

CLAIMS

1. A high-throughput assay system for identifying one or more compounds from a group of compounds wherein the one or more compounds positively modulates the activity of an olfactory receptor induced by an agonist odorant compound, comprising:

a) one or more isolated cells, each cell expressing one mammalian olfactory receptor, wherein the one or more cells comprises a target olfactory receptor that is activated by one or more odorant,

b) a first compound that binds to the olfactory receptor and that activates the olfactory receptor, and

c) at least one second compound selected from the one or more compounds that binds to the receptor non-competitively relative to the first compound and that enhances the activity of the receptor exposed to the first compound when compared to the activity of the receptor exposed to the first compound in the absence of the second compound,

wherein the olfactory receptor is selected from the group consisting of OR5AN1, Olfr1440, OR10J5, or Olfr16,

wherein the first compound is an agonist odorant compound of the olfactory receptor, and a musk or a floral muguet compound,

wherein the first compound and the second compound are different compounds,

wherein the second compound is not an agonist of the receptor,

~~wherein the second compound is a positive allosteric modulating compound,~~
and

wherein the activity of the receptor is synergistically enhanced by the combination of the first compound and the second compound,

wherein the second compound is at least one positive allosteric modulator selected from the group consisting of (E)-2-undecenal, (E,E)-2,4-nonadienal, (E)-2-tridecenal, (2E)-2-dodecenal, (2E,6Z)-2,6-nonadienal, (E,E)-2,6-nonadienal, (2E)-2,4-undecadienal, (2E)-2,4-dodecadienal, (E)-2-nonenal, (2E,4E,7Z)-decatrienal, (Z)-2-decenal, (E)-2-decenal, (Z)-4-(benzyloxy)but-2-enal, (E)-4-(benzyloxy)but-2-enal, (E)-4-cyclohexylidenebut-2-enal, (2E)-3-(4-methylphenyl)-2-propenal, (2E,5E)-6,10-dimethyl-2,5,9-undecatrienal, (2E)-7,8-dimethyl-2,7-nonadienal, methyl(5E)-7-oxo-5-heptenoate, (2E)-7-methyl-2,6-

octadienal, (2E)-6,6-dimethyl-2-heptenal, (+-)-(2E)-5,9-dimethyl-2,8-decadienal, (E)-2-tetradecenal, (E)-8-methoxy-4,8-dimethylnon-2-enal, and combinations thereof, or the second compound is at least one positive allosteric modulator selected from the group consisting of (E)-dec-2-enal, 2-phenylpropanal; (E)-but-2-enal; 3-methylbenzaldehyde; 3-(1,3-benzodioxol-5-yl)-2-methylpropanal; (+-)-3-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, (+-)-4-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, and mixtures thereof; heptanal, 4-Propan-2-ylbenzaldehyde; (3R)-3,7-dimethyloct-6-enal; (+)-(3S)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, (+)-(3R)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, and mixtures thereof; hexanal; 2,6-dimethylhept-5-enal; benzaldehyde; 2-methyl-3-(4-methylphenyl)propanal; 3,5,6-trimethyl-3-cyclohexene-1-carbaldehyde, 2,4,6-trimethyl-3-cyclohexene-1-carbaldehyde, and mixtures thereof; 4-ethylbenzaldehyde; 6-methoxy-2,6-dimethylheptanal; (E)-non-2-enal; and combinations thereof.

2. The assay system of claim 1, wherein the at least one second compound is selected from a pool of compounds based on structure-activity relationship (SAR) analysis.
3. The assay system of claim 1 or claim 2, wherein the second compound binds to a site on the olfactory receptor distinct from the site where the first compound binds.
4. The assay system of any one of claims 1 to 3, wherein the one or more isolated cells in part (a) comprised one or more isolated cells that were transformed to express the target olfactory receptor.
5. The assay system of claim 1, wherein at least one of the olfactory receptors comprises a polypeptide
 - a) comprises an amino acid sequence having at least ~~70~~99% sequence identity to SEQ ID NO: 2, 4, 6, or 8; and/or
 - b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least ~~70~~99% sequence identity to SEQ ID NO: 1, 3, 5, or 7, or the reverse complement thereof.

6. The assay system of claim 5, wherein the polypeptide

a) comprises an amino acid sequence having at least 90%, 95%, 98%, 99% or 100% sequence identity to SEQ ID NO: 2, 4, 6, or 8; and/or

b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least 90%, 95%, 98%, 99% or 100% sequence identity to SEQ ID NO: 1, 3, 5, or 7, or the reverse complement thereof.

7. The assay system of claim 1, wherein the mammalian olfactory receptor is a mouse olfactory receptor or a human olfactory receptor.

