Cryo-EM structure of the serotonin 5-HT\textsubscript{1B} receptor coupled to heterotrimeric G\textsubscript{o}

Javier García-Nafría\textsuperscript{1,2}, Rony Nehmé\textsuperscript{1,2}, Patricia C. Edwards\textsuperscript{1} & Christopher G. Tate\textsuperscript{1,\#}

G-protein-coupled receptors (GPCRs) form the largest family of receptors encoded by the human genome (around 800 genes). They transduce signals by coupling to a small number of heterotrimeric G proteins (16 genes encoding different \(\alpha\)-subunits). Each human cell contains several GPCRs and G proteins. The structural determinants of coupling of G\textsubscript{o} to four different GPCRs have been elucidated\textsuperscript{1–4}, but the molecular details of how the other G-protein classes couple to GPCRs are unknown. Here we present the cryo-electron microscopy structure of the serotonin 5-HT\textsubscript{1B} receptor (5-HT\textsubscript{1B}R) bound to the agonist donitriptan and coupled to an engineered G\textsubscript{o}, heterotrimer. In this complex, 5-HT\textsubscript{1B}R is in an active state; the intracellular domain of the receptor is in a similar conformation to that observed for the \(\beta_3\)-adrenoceptor (\(\beta_3\)AR)\textsuperscript{5} or the adenosine A\textsubscript{2A} receptor (A\textsubscript{2A}R)\textsuperscript{6} in complex with G\textsubscript{o}. In contrast to the complexes with G\textsubscript{o}, the gap between the receptor and the G\(\gamma\) subunit in the G\textsubscript{o}–5-HT\textsubscript{1B}R complex precludes molecular contacts, and the interface between the G\(\alpha\)-subunit of G\textsubscript{o} and the receptor is considerably smaller. These differences are likely to be caused by the differences in the interactions with the C terminus of the G\(\alpha\) \(\alpha\)-subunit. The molecular variations between the interfaces of G\textsubscript{o} and G\textsubscript{\textsubscript{\gamma}} in complex with GPCRs may contribute substantially to both the specificity of coupling and the kinetics of signalling.

Heterotrimeric G proteins can be divided into four subfamilies\textsuperscript{6}, G\textsubscript{o}, G\textsubscript{i/o}, G\textsubscript{s} and G\textsubscript{\textsubscript{\gamma}}2\textsubscript{13}, each containing \(\alpha\), \(\beta\) and \(\gamma\)-subunits. When an agonist binds to a GPCR, the receptor couples to a G protein, predominantly through the \(\alpha\)-subunit (G\(\alpha\)); there are relatively few contacts with the \(\beta\)-subunit and none with the \(\gamma\)-subunit. The overall structure of \(\alpha\)-subunits in the inactive GDP-bound state is highly conserved\textsuperscript{6}, and they undergo similar conformational changes upon coupling to GPCRs\textsuperscript{6}. This is characterized by a disorder-to-order transition of the C-terminal half of the \(\alpha\)-helix, which adopts an \(\alpha\)-helical conformation upon binding in the cytoplasmic cleft of an activated GPCR\textsuperscript{6}. The key role of the \(\alpha\)-helix in G-protein coupling to GPCRs has been confirmed through mutagenesis studies, which are consistent with the \(\alpha\)-helix accounting for 70% of the interactions between G\(\alpha\) and \(\beta_2\)AR\textsuperscript{5}. The amino acid sequences of the C terminus of the \(\alpha\)-helix are highly conserved within G-protein subfamilies, but are distinct between different subfamilies. The key role of this region in determining specificity is indicated by the ability to change the coupling specificity of a G protein by mutating the C-terminal region to match that of a different G protein\textsuperscript{6}. However, other regions of the \(\alpha\)-subunit also contribute to specificity, and mutations often do not map directly to specificity when transferred from one G protein to another\textsuperscript{10}. Many receptors couple to more than one G protein, and this coupling can appear different depending upon whether coupling is measured dynamically or in an end-point assay\textsuperscript{11}. The reported structure of \(\beta_3\)AR coupled to heterotrimeric G\textsubscript{\textsubscript{\gamma}} changed the model of how G proteins couple to and are activated by GPCRs\textsuperscript{5}, but it does not address the issue of G-protein specificity. We have therefore determined the structure of 5-HT\textsubscript{1B}R in complex with heterotrimeric G\textsubscript{o} to enable comparisons of receptor coupling to G\textsubscript{i/o} and G\textsubscript{o}.

