Cryo-EM structure of the activated GLP–1 receptor in complex with a G protein

Yan Zhang1*, Bingfa Sun2*, Dan Feng2, Hongli Hu1, Matthew Chu2, Qianhui Qu1, Jeffrey T. Tarrasch1, Shane Li2, Tong Sun Kobilka2, Brian K. Kobilka2,3 & Georgios Skiniotis4†

Glucagon–like peptide 1 (GLP–1) is a hormone with essential roles in regulating insulin secretion, carbohydrate metabolism and appetite. GLP–1 effects are mediated through binding to the GLP–1 receptor (GLP–1R), a class B G–protein–coupled receptor (GPCR) that signals primarily through the stimulatory G protein \(G_s\). Class B GPCRs are important therapeutic targets; however, our understanding of their mechanism of action is limited by the lack of structural information on activated and full–length receptors. Here we report the cryo–electron microscopy structure of the peptide–activated GLP–1R–\(G_s\) complex at near atomic resolution. The peptide is clasped between the \(N\)–terminal domain and the transmembrane core of the receptor, and further stabilized by extracellular loops. Conformational changes in the transmembrane domain result in a sharp kink in the middle of transmembrane helix 6, which pivots its intracellular half outward to accommodate the \(\alpha_5\)–helix of the Ras–like domain of \(G_s\). These results provide a structural framework for understanding class B GPCR activation through hormone binding.

GLP–1 is a hormone released from the gut in response to food intake and acts on GLP–1R to stimulate glucose–dependent insulin secretion from pancreatic \(\beta\) cells, reduce glucagon secretion from pancreatic \(\alpha\) cells, and decrease gastric motility and appetite. Given its physiological effects, GLP–1R represents an important drug target for type 2 diabetes and obesity, with GLP–1 and its analogues already serving as approved therapeutics.

GLP–1R is a prototypical member of the class B GPCRs and forms the glucagon receptor subfamily along with the glucagon receptor (GCGR), the glucagon–like peptide 2 receptor (GLP–2R), and the gastrin–inhibiting polypeptide receptor (GIPR). Class B receptors share common structural features including an extracellular N–terminal domain (NTD) that binds to the C–terminal half of the peptide hormone, and a seven transmembrane domain (7TM) that binds the N–terminal half of the peptide (Fig. 1a). The NTDs of class B GPCRs share a common fold stabilized by three disulfide bonds, and contribute most of the binding affinity of the receptor for the peptide. After this initial binding event, the N–terminal end of the peptide engages and activates the 7TM through a mechanism that is currently unknown.

To date, nine NTD structures in complex with short hormone peptides have been reported (reviewed in ref. 7), including the GLP–1R NTD bound to GLP–1 (ref. 4). More recently, the transmembrane structure of GCGR8,9 and the corticotropin–releasing factor receptor 1 (ref. 10) in their inactive state provided the first insights into the configuration of the 7TM of class B receptors. However, our understanding of the class B signal transduction mechanism remains limited, primarily owing to the lack of structural information on active–state receptors that include both the 7TM and NTD in complex with a peptide ligand.

GPCRs and their complexes have proven to be difficult targets of X–ray crystallography, often necessitating extensive engineering for conformational stabilization and crystallogenesis. Cryo–electron microscopy (cryo–EM) has recently emerged as a cutting–edge method for structure determination, yielding structures of macromolecular complexes that were otherwise unobtainable with traditional approaches. Nevertheless, high–resolution cryo–EM of asymmetric and relatively small membrane proteins (<200 kDa) remains challenging owing to the low signal–to–noise ratio that hampers the accuracy of angular determination for 3D reconstructions. This problem is compounded by the intrinsically dynamic character of the 7TM bundle and the relative instability of GPCR complexes, such as with G proteins or arrestins, often resulting in conformational variability or even dissociation during cryo–EM specimen preparation. Notwithstanding these challenges, cryo–EM visualization for GPCR complexes holds tremendous potential for uncovering the various molecular mechanisms involved in signal transduction and regulation of GPCRs and their effector proteins. Here, we applied single–particle cryo–EM to determine the structure of the 150 kDa complex between the active–state GLP–1R and heterotrimeric G protein \(G_s\), obtaining insights into class B GPCR activation.