~~8. The assay system of any one of claims 1 to 7, wherein the first compound is a musk or a floral compound.~~

~~9.8.~~ The assay system of any one of claims 1 to ~~8~~7, wherein the first compound ~~comprises~~is a musk compound and the olfactory receptor is OR5AN1 or Olfr1440; chimera and functional fragments thereof; and wherein the olfactory receptor comprises a polypeptide that

a) comprises an amino acid sequence having at least ~~7099~~7090% sequence identity to SEQ ID NO: 2 or 6; and/or

b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least ~~7099~~7090% sequence identity to SEQ ID NO: 1 or 5, or the reverse complement thereof.

~~10.9.~~ The assay system of any one of claims 1 to claim 8~~or 9~~, wherein the musk compound ~~is~~comprises a macrocyclic ketone or a nitromusk compound.

~~11.10.~~ The assay system of any one of claims 1 to ~~7~~8, wherein the first compound ~~is~~comprises a floral muguet compound and the olfactory receptor is OR10J5 or Olfr16; chimera and functional fragments thereof; and wherein the olfactory receptor comprises a polypeptide that

a) comprises an amino acid sequence having at least ~~7099~~7090% sequence identity to SEQ ID NO: 4 or 8; and/or

- b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least ~~70~~90% sequence identity to SEQ ID NO: 3 or 7, or the reverse complement thereof.

5 ~~12,11.~~ 11. The assay system of any one of claims 1 to ~~10~~11, wherein the modulated activity comprises

- a) modulating the binding affinity of the first compound to the orthosteric site,
 b) modulating the binding activation efficacy of the first compound to the orthosteric site, and/or
 10 c) modulating the G-protein binding site.

~~13,12.~~ 12. A method for identifying at least one positive allosteric modulating compound that enhances the activity of an olfactory receptor in the presence of an agonist of the receptor, comprising

- 15 a) providing one or more isolated cells, each cell expressing one mammalian olfactory receptor,
 b) exposing the one or more cells to a first compound that activates the olfactory receptor,
 c) exposing the one or more cells to one or more test ~~compound~~compounds, and
 20 d) selecting at least one second compound from the one or more test compounds when the at least one second compound in combination with the first compound has a synergistic effect on the receptor activity as a positive allosteric modulating compound;

25 wherein the olfactory receptor is selected from the group consisting of OR5AN1, Olfr1440, OR10J5, or Olfr16,

wherein the first compound is an agonist odorant compound of the olfactory receptor, and a musk or a floral muguet compound,

wherein the first compound and the second compound are different compounds, and

30 wherein the second compound is not an agonist of the receptor.

wherein the second compound is at least one positive allosteric modulator selected from the group consisting of (E)-2-undecenal, (E,E)-2,4-nonadienal, (E)-2-tridecenal, (2E)-2-dodecenal, (2E,6Z)-2,6-nonadienal, (E,E)-2,6-nonadienal, (2E)-2,4-undecadienal, (2E)-2,4-dodecadialenal, (E)-2-nonenal, (2E,4E,7Z)-decatrienal, (Z)-2-decenal, (E)-2-decenal, (Z)-4-(benzyloxy)but-2-enal, (E)-4-(benzyloxy)but-2-enal, (E)-4-cyclohexylidenebut-2-enal, (2E)-3-(4-methylphenyl)-2-propenal, (2E,5E)-6,10-dimethyl-2,5,9-undecatrienal, (2E)-7,8-dimethyl-2,7-nonadienal, methyl(5E)-7-oxo-5-heptenoate, (2E)-7-methyl-2,6-octadienal, (2E)-6,6-dimethyl-2-heptenal, (+-)-(2E)-5,9-dimethyl-2,8-decadialenal, (E)-2-tetradecenal, (E)-8-methoxy-4,8-dimethylnon-2-enal, and combinations thereof, or the second compound is at least one positive allosteric modulator selected from the group consisting of (E)-dec-2-enal, 2-phenylpropanal; (E)-but-2-enal; 3-methylbenzaldehyde; 3-(1,3-benzodioxol-5-yl)-2-methylpropanal; (+-)-3-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, (+-)-4-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, and mixtures thereof; heptanal, 4-Propan-2-ylbenzaldehyde; (3R)-3,7-dimethyloct-6-enal; (+)-(3S)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, (+)-(3R)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, and mixtures thereof; hexanal; 2,6-dimethylhept-5-enal; benzaldehyde; 2-methyl-3-(4-methylphenyl)propanal; 3,5,6-trimethyl-3-cyclohexene-1-carbaldehyde, 2,4,6-trimethyl-3-cyclohexene-1-carbaldehyde, and mixtures thereof; 4-ethylbenzaldehyde; 6-methoxy-2,6-dimethylheptanal; (E)-non-2-enal; and combinations thereof.