There are 13 GPCRs in the serotonin receptor family\textsuperscript{12}, all of which are expressed in the central nervous system where they have key roles in all aspects of behaviour\textsuperscript{13}. Structures of several 5-HT receptors have been determined in either inactive or active-intermediate states\textsuperscript{14–16}. 5-HT\textsubscript{1B}R binds the agonist donitriptan with high affinity and couples to G\textsubscript{i/o}\textsuperscript{17}. G\textsubscript{i/o} is the most abundant G protein in the brain and an engineered G\textsubscript{i/o} mini-G\textsubscript{i/o} was developed to form a heterotrimer with the G\(\gamma3\) and G\(\gamma7\) subunits, which can bind and stabilize the agonist-activated 5-HT\textsubscript{1B}R\textsuperscript{18}. We expressed and purified these proteins and assembled them into a complex containing 5-HT\textsubscript{1B}R, donitriptan, mini-G\textsubscript{i/o} \(\beta_1\) and \(\gamma_2\) subunits (see Methods). The purified complex was vitrified on electron microscopy grids and the structure was determined by cryo-electron microscopy (cryo-EM) and single-particle analysis to an overall resolution of 3.8 Å (Extended Data Figs. 1–4, Extended Data Table 1), with clear density for the majority of side chains and the agonist donitriptan (Fig. 1, Extended Data Fig. 2). Donitriptan occupies the orthosteric binding site, and the serotonin-like moiety of the ligand binds in a region analogous to that identified for the native agonists adrenaline\textsuperscript{19} and adenosine\textsuperscript{10} (Fig. 1, Extended Data Fig. 5). Donitriptan binds 5-HT\textsubscript{1B}R in a different mode to the ergot family of alkaloids, such as ergotamine and dihydroergotamine\textsuperscript{15} (Fig. 1). The donitriptan-binding site is formed by amino acid residues in transmembrane helices 3, 5, 6 and 7 (H3, H5, H6 and H7) and extends into the extracellular region to make contacts with H6, H7 and extracellular loop 2 (ECL2). Donitriptan is bound primarily by van der Waals contacts and limited polar interactions with Thr\textsubscript{134} and Asp\textsubscript{129}.

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\section*{Fig. 1} Overall cryo-EM reconstruction of the 5-HT\textsubscript{1B}R–G\textsubscript{o} heterotrimer complex. The density of the cryo-EM map (sharpened with a \(\lambda\) factor of −200) is coloured according to the subunit. The inset shows the orthosteric binding pocket in 5-HT\textsubscript{1B}R (light blue) with donitriptan depicted as sticks (green, carbon) and its density in the cryo-EM map. The lower panel shows a superposition of ergotamine-bound 5-HT\textsubscript{1B}R (red, PDB code 4AR)\textsuperscript{15} and donitriptan-bound 5-HT\textsubscript{1B}R (blue, PDB code 4AR)\textsuperscript{15}. Donitriptan (green, carbon) and ergotamine (orange, carbon) are depicted as sticks.
The conformation of the extracellular region of the receptor that forms and changes in rotamer of Arg 3.50, Tyr 5.58 and Tyr 7.53 (Fig. 2). Of note, αH6 (Cαmine-bound 5-HT1BR shows an 8 Å shift of the cytoplasmic end of residues 15. Comparison of the Gọ-coupled 5-HT1BR with the ergot-partial movement of H6 and partial rotamer changes of key amino acid and was suggested to be in an active intermediate state owing to the BRIL fusion bound to the agonist ergotamine has been determined are almost identical to those in the active-state structures, suggesting preservation of the cytoplasmic region of the receptors (Fig. 2). In addition, the orthosteric binding pocket does not exhibit any marked change in conformation in the transition from the active intermediate state to the active G-protein-coupled state. This resembles the transition of the A2AR21, but different to that observed in β2AR3, which has a different energy landscape22.

The overall architecture of 5-HT1BR coupled to Gọ is similar to that of the β2AR–Gọ and A2AR–Gs complexes1,3, but there are critical differences in the details. The interface between 5-HT1BR and Gọ consists of 9 amino acid residues from 5-HT1BR and 13 from the α-subunit of Gọ. This compares with 24 residues from β2AR and 20 in A2AR that make contact with 17 and 22 residues in Gọ, respectively (Fig. 3). The surface area of Gọ in contact with 5-HT1BR is 822 Å2, whereas the areas of Gọ in contact with β2AR and A2AR are 1260 Å2 and 1135 Å2, respectively. All the contacts made by Gọ with the receptor are through residues in the α5-helix. By contrast, contacts made by Gọ to β2AR and A2AR also involve regions in S1, S2–S3 and H4–S6 (Extended Data Fig. 6). The overall conformations of Gọ and Gọ are very similar when coupled to the receptors (Extended Data Fig. 7). However, alignment of the cytoplasmic regions of 5-HT1BR, β2AR and A2AR shows that the α5-helix of Gọ is positioned differently within the receptor compared to Gọ (Fig. 3, Extended Data Fig. 7). There is a 9° or 11° tilt of the N-terminal end of the α5-helix away from the plane of the membrane when compared to Gọ coupled to β2AR or A2AR, respectively.
(the pivot point is in the region of I344–N347 in G\(_{\alpha}\)). The different tilt angles are probably the result of the different positions of the approximately eight C-terminal amino acid residues of the \(\alpha 5\)-helices that are located within the receptor. This region contains the major determinants of specificity for different G proteins.\(^7\). The final four amino acid residues in the \(\alpha 5\) helix of G\(_{\alpha}\) are Y\(_{H5.23}\)E\(L\)H\(_{5.26}\) (superscripts refer to the CGN numbering system).\(^7\) The equivalent residues in G\(_s\) are Y\(H_{5.23}\)E\(L\)H\(_{5.26}\) (superscripts refer to the CGN numbering system).\(^7\) These residues form a ‘wavy hook’ structure at the end of the \(\alpha 5\)-helix in G\(_{\alpha}\). In G\(_{\alpha}\), the \(\pi\)-electrons of Tyr\(H_{5.23}\) form extensive contacts with the positively charged Arg\(3.50\), which forms the boundary between the cytoplasmic cleft where the \(\alpha 5\)-helix binds and the hydrophobic core of the receptor.\(^21\) Similarly, in G\(_o\), Cys\(354\)H\(_{5.26}\) interacts with Arg\(147.50\), although only through van der Waals interactions. Therefore, the \(\alpha 5\)-helix of both G\(_s\) and G\(_o\) penetrate GPCRs to the same degree. In contrast to G\(_s\), the single amino acid residue in G\(_o\) that makes most contacts with the receptor is the C-terminal Tyr\(354\)H\(_{5.26}\), the side chain of which stacks against Arg\(308.29\) in 5-HT\(_1\)AR and also makes a weak polar interaction with the same residue. In G\(_o\), the terminal amino acid Leu\(394\)H\(_{5.26}\) makes only very few contacts with \(\beta 2\)AR and is disordered in the A\(_{32}\)R−G\(_s\) structure. In the A\(_{32}\)R−mini-G\(_{\alpha}\) crystal structure, there are extensive contacts between Glu\(394\)H\(_{5.24}\) and three Arg residues in the H\(7\)/H\(8\) region of the receptor;\(^21\) the equivalent residue in G\(_s\) is Gly\(354\)H\(_{5.24}\), which makes only minor contacts with the receptor. Although it appears from the cryo-EM structure that all the major contacts between 5-HT\(_1\)AR and G\(_{\alpha}\) are mediated by the \(\alpha 5\)-helix of G\(_{\alpha}\), there is weak density for H5 and H6 of 5-HT\(_1\)AR that extends towards the \(\alpha 4\) helix in G\(_{\alpha}\) (Extended Data Fig. 1). It is known that mutations in the \(\alpha 4\) helix can affect coupling to 5-HT\(_1\)AR,\(^31\) but it is unclear from the structure whether this is because direct contacts to the receptor are absent from the mutated \(\alpha 4\) helix, or because there is a secondary effect of the mutation on the structure of G\(_{\alpha}\).