Cryo–EM structure determination

For cryo–EM studies we used rabbit GLP–1R, which shares 92% identity with the human receptor, as it expressed at higher levels in insect cells than the human or mouse homologue. GLP–1R is very unstable to extraction from membranes using conventional detergents such as DDM and MNG; therefore, we formed the complex between receptor and purified \(G_s\) in insect cell membranes before extraction of the GLP–1R–\(G_s\) complex with MNG and purification by antibody affinity and size–exclusion chromatography (Extended Data Fig. 1).

Sample evaluation by negative–stain EM and single–particle averaging confirmed a monodisperse particle population and stable complex formation. Initial cryo–EM experiments suggested that the protein complex avoided areas of thin vitreous ice, and we thus had to image the specimen in relatively thick ice at the expense of increased background...
noise in the micrographs (Extended Data Fig. 2a). Nevertheless, 2D classification revealed class averages with clear secondary structure features for the complex, including the GLP-1R 7TM region embedded in detergent micelle (Extended Data Fig. 2b). Subsequent 3D classification led to the identification of a particle partition showing well-defined and stable features for the complex, apart from the α-helical domain of Goα whose density was averaged out (Extended Data Fig. 3). This is in agreement with our previous studies showing that the α-helical domain gets delocalized from the Goα Ras-like domain and becomes flexible in the absence of nucleotide19. Refinement and reconstruction of the selected particle projections after subtracting densities for the detergent micelle and the mobile α-helical domain enabled us to obtain a 3D reconstruction of the complex at 4.1 Å global resolution, with 3.9 Å nominal resolution in the core region that includes GLP-1, TM1, TM2, TM5, TM7, ECL1 and ECL2, as well as the NTD (Fig. 2, Extended Data Figs 3–5). The cryo-EM map shows a stable and well-defined density for the GLP-1 peptide that is observed between the receptor NTD and the GLP-1 peptide agonist via a ‘two-domain’ binding mechanism. AH, α-helical bundle with omitted NTD.

The interface between the C-terminal half of the peptide hormone and NTD appears identical to the one in the crystal structure of NTD–GLP-1 (Extended Data Fig. 8). Notably, the cryo-EM map suggests that the NTD is not in contact with the 7TM region, although based on our refined structure we raise the possibility of Gln213 of the GLP-1 peptide that is observed between the receptor NTD and the GLP-1 peptide agonist via a ‘two-domain’ binding mechanism. AH, α-helical bundle with omitted NTD.

GLP-1 recognition by GLP-1R

The activated GLP-1R structure shows that the GLP-1 peptide is stably anchored in its position through an extensive network of interactions that involves TM1, 2, 5, 7, ECL1 and 2, as well as the NTD (Fig. 2, Extended Data Fig. 7). Even though the cryo-EM map is limited in resolution, the α-helical nature of the peptide and the stability of its position have allowed us to confidently establish its main interactions with the receptor. The majority of these observations, summarized in Supplementary Table 1, are confirmed through extensive mutagenesis studies on GLP-1R and other class B GPCRs30–31.

GLP-1 recognition by GLP-1R

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GLP-1 recognition by GLP-1R

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is flexible, which is probably important for the initial engagement of the peptide.

For some class B family members, the binding of the peptide to the TM core may stabilize the formation of an α-helix at the N terminus, which can then activate the receptor33. Consistent with this, the cryo-EM structure reveals that the N terminus of GLP-1 forms at least one additional helical turn in the core of the receptor compared to its structure when bound only to the NTD4. Thus, the N terminus of GLP-1 penetrates into the receptor core (Fig. 2a) to a depth comparable to the orthosteric agonist BI-167107 in the structure of activated β2 adrenergic receptor (β2AR)34, a class A GPCR (Extended Data Fig. 9b). An important interaction in this region is mediated by conserved H7P (6 indicates that the residue belongs to the GLP-1 peptide), which is in position to establish hydrogen bonding with R299 (Fig. 2b, c). This arginine resides in ECL2, and thus the long side chain points down towards the receptor core in order to reach close to the peptide N terminus (Extended Data Fig. 7). Another important contact at the N terminus of the peptide is mediated through E9P that participates in van der Waals interactions with Leu388 and Ser392 of TM7, while it is also positioned to potentially form hydrogen bonding with conserved polar residues R190 on TM2 and Y145 on TM1 (Fig. 2c). Position 190 is highly conserved, with strict occupancy by Arg or Lys, as is also the case for K197 (GCGR is exception) that is hydrogen bonded to T13P in GLP-1 (Fig. 2d).

