrane protein is a G-protein coupled receptor (GPCR).

48. The method of claim 45, wherein the native membrane protein is an integral membrane protein.

49. The method of claim 45, wherein the hydrophilic residues are selected from the group consisting of glutamine (Q), threonine (T), tyrosine (Y) and any combination thereof.

50. The method of claim 45, wherein one or more of the hydrophobic residues leucine (L), isoleucine (I), valine (V) and phenylalanine (F) are replaced.

51. The method of claim 45, wherein one or more phenylalanine residues of the α-helical domain of the native membrane protein are replaced with tyrosine.

52. The method of any one of claims 45 and 50, wherein one or more isoleucine and/or valine residues of the α-helical domain of the protein are replaced with threonine.

53. The method of any one of claims 45 and 50, wherein one or more leucine residues of the α-helical domain of the native membrane protein are replaced with glutamine.

54. The method of claim 45, wherein said native membrane protein is abnormally activated, up-regulated, or over-expressed in a disorder or disease.

55. The method of claim 45, wherein the disease is cancer.

56. The method of claim 55, wherein the cancer is small cell lung cancer.

57. The method of claim 55, wherein the cancer is melanoma.

58. The method of claim 55, wherein the cancer is breast cancer.

59. The method of claim 45, wherein the disease is Parkinson's disease.

60. The method of claim 45, wherein the disease is cardiovascular disease.

61. The method of claim 45, wherein the disease is hypertension.

62. The method of claim 45, wherein the disease is asthma.

63. The method of claim 45, wherein the native membrane protein is selected from the group comprising purinergic receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$), M_1 and M_3 muscarinic acetylcholine receptors, receptors for thrombin [protease-activated receptor (PAR)-1, PAR-2], thromboxane (TXA_2), sphingosine 1-phosphate ($S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$), lysophosphatidic acid (LPA_1, LPA_2, LPA_3), angiotensin II (AT_1), serotonin (5-HT_{2c} and 5-HT_4), somatostatin (sst_5), endothelin (ET_A and ET_B), cholecystokinin (CCK_1), V_{1a} vasopressin receptors, D_5 dopamine receptors, fMLP formyl peptide receptors, GAL_2 galanin receptors, EP_3 prostanoid receptors, A_1 adenosine receptors, α_1 adrenergic receptors, BB_2 bombesin receptors, B_2 bradykinin receptors, calcium-sensing receptors, chemokine receptors, KSHV-ORF74 chemokine receptors, NK_1 tachykinin receptors, thyroid-stimulating hormone (TSH) receptors,

protease-activated receptors, neuropeptide receptors, adenosine A2B receptors, P2Y purinoceptors, metabolic glutamate receptors, GRK5, GPCR-30, and CXCR4.

64. The method of any one of claims 45 to 63, wherein said water-soluble polypeptide retains the activity of binding the ligand of the native membrane protein.

65. The method of any one of claims 45 to 64, wherein said water-soluble polypeptide retains the ability to bind the ligand which normally binds to the native membrane protein.

66. The method of any one of claims 45 to 65, wherein one or more amino acids within potential ligand binding sites are not replaced.

67. The method of claim 45 wherein the mammal is a human.

68. The method according to any of claims 45-67, wherein said hydrophobic amino acid is present in the hydrophilic face of the α-helical domain.

69. The method according to any of claims 45-67, wherein said hydrophobic amino acid is present in the b, c or f position on a helical diagram.

70. The method according to any of claims 19-29, wherein about 10% to about 100% of the hydrophobic amino acid residues present in the hydrophilic face of α-helical domain is replaced.

71. The method according to claim 70, wherein at least about 15%, or about 25%, or about 50, or about 70% of the hydrophobic amino acid residues of the α-helical domain is replaced.

72. The method according to claim 70 or 71, wherein the helical content of the modified helical domain is at least about 30% or about 50% or about 75% or about 95% of the helical domain of the native protein.

73. A method for producing a native ligand binding modified polypeptide of a transmembrane protein comprising the step of replacing or mutating at least some of the hydrophobic amino acid residues by hydrophilic amino acid residues wherein said

modified polypeptide retains at least some of the ligand binding properties of said transmembrane protein.