The determinants of coupling specificity of G proteins are found predominantly at the C terminus of G\(_s\) in the \(\alpha 5\)-helix and the wavy hook. The architecture of this region is virtually identical in G\(_s\) and G\(_o\), but the differences in amino acid sequence (Extended Data Figs 6, 8) result in G\(_{\alpha}\) being bulkier than G\(_{\alpha}\) in the terminal five residues (Extended Data Fig. 9). This may be sufficient to prevent coupling of G\(_s\) to some G\(_{\alpha}\)-specific GPCRs as the narrower crevice in these GPCRs may exclude the bulkier C terminus of G\(_s\). Conversely, the wider crevice in G\(_s\)-coupled receptors may allow coupling of G\(_s\) provided that there are suitable residues lining the crevice to form a good interface. This last caveat raises the problem of predicting G-protein-coupling specificity. Although the structure and mechanism of GPCRs are highly conserved, human GPCRs show considerable sequence heterogeneity; therefore, there is little or no specific amino acid conservation correlating with the subtype of G protein that a receptor couples to.\(^25\) In addition, there is potential for different GPCR conformations,\(^26\) which suggests that the mode of G-protein coupling could be different between different receptors. This is the case in the complex of transducin peptide and opsin,\(^27\) in which the \(\alpha 5\)-helix is tilted by around 30° in comparison to the \(\alpha 5\)-helix of G\(_s\), even though the G proteins are in the same family. More structures need to be determined to evaluate the diversity of G-protein coupling.
The specific differences in packing at the C terminus of $G_o$ compared to $G_i/o$ have a disproportionate effect on the whole $G$ protein owing to their amplification as a result of the different insertion angle of the $\alpha_5$-helix. This results in a change in the tilt of the whole $G$ protein, which moves away from the plane of the membrane and results in a gap between the rest of the $G$ protein and 5-HT$_{1B}$R. Therefore, there are no contacts between 5-HT$_{1B}$R and $G_o$ subunits, and the only contacts made to $G_o$ are with the $\alpha_5$-helix. This is in marked contrast to the relatively close packing of $G_i$ to both $A_3\beta$R and $\beta_2$AR (Fig. 4). Given that the mechanism of GPCR44 and $G$-protein activation is conserved, it is likely that the small interface between 5-HT$_{1B}$R and $G_i/o$ is a common feature of receptor coupling with $G_o$ family, and will be seen in other GPCRs that are activated by diffusible ligands. A likely consequence of the small interface in the receptor–$G_o$ complex is that $G_i/o$ may have a faster rate of dissociation than $G_i$ in the same GPCR. The kinetics of the steps in GPCR signaling pathways are thought to have a profound effect on which particular signaling event results from agonist binding to a receptor in a specific cell type.23,24 A combination of structural data and kinetic analyses will be essential to unravel the complexities of this system.