Moving away from the peptide N terminus, notable interactions with GLP-1R involve ECL1 and ECL2. A cluster of neighbouring serines (S145, S179, S185) forms polar interactions with T298 and the backbone oxygen of W297 and nitrogen of R299 on ECL2 (Fig. 2c). Thus, ECL2 plays a prominent role in GLP-1 binding, in agreement with previous studies20,21,28. Towards the peptide C terminus, we observe W31P interacting with ECL1, packing against residues Q211 and H212 (Fig. 2d). ECL1 has been implicated in peptide binding based on hydrogen–deuterium exchange experiments and molecular dynamics simulations also indicate that the peptide binding event stabilizes the conformation of ECL1 in the glucagon–GCGR complex32.

Comparison with inactive family B receptors
GLP-1R and GCGR share an overall 44% sequence homology that extends to 55% for the transmembrane domains. We thus chose the crystal structure of GCGR in complex with an antagonist5 as a reference to examine the structural changes associated with class B GPCR activation. Comparison between activated GLP-1R and inactive GCGR reveals that the transmembrane bundle undergoes extensive conformational transitions induced by the multiple peptide–receptor interactions (Fig. 2). In the extracellular half of the receptor, TM7 bends towards TM6 using as a pivot point the kink at G395 (Wooten numbering in superscript)5,6b,7,8b (Ballesteros–Weinstein numbering for class A GPCRs in superscript15) in class A GPCRs26. In the inactive class B GPCR structures, the extracellular halves of TM6 and TM7, including ECL3, exhibit high crystallographic temperature factors, indicating structural flexibility. In the active GLP-1R complex, the extracellular half of TM6 unwinds and moves outwards to allow interactions between the peptide amino terminus and the transmembrane domain binding pocket. The extracellular half of TM1 follows the movement of TM7, dictated by the hydrogen bond between the highly conserved S1551.50b and the backbone of L3967.51b, which was found to stabilize the kink in TM7 in both apo and inactive class B GPCRs8–10. In addition, more limited changes are observed in the extracellular halves of TM3, TM4 and TM5, which appear to move in tandem with the same orientation in response to peptide binding (Fig. 3b). Notably, the extracellular end of TM2 is extended by ten residues in activated GLP-1R compared to inactive GCGR (Fig. 3a, b). This α-helical extension elevates the position of ECL1, which is ordered against the C-terminal half of the peptide, in contrast to its apparent disorder in all available class B GPCR structures8–10. Consistent with this observation, hydrogen–deuterium exchange studies and molecular dynamics simulations also indicate that the peptide binding event stabilizes the conformation of ECL1 in the glucagon–GCGR complex12.

In the intracellular half of the receptor we observe a remarkable agreement in the positions of TM1–4 and TM7 between the inactive GCGR and active GLP-1R (Fig. 3c). The most profound structural difference is the sharp kink in the middle of TM6, which pivots its intracellular half to move outwards by approximately 18 Å when measured at the Cα carbon of K3463.54b. The TM6 kink locates in the extracellular side is TM2 extended by three helical turns stabilized by peptide ligand binding. The disordered extracellular loops in the inactive GCGR structure are stabilized and structurally ordered in the activated GLP-1R structure. Comparison of HETX motif networking (in stick representation, Wooten numbering in superscript) between inactive GCGR and active GLP-1R shows that the outwards movement of TM6 removes T4b4.27b from the polar network.