74. A method for treating a disease or disorder mediated by ligand binding of a transmembrane protein comprising the step of administering a modified polypeptide wherein said polypeptide corresponds to a mutated amino acid sequence of said protein wherein transmembrane region of the sequence is modified so that at least some of the hydrophobic amino acid residues are replaced by hydrophilic amino acid residues.

75. The method according to claim 73 or 74, wherein said transmembrane region is α-helical.

76. The method according to claim 73 or 74, wherein hydrophobic amino acid residues corresponding to at least one of b, c or f positions of the helical wheel is replaced by hydrophilic amino acid residues.

77. The method according to claim 76, wherein said hydrophilic residues are selected from glutamine (Q), threonine (T), tyrosine (Y) and any combination thereof.

78. The method according to claims 75 or 76, wherein said hydrophobic residues are selected from leucine (L), isoleucine (I), valine (V) and phenylalanine (F).

79. The method according to any of claims 73-78, wherein said modified polypeptide retains ligand binding affinity of said transmembrane protein.

80. The method according to claim 79, wherein said ligand binding affinity of said polypeptide is substantially similar or better than the ligand binding affinity of said transmembrane protein.

81. The method according to claim 79, wherein said ligand binding affinity of said polypeptide is about 10%, or about 20% or about 30% or about 40% or about 50% or about 60% or about 70% or about 80% or about 90% of the ligand binding affinity of said transmembrane protein.

82. The method according to any of claims 73-81, wherein said modified polypeptide is water soluble.

83. The method according to any of claims 73-82, wherein said transmembrane protein is a G-protein coupled receptor (GPCR).

84. The method according to any of claims 73-82, wherein said transmembrane protein is selected from purinergic receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$), M_1 and M_3 muscarinic acetylcholine receptors, receptors for thrombin [protease-activated receptor (PAR)-1, PAR-2], thromboxane (TXA_2), sphingosine 1-phosphate ($S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$), lysophosphatidic acid (LPA_1, LPA_2, LPA_3), angiotensin II (AT_1), serotonin ($5\text{-}HT_{2c}$ and $5\text{-}HT_4$), somatostatin (sst_5), endothelin (ET_A and ET_B), cholecystokinin (CCK_1), V_{1a} vasopressin receptors, D_5 dopamine receptors, fMLP formyl peptide receptors, GAL_2 galanin receptors, EP_3 prostanoid receptors, A_1 adenosine receptors, α_1 adrenergic receptors, BB_2 bombesin receptors, B_2 bradykinin receptors, calcium-sensing receptors, chemokine receptors, KSHV-ORF74 chemokine receptors, NK_1 tachykinin receptors, thyroid-stimulating hormone (TSH) receptors, protease-activated receptors, neuropeptide receptors, adenosine A2B receptors, P2Y purinoceptors, metabolic glutamate receptors, GRK5, GPCR-30, and CXCR4.

85. The method according to claim 84, wherein said modified polypeptide binds to the corresponding native ligand.

86. The method according to claim 84 or 85, wherein said modified polypeptide is CXCR4 QTY.

87. The method according to any of claims 73-86, wherein said modified polypeptide is at least about 10% or about 20% or about 30% or about 40% or about 50% or about 60% or about 70% or about 80% or about 90% or about 100% or about 125% or about 150% or about 175% or about 200% or about 225% or about 250% or about 300% more water soluble than said transmembrane protein.

88. The method according to any of claims 45-54 or 63-87, wherein said disease or disorder is selected from arthritis, lymphoma, non-small lung cancer, lung cancer, breast cancer, prostate cancer, multiple sclerosis, central nervous system

developmental disease, dementia, Parkinson's disease, Alzheimer's disease, tumor, fibroma, astrocytoma, myeloma, glioblastoma, an inflammatory disease, an organ transplantation rejection, or angiogenesis.

89. A modified polypeptide produced by the method according to any of claims 83-87.

90. A pharmaceutical composition comprising an effective amount of a water-soluble polypeptide of any one of claims 1 to 9, 11 or 88, and a pharmaceutically acceptable diluent or carrier.