Online content

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0241-9

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1. García-Nafría, J., Lee, Y., Bai, X., Carpenter, B. & Tate, C. G. Cryo-EM structure of the adenosine $A_2_b$ receptor coupled to an engineered heterotrimetric $G$ protein. eLife 7, e35946 (2018).
2. Liang, Y. L. et al. Phase-plate cryo-EM structure of a class B GPCR–$G_o$ complex. Nature 546, 118–123 (2017).
3. Rasmussen, S. G. et al. Crystal structure of the $\beta_2$ adrenergic receptor–$G_o$ protein complex. Nature 477, 549–555 (2011).
4. Zhang, Y. et al. Cryo-EM structure of the activated GLP-1 receptor in complex with a $G$ protein. Nature 546, 248–253 (2017).
5. Syrovatkina, V., Alegre, K. O., Dey, R. & Huang, X. Y. Regulation, signaling, and physiological functions of $G$ proteins. J. Mol. Biol. 428, 3850–3868 (2016).
6. Oldham, W. M. & Hamm, H. E. Structural basis of function in heterotrimeric $G$ proteins. Q. Rev. Biophys. 39, 117–166 (2006).
7. Flock, T. et al. Universal allosteric mechanism for $G_o$ activation by GPCRs. Nature 524, 173–179 (2015).
8. Venkataraman, A. J. et al. Molecular signatures of $G$ protein-coupled receptors. Nature 494, 185–194 (2013).
9. Oldham, W. M. & Hamm, H. E. HeterotrimERIC $G$ protein activation by $G$ protein-coupled receptors. Nat. Rev. Mol. Cell Biol. 9, 60–71 (2008).
10. Nehme, R. et al. Mini-$G$ proteins: novel tools for studying GPCRs in their active conformation. PLoS One 12, e0175642 (2017).
11. Masuho, I. et al. Distinct profiles of functional discrimination among $G$ proteins determine the actions of $G$ protein-coupled receptors. Sci. Signal. 8, ra123 (2015).
12. McCray, J. D. & Roth, B. L. Structure and function of serotonin $G$ protein-coupled receptors. Pharmacol. Ther. 150, 129–142 (2015).
13. Berger, M., Gray, J. A. & Roth, B. L. The expanded biology of serotonin. Annu. Rev. Med. 60, 355–366 (2009).
14. Becker, D. et al. Structural features for functional selectivity at serotonin receptors. Science 340, 610–614 (2013).
15. Wang, C. et al. Structural basis for molecular recognition at serotonin receptors. Science 340, 610–614 (2013).
16. Yin, W. et al. Crystal Structure of the human 5-HT$_{1B}$R serotonin receptor bound to an inverse agonist. Cell Discovery 4, 12 (2018).
17. Albert, P. R. & Tiberi, M. Receptor signaling and structure: insights from serotonin-1 receptors. Trends Endocrinol. Metab. 12, 458–460 (2001).
18. Ring, A. M. et al. Adrenaline-activated structure of $\beta_2$-adrenoreceptor stabilized by an engineered nanobody. Nature 502, 575–579 (2013).
19. Lebon, G. et al. Agonist-bound adenosine $A_2_b$ receptor structures reveal common features of GPCR activation. Nature 474, 521–525 (2011).
20. Balligand, J.-L., Huaux, G. & Desvergne, B. Kinetic pathways for the conformational changes of G proteins. Annu. Rev. Pharmacol. Toxicol. 35, 473–524 (1995).
21. Carpenter, B., Nehme, R., Warne, T., Leslie, A. G. & Tate, C. G. Structure of the adenosine $A_2_b$ receptor bound to an engineered $G_o$. Nature 536, 104–107 (2016).
22. Lebon, G., Warne, T. & Tate, C. G. Agonist-bound structures of $G$ protein-coupled receptors. Curr. Opin. Struct. Biol. 22, 482–490 (2012).
23. Bae, H., Cabrera-Vera, T. M., Depree, K. M., Gruber, S. G. & Hamm, E. H. Two amino acids within the $\alpha_4$ helix of $G_{oi}$ mediate coupling with the hydroxylamine of $G_{oi}$. J. Biol. Chem. 274, 14963–14971 (1999).
24. Venkataraman, A. J. et al. Diverse activation pathways in class A GPCRs converge near the $G$ protein-coupling region. Nature 536, 484–487 (2016).
25. Flock, T. I. et al. Selectivity determinants of $G$ protein-$G$ protein binding. Nature 545, 317–322 (2017).
26. Kobalik, B. K. & Deupi, X. Conformational complexity of $G$ protein-coupled receptors. Trends Pharmacol. Sci. 28, 397–406 (2007).
27. Scheerer, P. et al. Crystal structure of opioid in its G protein-interacting conformation. Nature 455, 497–502 (2008).
28. Grundmann, M. & Kostenis, E. Temporal bias: time-encoded dynamic GPCR signaling. Trends Pharmacol. Sci. 38, 1110–1124 (2017).
29. Lane, J. R., May, L. T., Parton, R. G., Sexton, P. M. & Christophoulopoulou, A. A kinetic view of GPCR allostery and biased agonism. Nat. Chem. Biol. 13, 929–937 (2017).
30. Isberg, V. et al. GPCRdb: an information system for $G$ protein-coupled receptors. Nucleic Acids Res. 44, D356–D364 (2016).
METHODS
Expression and purification of 5-HT1B-R. N-terminally truncated wild-type human 5-HT1B-R (residues 34–390) was modified to contain a C-terminal histidine tag (His10) and TEV protease cleavage site. The L138W mutation was introduced to increase thermostability. Recombinant baculoviruses expressing 5-HT1B-R were prepared using the flashBAC ULTRA system (Oxford Expression Technologies). Trichoplusia ni cells (Expression Systems) were grown in suspension in PSP92 media (Expression Systems) to a density of 10^6 cells/ml infected with 5-HT1B-R baculovirus and incubated for 48 h. Cells were harvested and membranes prepared by two ultracentrifugation steps in 20 mM HEPES pH 7.5, 1 mM EDTA, 1 mM PMSE. Membranes were resuspended finally in 20 mM HEPES pH 7.5, 500 mM NaCl, 5 mM MgCl2, 10 mM imidazole and Complete protease inhibitors (Roche) and flash frozen in liquid nitrogen and stored at −80 °C.