13. The method of claim 12, wherein the activation of the olfactory receptor to the first compound is measured by measuring the response of said olfactory receptor in the presence and absence of the first compound.

14. The method of claim 12 or claim 13, wherein the at least one second compound is selected from one or more test compounds based on structure-activity relationship (SAR) analysis.

15. The method of any one of claims 12 to 14, wherein the second compound binds to a site on the olfactory receptor distinct from the site where the first compound binds.

16. The method of any one of claims 12 to 15, wherein prior to step a) the one or more cells is transformed to express the olfactory receptor.

5 17. The method of any one of claims 12 to 16, wherein the olfactory receptor is heterologous to the one or more cells.

18. The method of any one of claims 12 to 17, wherein the cell is selected from the group consisting of HEK293, CHO, Xenopus oocytes, COS, insect, yeast and cells derived from the olfactory placode.

10 19. A method for identifying a compound that enhances the activity of an olfactory receptor induced by an agonist odorant compound comprising:

(i) screening one or more compounds in a binding assay in the presence of the agonist odorant compound;

15 (ii) selecting one or more of the compounds screened that specifically enhances the specific binding or the effect of the binding of an agonist odorant compound to a mammalian olfactory receptor; and

(iii) identifying compounds that potentially modulate the perception of the agonist odorant compound based on their enhancement of the specific binding or the effect of the binding of the agonist odorant compound to the olfactory receptor,

20 wherein the olfactory receptor is OR5AN1, Olfr1440, OR10J5, or Olfr16,

wherein the agonist odorant compound is a musk or a floral muguet compound,

wherein the selected compound is at least one positive allosteric modulator selected from the group consisting of (E)-2-undecenal, (E,E)-2,4-nonadienal, (E)-2-tridecenal, (2E)-2-

dodecenal, (2E,6Z)-2,6-nonadienal, (E,E)-2,6-nonadienal, (2E)-2,4-undecadienal, (2E)-2,4-dodecadienal, (E)-2-nonenal, (2E,4E,7Z)-decatrienal, (Z)-2-decenal, (E)-2-decenal, (Z)-4-(benzyloxy)but-2-enal, (E)-4-(benzyloxy)but-2-enal, (E)-4-cyclohexylidenebut-2-enal, (2E)-3-(4-methylphenyl)-2-propenal, (2E,5E)-6,10-dimethyl-2,5,9-undecatrienal, (2E)-7,8-dimethyl-2,7-nonadienal, methyl(5E)-7-oxo-5-heptenoate, (2E)-7-methyl-2,6-octadienal, (2E)-6,6-dimethyl-2-heptenal, (+-)-(2E)-5,9-dimethyl-2,8-decadienal, (E)-2-tetradecenal, (E)-8-methoxy-4,8-dimethylnon-2-enal, and combinations thereof, or the selected compound is at least one positive allosteric modulator selected from the group consisting of (E)-dec-2-enal, 2-phenylpropanal; (E)-but-2-enal; 3-methylbenzaldehyde; 3-(1,3-benzodioxol-5-yl)-2-methylpropanal; (+-)-3-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, (+-)-4-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, and mixtures thereof; heptanal, 4-Propan-2-ylbenzaldehyde; (3R)-3,7-dimethyloct-6-enal; (+)-(3S)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, (+)-(3R)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, and mixtures thereof; hexanal; 2,6-dimethylhept-5-enal; benzaldehyde; 2-methyl-3-(4-methylphenyl)propanal; 3,5,6-trimethyl-3-cyclohexene-1-carbaldehyde, 2,4,6-trimethyl-3-cyclohexene-1-carbaldehyde, and mixtures thereof; 4-ethylbenzaldehyde; 6-methoxy-2,6-dimethylheptanal; (E)-non-2-enal; and combinations thereof.

20. The method of any one of claims 12 to 19, wherein the mammalian olfactory receptor is a mouse olfactory receptor or a human olfactory receptor.

21. The method of any one of claims 12 to 20, wherein at least one of the olfactory receptors comprises a polypeptide that

a) comprises an amino acid sequence having at least 70 % sequence identity to SEQ ID NO: 2, 4, 6, or 8; and/or

b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at

least 70 % sequence identity to SEQ ID NO: 1, 3, 5, or 7, or the reverse complement thereof.

22. The method of any one of claims 12 to 21, wherein the polypeptide

a) comprises an amino acid sequence having at least 90 %, 95 %, 98 %, 99 % or 100 % sequence identity to SEQ ID NO: 2, 4, 6, or 8; and/or

b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least 90 %, 95 %, 98 %, 99 % or 100 % sequence identity to SEQ ID NO: 1, 3, 5, or 7, or the reverse complement thereof.