Figure 3 | Comparison of active-state GLP-1R with inactive GCGR. a–c, Side (a), extracellular (b) and cytoplasmic (c) views of the activated GLP-1R transmembrane bundle (light green) in superposition to the inactive glucagon receptor bound to allosteric antagonist (not shown; PDB code: 3EE7, blue). Significant conformational changes are observed on the cytoplasmic face of TM5 and TM6. TM6 moves outwards by 18 Å as measured at the Cα of Lys346, while TM5 moves a smaller distance by 7 Å when measured at the Cα of Lys334. A notable difference on the
Pro6-Glu-Leu-Val-Leu-Gly6 sequence, representing the PXXG motif that is conserved throughout the mammalian class B receptors, with the proline playing a key role in receptor activation.57,58 The presence of proline and glycine, having poor helix-forming propensities, makes this motif flexible and susceptible to unwinding, which is required for signal transduction through the 7TM bundle. A smaller outwards movement of about 7 Å is observed for the cytoplasmic end of TM5 as it repositions to facilitate the outward opening of TM6. As a result of the TM6 outward movement, the polar interaction network involving H2–E42 and Y7–Q384 is disrupted (Fig. 3d). The HETX motif is highly conserved in family B members and is equivalent to the conserved polar network in the GLP-1R–Gs interaction.40-41

Interactions between activated GLP-1R and Gs

The 18 Å outwards opening of the cytoplasmic half of TM6 and more limited associated movement of TM5 forms a cavity together with TM2, 3 and TM7 that serves as the main binding site for the Goαs Ras-like domain of heterotrimeric Gs (Fig. 4a, b). Compared to the β2AR–Gs crystal structure,18 the interface of Gs with GLP-1R additionally involves direct interactions of Gs with ICL1 and α-helix 8, which is tilted towards the G protein (Fig. 4c, d, Extended Data Fig. 6b). The connection between helix 8 and Gs is clearly visible in maps generated without masking the detergent micelle, revealing that R419 of helix 8 is in close proximity to the 310–311 backbone of Gs (Extended Data Fig. 6b). Furthermore, the structure suggests that additional electrostatic contacts may be present between E412 of helix 8 and H171 of ICL1 with D312 of Gs, K415 of helix 8 and D291 backbone of Gs (Fig. 4d). In contrast to its G-protein-proximal face that displays polar residues, the membrane-proximal face of helix 8 includes conserved bulky aromatic residues, consistent with our observation that this helix is found buried in the detergent micelle of the GLP-1–GLP-1R–Gs complex (Extended Data Fig. 6b). We thus conclude that helix 8 is in close contact with the membrane, which may enhance receptor stabilization in the lipid bilayer.

The GLP-1R–Goαs Ras-like domain interface is primarily stabilized by both hydrophobic and polar interactions involving TM2, TM3–4, TM5–6 and the TM7–helix 8 junction of the receptor with α4-helix, α5-helix, αN-helix, and the beginning of the 36-strand of the Gβγ Ras-like domain (Fig. 4). The carboxyl terminal α5-helix of the Goαs Ras-like domain overlaps the position of the cytoplasmic end of TM6 in the inactive state, explaining the requirement for TM6 opening in order to accommodate the G protein. The conformational transition of TM6 results in breaking up the highly conserved polar network within TM2–6–7–helix 8 (ref. 42), thereby releasing R176 in TM2, N406 and E408 in the TM7–helix 8 junction. The cryo-EM density map suggests that these residues may be stabilized again in the GLP-1R–Gs complex through electrostatic interactions or hydrogen bonds with Q390 and E392 of the Goαs α5-helix (Fig. 4a).

Comparison with activated class A GPCRs

The only available structure of a GPCR–G protein complex to date is from the complex of heterotrimeric Gs with activated β2AR, a class A GPCR.16 Structural superposition of GLP-1R–Gs with β2AR–Gs reveals that the G protein conformation is almost identical (Extended Data Fig. 10a). The overall root mean square deviation in Gs between the two complexes is approximately 1.0 Å, with the main differences located adjacent to the α-helical domain that becomes delocalized in the absence of nucleotide in Goαs. Goγ3 in the GLP-1R–Gs structure positions closer to the receptor by about 2 Å, a spatial proximity facilitated through the interaction between helix 8 and Gs.19,20

Superposition of the transmembrane domains in both G protein complexes shows that active GLP-1R and β2AR share similar folds in regards to the global conformation of the 7TM helices (Fig. 5a). One notable difference is the substantially longer α-helical extensions of TM2, resulting in the elevation of ECL1 that engages the C-terminal half of the peptide. Viewing towards the membrane plane from the extracellular side, we observe divergence in the position of the α-helical tips of all transmembrane domains apart from TM3 and TM4 (Fig. 5b).
This divergence primarily reflects the differences in the type of ligand engaging the receptor, with the larger peptide ligand requiring additional space for accommodation by the transmembrane bundle.