Membranes from 21 of cells were solubilised with 2% n-decyl-β-D-maltopyranoside (DM) on ice for 30 min in the presence of 1 μM donitriptan hydrochloride. The sample was clarified by ultracentrifugation and loaded onto a 5 ml Ni-NTA column (Qiagen). The column was equilibrated and sample was loaded in buffer A (20 mM HEPES pH 7.5, 500 mM NaCl, 1 mM MgCl2, 50 mM imidazole, 1 μM donitriptan hydrochloride, 0.15% DM), and eluted with buffer B (20 mM HEPES pH 7.5, 100 mM NaCl, 1 mM MgCl2, 300 mM imidazole, 1 μM donitriptan hydrochloride, 0.15% DM). The eluate was concentrated using a 50-kDa cut-off Amicon centrifugal ultrafiltration unit (Millipore), and exchanged into desalting buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 1 mM MgCl2, 1 μM donitriptan hydrochloride, 0.15% DM) using a PD10 column (GE Healthcare). Then, 2.5 mg TEV protease was added, and the sample was incubated on ice overnight. TEV protease was then removed by an application on Ni-NTA resin. The sample was concentrated to ~1 ml and loaded onto a Superdex 200 column (GE Healthcare). Peak fractions corresponding to monomers of receptors were pooled and concentrated. A typical yield was 1–2 mg pure 5-HT1B-R per litre of culture.

Formation of a 5-HT1B-R heterotrimeric mini-Gi complex. Purified 5-HT1B-R was mixed with a 1.2-fold molar excess of mini-Gi13,14β/γ complex in the presence of apyrase (0.2 U/ml) and the mixture was incubated on ice overnight. The sample was loaded on to a Superdex 200 column. Peak fractions containing the 5-HT1B-Rβ/γ mini-Gi13,14β/γ complex were pooled and concentrated to 4 mg/ml.

Cryo-grid preparation and data collection. Cryo-EM grids were prepared by applying 3 μl sample (at a protein concentration of 2.2 mg/ml) on glow-discharged holey gold grids (Quantifoil Au 1.2/1.3 300 mesh). Excess sample was removed by blotting with filter paper for 3–4 s before plunge-freezing in liquid ethane using a FEI Vitrobot Mark IV at 100% humidity and 4 °C. Images were collected on a FEI Titan Krios microscope at 300 kV using a Falcon III detector in electron counting mode and a Volta phase plate. EPU software (FEI) was used for automatic data collection. Data were collected in nine independent sessions to give a total of 5,737 movies. Each micrograph was collected as 75 movie frames at a dose rate of 0.04 e−/Å2·sec (60 e−/Å2 per frame) for 60 s, with a total accumulated dose of ~30 e−/Å2. The map calculation was 75,000 ×, yielding a pixel size of 1.06 Å/pixel.

Data processing and model building. RELION-2.1 was used for all data processing unless otherwise specified. Since data were pooled from nine independent sessions we provide here the general strategy for data processing and collection, while precise particle numbers for a representative dataset are presented in Extended Data Fig. 3. Overall, drift, beam induced motion and dose weighting were corrected with MotionCor2 using 5 × 5 patches. CTF fitting and phase shift estimation were performed using GCTf v0.11.03, which yielded the characteristic pattern of phase shift accumulation over time for each position. Generally, 40 images were taken at each Volta phase plate position. Auto-picking was performed with a Gaussian blob as a template which readily resulted in optimal particle picking. Particles were extracted in a box of 150 pixels (159 Å) and inputted into a one or two reference-free 2D classification (if the majority of 2D classes had non-recognizable or low-quality features, then the selected particles belonging to quality classes were taken to a second round of 2D classification). An ab initio model was generated using 10,000 particles with RELION 2.1 in the first data collection and used throughout. The resulting particles after 2D classification where then used for 3D classification in both three and four classes simultaneously in order to check for consistency in 3D classification and to generate models with different numbers of particles. The models with the best defined features were selected for refinement either on their own or together with a second class from the same 3D classification (if more than one quality model was present). The particles that reached the highest resolution after gold-standard resolution estimation were saved. Particles obtained in a similar fashion from the different sessions were then merged and refined together. During refinement, the low-pass filter effect of the Wiener filter in the refinement protocol was optimised using the map subtraction of the decimal parameters (τ = 3). This allowed the refinement algorithm to consider higher spatial frequencies in the alignment of the individual particles. Nevertheless, both half-reconstructions were kept completely separately, and the final resolution estimate (at the post-processing stage in RELION) was based on the standard Fourier shell correlation (FSC) between the two unfiltered half-reconstructions. The final model contained 730,118 particles and reached an overall resolution of 3.78 Å with side chains visible for most of the complex (Extended Data Figs. 1, 2). Local resolution estimates were calculated with Resmap showing a core of the protein at ~3.5 Å resolution and an extracellular region of the receptor and βγ N termini at poorer resolution with the worst regions reaching ~5 Å (Extended Data Fig. 1). A manual subtraction of the DM micelle did not improve the quality of the map upon refinement.