23. The method of any one of claims 12 to 22, wherein the first compound is a musk

compound and the olfactory receptor is OR5AN1 or Olfr1440; chimera and functional fragments thereof; and wherein the olfactory receptor comprises a polypeptide that

a) comprises an amino acid sequence having at least 70 % sequence identity to SEQ ID NO: 2 or 6; and/or

b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least 70 % sequence identity to SEQ ID NO: 1 or 5, or the reverse complement thereof.

24. The method of any one of claims 12 to 23, wherein the musk compound comprises a macrocyclic ketone or a nitromusk compound.

25. The method of any one of claims 12 to 22, wherein the first compound is a floral muguet compound and the olfactory receptor is OR10J5 or Olfr16; chimera and functional

fragments thereof; and wherein the olfactory receptor comprises a polypeptide that

a) comprises an amino acid sequence having at least 70 % sequence identity to SEQ ID NO: 4 or 8; and/or

b) is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least 70 % sequence identity to SEQ ID NO: 3 or 7, or the reverse complement thereof.

26. The method of any one of claims 12 to 25, wherein the modulated activity comprises

a) modulating the binding affinity of the first compound to the orthosteric site,

b) modulating the binding activation efficacy of the first compound to the
orthosteric site, and/or

c) modulating the G-protein binding site.

27. The method of any one of claims 12 to 26, wherein the enhancement of the effect of the binding of the agonist odorant compound to the olfactory receptor comprises enhancing the potency and/or efficacy of the activity of the receptor compared to the activity of the receptor in the absence of the selected compound.

28. The method of any one of claims 12 to 24, 26 or 27, wherein the receptor is OR5AN1, the agonist odorant compound is a macrocyclic ketone or a nitromusk compound; and the selected compound is at least one positive allosteric modulator selected from the group consisting of (E)-2-undecenal, (E,E)-2,4-nonadienal, (E)-2-tridecenal, (2E)-2-dodecenal, (2E,6Z)-2,6-nonadienal, (E,E)-2,6-nonadienal, (2E)-2,4-undecadienal, (2E)-2,4-dodecadialenal, (E)-2-nonenal, (2E,4E,7Z)-decatrienal, (Z)-2-decenal, (E)-2-decenal, (Z)-4-(benzyloxy)but-2-enal, (E)-4-(benzyloxy)but-2-enal, (E)-4-cyclohexylidenebut-2-enal, (2E)-3-(4-methylphenyl)-2-propenal, (2E,5E)-6,10-dimethyl-2,5,9-undecatrienal, (2E)-7,8-dimethyl-2,7-nonadienal, methyl(5E)-7-oxo-5-heptenoate, (2E)-7-methyl-2,6-octadienal, (2E)-6,6-dimethyl-2-heptenal, (+-)-(2E)-5,9-dimethyl-2,8-decadialenal, (E)-2-tetradecenal, (E)-8-methoxy-4,8-dimethylnon-2-enal, and combinations thereof.

29. The method of any one of claims 12 to 28, wherein the one or more selected compound is selected based on structure-activity relationship (SAR) analysis specifically designed to identify the chemical determinants of putative enhancement.

30. The method of any one of claims 12 to 22 or 25 to 27, wherein the receptor is OR10J5, the agonist odorant compound is a floral muguet compound; and the selected compound is at least one positive allosteric modulator selected from the group consisting of (E)-dec-2-enal, 2-phenylpropanal; (E)-but-2-enal; 3-methylbenzaldehyde; 3-(1,3-benzodioxol-5-yl)-2-methylpropanal; (+)-3-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, (+)-4-(4-methyl-3-penten-1-yl)-3-cyclohexene-1-carbaldehyde, and mixtures thereof; heptanal, 4-Propan-2-ylbenzaldehyde; (3R)-3,7-dimethyloct-6-enal; (+)-(3S)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, (+)-(3R)-3-[(1R)-4-methyl-3-cyclohexen-1-yl]butanal, and mixtures thereof; hexanal; 2,6-dimethylhept-5-enal; benzaldehyde; 2-methyl-3-(4-methylphenyl)propanal; 3,5,6-trimethyl-3-cyclohexene-1-carbaldehyde, 2,4,6-trimethyl-3-cyclohexene-1-carbaldehyde, and mixtures thereof; 4-ethylbenzaldehyde; 6-methoxy-2,6-dimethylheptanal; (E)-non-2-enal; and combinations thereof.

31. The method of claim 19, wherein the screening comprises calculating an activation window to measure the synergistic effect enhancement at EC₀₅₋₅₀.