In contrast to the extracellular half, the transmembrane domains at the cytoplasmic half of activated GLP-1R and β2AR assume similar topology (Fig. 5c). Despite the sharp kink and unwinding in the middle of TM6 of GLP-1R compared to its preserved helicity in β2AR, the cytoplasmic ends reach the same position. Thus, both GLP-1R and β2AR form a similar cavity recognizing the C-terminal of the α5-helix of the Goα-like domain, consistent with the fact that for both receptors the interactions with the α5-helix are primarily non-polar and also involve some position equivalent residues, for example, X3.50b, X3.53, X5.65 and X6.36 (Extended Data Fig. 10b, c). It is notable however, that even though the overall structural landscape is very similar, the molecular details of the recognition pattern on the receptor are very different between β2AR and GLP-1R (Extended Data Fig. 10b, c). This observation also highlights some flexibility in the configuration of the α5-helix of the Goα Ras-like domain, enabling it to form diverse interactions through the same set of C-terminal amino acids.

Implications for family B GPCR activation

The cryo-EM structure of the activated GLP-1R–Gs complex facilitates the integration of a large body of biochemical and biophysical data towards understanding the activation of class B GPCRs through peptide binding. Current evidence suggests that in the absence of ligand the NTD is mobile through flexibility in the linker connecting it to TM1 (ref. 32), thereby increasing the probability of the initial recruiting interactions with the C-terminal region of the peptide. After an initial, high-affinity recruitment by the NTD, the captured peptide engages the 7TM region, with its N-terminal region establishing interactions with the transmembrane core. The structure shows that GLP-1 binding destabilizes the helicity of TM6 with simultaneous rearrangements in the central polar network of the transmembrane bundle (Fig. 6). This network facilitates the stabilization of the TM core and is positioned to detect peptide binding through potential interactions of R2.46b-X-X-Gly6.30b in GCGR with residue E9 at the peptide N terminus. The middle of TM6 includes the helically unstable Pro6.47b-X-X-Gly6.30b motif, which appears to unravel as a consequence of TM7 bending due to steric hindrance with the bound peptide. While the resolution of the cryo-EM map does not allow us to clearly resolve the interactions in this region, our model suggests that the exposure of the carbonyl oxygen atoms in the backbone of Pro6.47b-Leu-Leu6.49b enables interactions with polar residues in TM3, 5 and 7 that stabilize the outwards opening of the cytoplasmic half of TM6. The rearrangement of TM6 subsequently breaks apart polar interactions of the conserved HETX motif and TM2–6–7–helix 8 network, thereby creating a cavity and releasing residues that engage the α5-helix of the Goα Ras-like domain. Thus, peptide binding at the extracellular surface is communicated to the intracellular receptor side and results in G protein engagement and activation.

Despite the diversity between class A and B GPCRs, we observe that both receptor types converge topologically in the cytoplasmic side. This structural similarity near the G protein coupling sites reflects a convergence of activation pathways, which has been highlighted in class A GPCRs45, and enables this large family of receptors to bind and be activated by very diverse ligands, but signal intracellularly via a small common repertoire of G proteins. While further work is needed to provide a detailed picture of activation pathways in different GPCR classes, we anticipate that the application of the rapidly evolving cryo-EM technologies will transform structural studies of these challenging proteins.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Figure 6 | Polar network rearrangements upon GLP-1R activation. Comparison of polar network arrangements in the inactive-state GCGR (PDB code: 5EE7; the coordinates for residue R2.46b were obtained from the crystal structure of apo GCGR) and active-state GLP-1R. GLP-1 binding results in an outward movement of the cytoplasmic half of TM6 with simultaneous rearrangements of the central polar network. The rearrangement of TM6 breaks apart polar interactions of the conserved HETX and TM2–6–7–helix 8 networks, releasing residues for interactions with the α5-helix of the Goα-like domain. Peptide ligand GLP-1, TM6 and the α5-helix of Goα Ras-like are shown in ribbon representation and coloured as in Fig. 1. Polar network residues are shown in stick representation with Wootten numbering in superscript. The exposed backbone carbonyl oxygen atoms of Pro6.47b-Leu-Leu-Gly6.30b are shown as red spheres.
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Supplementary Information is available in the online version of the paper.