Model building and refinement was carried out using the CCP-EM software suite. The 5-HT1B-R-ergotamine crystal structure was used as a starting model (Protein Data Bank (PDB) accession 4AIR) for receptor building. 5-HT1B-R was modelled from residue L45 to R385. Although density was present from Y38 and this region seems to adopt a similar conformation to the 5-HT1B-R crystal structure, the poor resolution in this region prompted us to leave it unmodelled. Residues R188 to V196 in the ECL2 and L339 to C344 in IC1L3 were flexible with absent or very poor map density and were therefore, not modelled. For the same reason residues K241 to L304 forming the large 5-HT1B-R ICL3 loop were left unmodelled. Mini-Gi was modelled from residue LS to Y354 following native Go numbering. Modifications in Go were obtained from PDB accession 4AIR (Extended Data Fig. 7). Although β and γ subunits were modelled using the available crystal structures, poor density was found for both N termini, with the whole of the γ subunit having poor density. For this reason the worst regions of these subunits were modelled as poly-alanine. Initial manual model building was performed in Coot following a jelly-body refinement in RELION. The resulting models and library were created with LigandFit and manually fitted into the density using space real space refinement in Coot. Restraints were generated with ProSMART in order to maintain structural features in regions of poorer density. B factors were set to 40 Å2 before refinement. The model then followed cycles of manual modifications in Coot and restraint refinement in REFMAC5. The final model achieved good geometry (Extended Data Table 1) with validation of model performed in Coot, Molprobity and EMRinger. Goodness of fit of the model to the map was carried out using Phenix using a global model-vs-map FSC correlation (Extended Data Fig. 2). Overfitting in refinement was monitored throughout using FSCwork 1.26.6

Limit on the interpretation of the resolution limit is the ligand binding pocket. The ligand binding pocket of 5-HT1B-R is occupied by a single molecule of the agonist donitriptan. Despite the resolution varying between 3.8 Å to 4.3 Å in this region, estimated from the local resolution map (Extended Data Fig. 1), the density allowed modelling of the position and orientation of donitriptan and the majority of the amino acid side chains in the pocket. However, the resolution limits the accuracy of the refined coordinates and care must be taken when analysing the interactions of residues in any of these positions. The best resolution is towards the base of the pocket and Tyr550 is the only residue through the interface of the membrane bilayer and resolution gets worse towards the extracellular surface of the receptor. The ligand has been modelled using real space refinement, taking into account the location of nearby residues as well as using a library of restraints with allowed conformations of donitriptan. The density allowed modelling of the position and orientation of the donitriptan molecule, with the indole group buried deep in the orthosteric binding pocket and the remainder of the ligand protruding towards the extracellular surface.

Clear interpretable density is found for large aromatic groups, while density for poorer residues with smaller side chains such as Ser334 and Ser212 interacts with the orthosteric binding pocket and the remainder of the aromatic group of donitriptan. This was concluded based on interpretation of maps with different sharpening levels, but its rotamer cannot be assigned with confidence. The orientation of the primary amine on the serotonin moiety in donitriptan and the adjacent side chain of Asp129 cannot be confidently assigned owing to poor density. However, Asp129 is absolutely conserved in all the human serotonin GPCRs and forms a hydrogen bond with ergotamine in the high-resolution crystal structure of 5-HT1B-R. We have therefore modelled Asp129 and a second rotamer to make a potential hydrogen bond with this primary amine in donitriptan, despite the lack of density for both the primary amine and the carbonyl group of Asp129.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. All data generated or analysed during this study are included in the manuscript and supporting Information. The cryo-EM density map has been deposited in the Electron Microscopy Data Bank under accession code EMD–4358 and the coordinates have been deposited in the Protein Data Bank under accession number 6G79.
31. Kimanius, D., Forsberg, B. O., Scheres, S. H. & Lindahl, E. Accelerated cryo-EM structure determination with parallelisation using GPUs in RELION-2. eLife 5, https://doi.org/10.7554/eLife.18722 (2016).
32. Zheng, S. Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 331–332 (2017).
33. Zhang, K. Gctf: Real-time CTF determination and correction. J. Struct. Biol. 193, 1–12 (2016).
34. Fernandez-Leiro, R. & Scheres, S. H. W. A pipeline approach to single-particle processing in RELION. Acta Crystallogr D 73, 496–502 (2017).
35. Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. J. Struct. Biol. 180, 519–530 (2012).
36. Kucukelbir, A., Sigworth, F. J. & Tagare, H. D. Quantifying the local resolution of cryo-EM density maps. Nat. Methods 11, 63–65 (2014).
37. Burnley, T., Palmer, C. M. & Winn, M. Recent developments in the CCP-EM software suite. Acta Crystallogr D 73, 469–477 (2017).
38. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr D 60, 2126–2132 (2004).
39. Murshudov, G. N. et al. REFMACS for the refinement of macromolecular crystal structures. Acta Crystallogr. D 67, 355–367 (2011).
40. Lebedev, A. A. et al. JLigand: a graphical tool for the CCP4 template-restraint library. Acta Crystallogr. D 68, 431–440 (2012).
41. Nicholls, R. A., Long, F. & Murshudov, G. N. Low-resolution refinement tools in REFMACS. Acta Crystallogr. D 68, 404–417 (2012).
42. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. D 66, 12–21 (2010).
43. Barad, B. A. et al. EMRinger: side chain-directed model and map validation for 3D cryo-electron microscopy. Nat. Methods 12, 943–946 (2015).
44. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D 66, 213–221 (2010).
45. Amunts, A. et al. Structure of the yeast mitochondrial large ribosomal subunit. Science 343, 1485–1489 (2014).
Extended Data Fig. 1 | Cryo-EM single particle reconstruction of the 5-HT$_{1B}$R–G$_{	ext{o}}$ complex structure. a, Representative micrograph (magnification $75,000 \times$, defocus $-0.6 \mu$m) of the 5-HT$_{1B}$R–G$_{	ext{o}}$ complex collected using a Titan Krios with the Falcon III detector and Volta phase plate. b, Representative 2D class averages of the 5-HT$_{1B}$R–G$_{	ext{o}}$ complex. c, FSC curve of the final reconstruction showing an overall resolution of 3.8 Å using the gold-standard FSC of 0.143. Both masked and unmasked FSC curves are shown to highlight the lack of masking artefacts. d, Final reconstruction coloured by subunit. Inset shows a magnified view of the weak density for ICL3. The magnified region corresponds to a map sharpened with $B = -50$ to remove noise from lower density levels. e, Local resolution estimation of the 5-HT$_{1B}$R map as calculated by Resmap.
Extended Data Fig. 2 | Cryo-EM map quality and model validation.

