**Supplementary Information**

**Ligand discrimination in hOR1A1 based on the capacitive response**

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**Figure S1.** Functional tests of the hOR1A1-transfected HEK293 cells using the cAMP-mediated luciferase assay (GloSensor assay). The hOR1A1 activity was measured using HEK293S GnTI- cells that had been stably transfected with the pcDNA4/To-FLAG-hOR1A1-rho1D4 plasmid. The cells were treated with tetracycline (1 mg/mL) 48 h before the test. Receptor activity was measured after the cells were stimulated with the corresponding ligand. Data were fitted with sigmoid dose-response curves and the binding affinity (EC50 value), and efficacy (maximal amplitude) values were determined using Sigma Plot software. The results are shown as the means ± S.E.M. N > 3.

**Table S1**. Specific capacitance (*Cs*) obtained from voltammetry with and without the presence of (*R*)-(-)-carvone1.

|  |  |  |
| --- | --- | --- |
| Scan rate (mV/s) | *CS* OR (F/m2) | *CS* OR + (*R*)-(-)-carvone (F/m2) |
| 581 | 0.365  0.381  0.384  0.383  0.382  0.452  0.448  0.440  0.435  0.431  0.618  0.614  0.606  0.614  0.595 | 0.301  0.298  0.292  0.289  0.286  0.365  0.360  0.353  0.349  0.345  0.470  0.469  0.463  0.458  0.455 |
| 1000 | 0.339  0.347  0.349  0.349  0.348  0.380  0.388  0.388  0.387  0.387  0.533  0.544  0.545  0.544  0.542 | 0.246  0.252  0.254  0.254  0.253  0.295  0.303  0.305  0.304  0.304  0.401  0.410  0.411  0.410  0.409 |
| 5800 | 0.220  0.224  0.226  0.227  0.228  0.232  0.237  0.239  0.239  0.240  0.331  0.341  0.344  0.345  0.345 | 0.167  0.170  0.171  0.172  0.172  0.184  0.187  0.189  0.190  0.190  0.253  0.258  0.260  0.261  0.261 |
| 10000 | 0.175  0.178  0.180  0.180  0.181  0.178  0.181  0.183  0.184  0.185  0.246  0.251  0.254  0.255  0.254 | 0.141  0.143  0.144  0.144  0.145  0.149  0.151  0.152  0.153  0.153  0.197  0.200  0.202  0.203  0.204 |

**Table S2.** Specific capacitance (Cs) obtained from voltammetry with and without the presence of (*S*)-(+)-carvone.

|  |  |  |
| --- | --- | --- |
| Scan rate (mV/s) | *CS* OR (F/m2) | *CS* OR + (*S*)-(+)-carvone (F/m2) |
| 581 | 0.575  0.572  0.564  0.559  0.555  0.544  0.542  0.535  0.529  0.525  0.570  0.568  0.560  0.554  0.549 | 0.439  0.435  0.428  0.422  0.418  0.421  0.417  0.409  0.404  0.400  0.430  0.426  0.419  0.414  0.410 |
| 1000 | 0.478  0.494  0.498  0.499  0.498  0.465  0.474  0.475  0.474  0.473  0.451  0.472  0.480  0.482  0.483 | 0.364  0.373  0.374  0.374  0.373  0.343  0.352  0.354  0.353  0.353  0.356  0.365  0.366  0.365  0.365 |
| 5800 | 0.297  0.303  0.306  0.308  0.309  0.282  0.288  0.291  0.292  0.293  0.286  0.293  0.297  0.300  0.301 | 0.226  0.231  0.233  0.234  0.235  0.212  0.216  0.219  0.220  0.220  0.223  0.228  0.230  0.231  0.231 |
| 10000 | 0.224  0.228  0.231  0.232  0.233  0.215  0.219  0.221  0.222  0.223  0.220  0.225  0.227  0.229  0.230 | 0.179  0.182  0.183  0.184  0.185  0.170  0.172  0.174  0.175  0.176  0.175  0.178  0.180  0.180  0.181 |

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**Figure S2.** Potentiostatic electrochemical impedance spectroscopy (PEIS) with zero polarization. **A.** Averaged Bode impedance and phase plots obtained for hOR1A1 before (purple) and after incubation with dihydrojasmone (black), (*R*)-(-)-carvone (orange) or (*S*)-(-)-carvone (green). Data were obtained in the 500 mHz-100 kHz range with an applied sinus amplitude of 10 mV. n = 10 and N = 2 for dihydrojasmone and N = 3 for carvones. **B.** Representative fitting of PEIS spectra to an equivalent electrical circuit with a constant phase element (CPE) and a resistor connected in series.

The impedance of a CPE is given by:

Where *Qdl* and *αdl* are the CPE parameters. *αdl* is dimensionless and takes values between 0-1. When *αdl* = 1, the system behaves as a pure capacitor. For *αdl* = 0.6-1, the Nyquist plots show a straight line tilted 90x *αdl* degrees with the *x*-axis. *Qdl* is expressed in .