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Author Contributions Y.Z. performed cryo-EM map calculation, model building and refinement; B.S. established GLP-1–GLP-1R–Gs complex formation strategy; B.S., D.F. and M.C. expressed and purified the complex; S.L. prepared and refined the chain structures.

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GLP-1R–Gs–Nb35 complex formation and purification. S9 cell pellets infected with virus containing GLP-1R were lysed in 10 mM Tris, pH 7.6, 1 mM EDTA, 4 mg ml\(^{-1}\) idoacetamide, 2.5 mg ml\(^{-1}\) leupeptin, 0.16 mg ml\(^{-1}\) benzamidine. The sample was centrifuged at 200g for 7 min to get rid of non-disrupted cells and large cell debris such as nuclei. The supernatant was then centrifuged at 37,800g for 25 min to collect the membranes. The membranes were washed by homogenization in 30 mM HEPES, pH 7.5, 150 mM NaCl, 100 μM TCEP, 2.5 mg ml\(^{-1}\) leupeptin, 0.16 mg ml\(^{-1}\) benzamidine then collected by centrifugation at 37,800g for 25 min.

The GLP-1R–G\(_\gamma\)–Nb35 complex was formed in membranes. The washed membranes were homogenized in 30 mM HEPES, pH 7.5, 150 mM NaCl, 100 μM TCEP, 2.5 mg ml\(^{-1}\) leupeptin, 0.16 mg ml\(^{-1}\) benzamidine. For every 6 litres of GLP-1R cell pellets, 10 mg of G\(_\gamma\) and 2 mg of Nb35 were added, along with 1 mM MnCl\(_2\), 10 mM MgCl\(_2\), 10 μM GLP-1 peptide, 4,000 units λ-phosphatase, and 1 unit apyrase. The sample was mixed overnight at 4°C. The membrane sample with GLP-1R–G\(_\gamma\)–Nb35 complex formed was collected by centrifugation at 37,800g and then solubilized by in a buffer comprised of 1% DDM, 0.5% CHAPS, 0.2% cholesterol hemisuccinate (CHS), 30 mM HEPES, pH 7.8, 150 mM NaCl, 30% glycerol, 25 μM TCEP, 2.5 mg ml\(^{-1}\) leupeptin, 0.16 mg ml\(^{-1}\) benzamidine, and 1 μM GLP-1. After 1 h of solubilization, the sample was centrifuged at 37,800g to remove the insoluble debris. The supernatant was diluted twofold by adding the same volume of buffer without DDM, CHAPS and CHS. Then anti-Flag M1 affinity resin was added and CaCl\(_2\) was added to final concentration of 2.5 mM. After 2 h of mixing, the M1 resin was collected by centrifugation at 200g and loaded into a column, and was extensively washed by Flag wash buffer (30 mM HEPES, pH 7.5, 500 mM NaCl, 10% v/v glycerol, 2.5 mg ml\(^{-1}\) CaCl\(_2\), 25 μM GLP-1, 25 μM TCEP, 0.1% DDM, 0.125% CHAPS, 0.02% CHS). The buffer was exchanged to 30 mM HEPES, pH 7.5, 150 mM NaCl, 100 μM TCEP, 0.25% BSA, 0.25% pentyl glycol (MNG, NG310 Anatrace), 0.25% GDN (GDN101, Anatrace), 0.048% 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-1′-rac-glycerol (POPG, Avanti), and 0.03% cholesterol (Sigma-Aldrich) in a stepwise manner, over a period of at least 1 h. The complex sample was eluted from an anti-Flag column by Flag elution buffer, 30 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM EDTA, 200 μM Flag peptide, 1 μM GLP-1, 25 μM TCEP, 0.01% MNG, 0.01% GDN, 0.00192% POPG, 0.00012% cholesterol. 3C protease and PNGaseF (New England Biolabs) was then added.