a, Transmembrane helices of 5-HT\(_{1B}\)R. b, The \(\alpha5\)-helix of G\(_{\alpha}\).
c, Donitriptan and the neighbouring side chains in the orthosteric binding site. d, FSC of the refined model versus the map (green curve) and FSC\(_{\text{work}}$/FSC_{\text{test}}$ validation curves (blue and red curves, respectively).
Extended Data Fig. 3 | Flow chart of data processing. Micrographs were collected during nine sessions on the Titan Krios (either 24 h or 48 h) and each session was processed independently. The number of images and particles from one 48-h session is indicated on the flowchart as a guide. At the bottom of the figure, the final number of particles is shown. Each dataset was corrected separately for drift, beam-induced motion and radiation damage. After CTF estimation, particles were picked using a Gaussian blob and submitted to either one or two rounds of reference-free 2D classification (see Methods). A 3D classification was performed on the selected particles using an ab initio model generated from ten thousand particles. Classification was performed in parallel in three and four classes. The models with best features were refined on their own; if there were two classes of similar high quality, these were then re-refined together (the resolution of the models refers to the resolution after refinement and calculation of gold-standard FSC = 0.143). The set of particles that obtained the best map quality and resolution were saved and merged with the best particles from other datasets. A final model with 730,118 particles was refined and achieved a global resolution of 3.78 Å.
**Extended Data Fig. 4 | Modelling quality of the 5-HT\textsubscript{1B}R structure.**

a. Amino acid sequence of the 5-HT\textsubscript{1B}R construct used for the cryo-EM structure determination. Residues are coloured according to how they have been modelled. Black, good density allows the side chain to be modelled; red, limited density for the side chain, therefore the side chain has been truncated to C\textsubscript{\beta}; blue, no density observed and therefore the residue was not modelled. Regions highlighted in grey represent the transmembrane \(\alpha\)-helices, and amphipathic helix 8 is highlighted in yellow. b. Model of 5-HT\textsubscript{1B}R showing the \(C_x\) positions of amino acid residues with poor density (spheres) and unmodelled regions (dotted lines).
Extended Data Fig. 5 | Superposition of donitriptan, adrenaline and adenosine bound to their respective receptors. 5-HT₁B, β₂AR and A₂AR were superimposed (using Pymol) over the whole of the receptor. Green, donitriptan; pink, adrenaline; blue, adenosine.
Extended Data Fig. 6 | Comparison of the amino acid sequences of the α-subunits of G_o and G_s. Diamonds above the sequences indicate amino acid residues in G_s in which the side chains make atomic contacts to residues in β_2AR (32 con) or A2A_R (2A con). Ovals indicate amino acid residues in G_o, in which only the main chain atoms make contacts with the receptor. Secondary structural elements are indicated as grey bars with positions numbered according to the CGN numbering system.

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Extended Data Fig. 7 | Similarity of Gα structures and the difference poses of the α5-helices in Gαo and Gαs coupled to receptors. a, The structures of the α-subunits in complex with 5-HT1B, β2AR and A2AR were superimposed over the whole of their sequence in Pymol. Blue, Gαo coupled to 5-HT1B; green, Gαs coupled to A2AR; Gαs coupled to β2AR. 

b, 5-HT1B (blue), β2AR (green) and A2AR (red) were superimposed based on H3, H5 and H6. Two different views are shown with the red arrows indicating differences in orientation of Gαo and Gαs.
Extended Data Fig. 8 | Alignment of the amino acid sequences of Gα and Gαi α-subunits. Residues in grey correspond to the α-helical region that does not make contact with GPCRs and was deleted during the construction of mini-Gα. Secondary structural elements are depicted as grey bars with the CGN numbers shown to aid comparisons. Amino acids are highlighted as follows: pink, stabilizing residues required to generate mini-Gα; yellow, residues in Gα that are different from residues conserved in all three Gαi sequences; blue, residues that are non-conserved in Gαi sequences. #, the affinity tag on mini-Gα used for purification (MGHHHHHENLYFQG).
Extended Data Fig. 9 | Comparison of the α5-helices of Gs and Go. The α5 helices in the cryo-EM structures of A2AR–Gs (carbon, green) and 5-HT1B–Go (carbon, light blue) were aligned (in Pymol) along their whole sequence and displayed in different poses: cartoon depiction (a); Gs (green spheres), Go (blue sticks) (b); Go (blue spheres), Gs (green sticks) (c).
Extended Data Table 1  |  Data collection and refinement statistics