For an ideally polarizable electrode, capacitance can be determined from CPE parameters using Brug’s equation2:

**Table S3.** CPE parameters obtained from the fitting of PEIS spectra at zero polarization, and calculated *CS* from Brug’s equation.

|  |  |  |
| --- | --- | --- |
| Dihydrojasmone | | OR |
| *R1* (ohm) | 136.7 | 118.5 |
| 138.0 | 117.1 |
| *Qdl* | 9.885E-6 | 1.141E-5 |
| 9.715E-6 | 9.783E-6 |
| *αdl* | 0.88757 | 0.89422 |
| 0.88802 | 0.89823 |
| *Cs* (F/m2) | 0.0157 | 0.0215 |
| 0.0155 | 0.0197 |
| (*R*)-(-)-carvone | | OR |
| *R1* (ohm) | 157.0 | 160.5 |
| 98.2 | 150.8 |
| *Qdl* | 5.927E-6 | 6.688E-6 |
| 8.777E-6 | 1.115E-5 |
| *αdl* | 0.91046 | 0.91226 |
| 0.89242 | 0.89484 |
| *Cs* (F/m2) | 0.0141 | 0.0166 |
| 0.0158 | 0.0206 |
| (*S*)-(+)-carvone | | OR |
| *R1* (ohm) | 125.2 | 118.3 |
| 123.0 | 151.1 |
| 97.3 | 143.5 |
| *Qdl* | 7.822E-6 | 9.733E-6 |
| 7.419E-6 | 8.963E-6 |
| 8.030E-6 | 9.965E-6 |
| *αdl* | 0.89538 | 0.88913 |
| 0.89733 | 0.89648 |
| 0.89095 | 0.89548 |
| *Cs* (F/m2) | 0.0143 | 0.0162 |
| 0.0141 | 0.0167 |
| 0.0139 | 0.0185 |

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**Figure S3.** Potentiostatic electrochemical impedance spectroscopy (PEIS). Polarization experiments. **A.** Averaged Bode impedance and phase plots obtained for hOR1A1 before (purple) and after incubation with dihydrojasmone (black), (*R*)-(-)-carvone (orange) or (*S*)-(-)-carvone (green), respectively, without polarizing (pale color) or polarizing the electrode +150 mV (strong color). Data were obtained in the 500 mHz-100 kHz range with an applied sinus amplitude of 10 mV. n = 5 and N = 2. **B.** Representative fitting of PEIS spectra to an equivalent electrical circuit with two CPEs and a resistor connected in series. CPE2-P parameter, attributed to the electric double layer resulting from the applied overpotential, was fixed ⁓ 0.6 to allow for modelling it as a CPE in the equivalent circuit2,3.

**Table S4.** CPE parameters obtained from the fitting of PEIS spectra at +150 mV polarization, and calculated *CS* from Brug’s equation.

|  |  |  |
| --- | --- | --- |
| Dihydrojasmone | | OR |
| *R1* (ohm) | 128.5 | 125.7 |
| 118.0 | 146.4 |
| *Qdl* | 2.000E-05 | 2.339E-05 |
| 2.848E-05 | 2.416E-05 |
| *αdl* | 0.94469 | 0.96259 |
| 0.91582 | 0.95547 |
| *Cs* (F/m2) | 0.102 | 0.163 |
| 0.0894 | 0.149 |
| (*R*)-(-)-carvone | | OR |
| *R1* (ohm) | 100.5 | 146.8 |
| 126.0 | 107.5 |
| *Qdl* | 1.901E-05 | 2.343E-05 |
| 1.804E-05 | 1.956E-05 |
| *αdl* | 0.94677 | 0.95777 |
| 0.93333 | 0.94680 |
| *Cs* (F/m2) | 0.101 | 0.150 |
| 0.0745 | 0.104 |
| (*S*)-(+)-carvone | | OR |
| *R1* (ohm) | 107.5 | 138.3 |
| 124.5 | 126.6 |
| *Qdl* | 1.847E-05 | 2.227E-05 |
| 2.009E-05 | 2.231E-05 |
| *αdl* | 0.94494 | 0.95154 |
| 0.93259 | 0.94442 |
| *Cs* (F/m2) | 0.0949 | 0.128 |
| 0.0826 | 0.114 |

The frequency dependent capacitance (*C*(*ω*)) can be extracted from AC impedance data, and it is a combination of real (*C’*) and imaginary (*C’’*) parts:

The imaginary part of the complex capacitance (*C’’*(*ω*)) relates to the irreversible energy dissipation, corresponding to the relaxation process, and can be obtained from:

where *Z’*(*ω*)is the imaginary part of the impedance and |*Z*(*ω*)| is the impedance modulus.

Relaxation time constant (τ*R*) is obtained from the peak frequency (relaxation frequency, *fR*) in the *C’’* vs. frequency plots according to the equation4:

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**Figure S4.** Kernel Density Estimation (KDE) plot showing **A.** the distribution of dipole moment magnitudes and **B.** the distribution of the dipole moment vector deviation from the *z*-axis for 100 models in the active conformation (solid line) and inactive conformation (dashed line). In red hOR1A1 with C-ter and N-ter part of OR1A1 were removed.

Adenosine A2a and β2 adrenergic receptor structure model were produced. Active and Inactive structures were taken from the PDB for A2a (PDB IDs: 5G53, 8GNG respectively active and inactive) and β2 receptor (PDB IDs: 3SN6, 4GBR respectively active and inactive). Conformational state was determined using GPCRdb procedure as reference5. From those two starting conformations, the distributions of electrical dipole (Figure S4), were obtained following the same procedure described for hOR1A1.

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**Figure S5.** Kernel Density Estimation (KDE) plot showing the distribution of **A.** dipole moment magnitudes and **B.** dipole moment vector deviation from the *z*-axis for 100 models in the active conformation (solid line) and inactive conformation (dashed line). Green lines represent models for the GPCR class A adenosine A2A receptor, in red the β2 receptor and in black hOR1A1.

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