| 5-HT₁B - MiniG<sub>0</sub>βγ<sub>γ</sub> (EMDB-4358) (PDB 6G79) |
|---------------------------------------------------------------|
| **Data collection and processing**                            |
| Magnification                                                 | 75,000x |
| Voltage (kV)                                                  | 300     |
| Electron exposure (e-/Å<sup>2</sup>)                         | 30      |
| Defocus range (µm)                                           | -0.3 to -1.0 |
| Pixel size (Å)                                                | 1.06    |
| Symmetry imposed                                             | C1      |
| Initial particle images<sup>a</sup> (no.)                     | 1,249,822 |
| Final particle images (no.)                                   | 730,118 |
| Map resolution (Å)                                           | 3.78    |
| FSC threshold                                                | 0.143   |
| Map resolution range<sup>b</sup> (Å)                         | ~3.4 to ~4.6 |

| **Refinement**                                                |
| Initial model used (PDB code)                                 | 5G53, 3SN6 |
| Model resolution<sup>c</sup> (Å)                              | 3.9      |
| FSC threshold                                                | 0.5      |
| Map sharpening B factor (Å<sup>2</sup>)                       | -200     |
| Model composition                                            |
| Non-hydrogen atoms                                           | 6053     |
| Protein residues                                             | 6023     |
| Ligands                                                      | 30       |
| B factors (Å<sup>2</sup>)                                     |
| Protein                                                      | 97       |
| Ligand                                                       | 108      |
| R.m.s. deviations                                            |
| Bond lengths (Å)                                             | 0.007    |
| Bond angles (°)                                              | 1.02     |
| Validation                                                   |
| MolProbity score                                             | 1.07     |
| Clashscore                                                   | 0.61     |
| Poor rotamers (%)                                            | 0.56     |
| EMRinger score                                               | 2.34     |
| Ramachandran plot                                            |
| Favored (%)                                                  | 94.64    |
| Allowed (%)                                                  | 4.88     |
| Disallowed (%)                                               | 0.48     |

<sup>a</sup>After 2D classification.
<sup>b</sup>Local resolution range.
<sup>c</sup>Resolution at which FSC between map and model is 0.5.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

☐ N/a

☐ Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ The statistical test(s) used AND whether they are one- or two-sided

☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ A description of all covariates tested

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

☐ Give P values as exact values whenever suitable.

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

☐ Clearly defined error bars

☐ State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

| Data collection | EPU 1.9, EPU 1.10 |

Data analysis

| Data analysis | RELION 2.1, MotionCor2, Gctf v0.1.06, CCP-EM v1, REFMAC5, ProSMART, Phenix 1.13, ResMap1.1.4, Coot 0.8.9.1, Chimera 1.8.1, Pymol 1.8.4.2, JUlgad 1.0.4, Molprobity webserver, EMRinger webserver |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data. All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The structure has been deposited in the PDB with accession code 6G79 and in the EMDB with accession code 4358.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Not applicable |
| Data exclusions | None |
| Replication | Structure determination does not require replication |
| Randomization | Not applicable |
| Blinding | Not applicable |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
| --- | --- |
| ☑ | Unique biological materials |
| ☑ | Antibodies |
| ☑ | Eukaryotic cell lines |
| | Palaeontology |
| | Animals and other organisms |
| | Human research participants |

Methods

| n/a | Involved in the study |
| --- | --- |
| | ChIP-seq |
| | Flow cytometry |
| | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials.

Obtaining unique materials

None used

Antibodies

Antibodies used

None

Validation

Not applicable
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) Trichoplusia ni: Expression Systems
Authentication The cell line was not authenticated
Mycoplasma contamination The cell line was not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)
None

Palaeontology

Specimen provenance n/a
Specimen deposition n/a
Dating methods n/a

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals n/a
Wild animals n/a
Field-collected samples n/a

Human research participants

Policy information about studies involving human research participants

Population characteristics n/a
Recruitment n/a

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirms that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.
Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
☐ All plots are contour plots with outliers or pseudocolor plots.
☐ A numerical value for number of cells or percentage (with statistics) is provided.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Methodology

Sample preparation

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

☐ Used

☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.
### Noise and artifact removal
Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

### Volume censoring
Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

### Statistical modeling & inference

#### Model type and settings
Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g., fixed, random or mixed effects; drift or auto-correlation).

#### Effect(s) tested
Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

#### Specify type of analysis:
- [ ] Whole brain
- [ ] ROI-based
- [ ] Both

#### Statistic type for inference
Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

#### Correction
Describe the type of correction and how it is obtained for multiple comparisons (e.g., FWE, FDR, permutation or Monte Carlo).

### Models & analysis

| n/a | Involved in the study |
|-----|-----------------------|
|     | Functional and/or effective connectivity |
|     | Graph analysis |
|     | Multivariate modeling or predictive analysis |

#### Functional and/or effective connectivity
Report the measures of dependence used and the model details (e.g., Pearson correlation, partial correlation, mutual information).

#### Graph analysis
Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g., clustering coefficient, efficiency, etc.).

#### Multivariate modeling and predictive analysis
Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.