After deglycosylation and digestion, the GLP-1R–G\(_\gamma\)–Nb35 complex sample was concentrated and loaded onto Superdex 200 10/300 GL column with running buffer 30 mM HEPES, pH 7.5, 150 mM NaCl, 1 μM GLP-1, 100 μM TCEP, 0.01% MNG, 0.01% GDN, 0.00192% POPG, 0.00012% cholesterol, and the fractions for monomeric complex was collected and concentrated individually for electron microscopy experiments.

Cryo-EM data acquisition. A sample of 3.5 μl of purified hGLP-1–rGLP-1R–G\(_\gamma\) complex at a concentration of approximately 1 mg ml\(^{-1}\) was applied to glow-discharged holey carbon grids (Quantifoil R2/2, 300 mesh), and subsequently vitrified using a Vitrobot Mark IV (FEI Company). The specimen was visualized with a Titan Krios electron microscope (FEI) operating at 300 kV accelerating voltage, at a nominal magnification of 29,000× using a K2 Summit direct electron detector (Gatan, Inc.) in counting mode, corresponding to a pixel size of 1.0 Å on the specimen level. In total, 17,322 images with defocus values in the range of −1.5 to −3.0 μm were recorded with a dose rate of about 9.0 electrons per Å\(^2\) per second. The total exposure time was set to 10 s with intermediate frames recorded every 0.2 s, resulting in an accumulated dose of about 90 electrons per Å\(^2\) and a total of 50 frames per movie stack.

Image processing and 3D reconstructions. A total of 1,460,600 dose-fractoned image stacks were subjected to beam-induced motion correction using MotionCor2 (ref. 44). A sum of all frames, filtered according to exposure dose, in each image stack was used for further processing. CTF parameters for each micrograph were determined by CTFIND4 (ref. 45). Particle selection, two-dimensional classification and three-dimensional classification were performed on a binned dataset with a pixel size of 2 Å using RELION54–66. In total, 2,675,742 particle projections were selected using semi-automated procedures and subjected to reference-free two-dimensional classification to discard false-positive particles or particles categorized in poorly defined classes, resulting in 620,626 particle projections for further processing. An ab initio map generated using VIPER49 was used as initial reference model for maximum-likelihood-based 3D classification. One stable class accounting for 139,299 particles showed detailed features for all subunits and was subsequently subjected to 3D refinement and reconstruction after subtracting densities for the mobile G\(_\alpha\)\_Gβγ Ras-like domain and the detergent micelle from the raw micrographs. The final map has a global nominal resolution of 4.1 Å, with nominal resolution of 3.9 Å in the core region that includes GLP-1, the transmembrane domain and the Ras-like domain.

Model building and refinement. The initial template of the rGLP-1R transmembrane domain was derived from a homology-based model calculated by I-TASSER63. The crystal structure of GLP-1R NTD–GLP-1 complex (PDB ID: 3IOL) and βγ–GS complex (PDB ID: 3SN6) were used as initial models for NTD–hGLP-1 and G, heterotrimer, respectively. All models were docked into the EM density map using Chimera62, following by iterative manual adjustment and real-space refinement using COOT63 and fragment-based refinement with Rosetta64. Sequence assignment was guided by bulky amino acid residues such as Phe, Tyr, Trp and Arg. The final model was subject to global refinement and minimization in real space using the module phenix.real_space_refine in PHENIX65. Model overfitting was evaluated through its refinement against one cryo-EM half map. FSC curves were calculated between the resulting model and the half map used for refinement as well as between the resulting model and the other half map for cross-validation (Extended Data Fig. 4). The final refinement statistics are provided in Supplementary Table 2.

Data availability. All data generated or analysed during this study are included in this published article and its Supplementary Information. The cryo-EM density map has been deposited in the Electron Microscopy Data Bank under accession code EMD-8653 and the coordinates have been deposited in the Protein Data Bank under accession number 5VAI.
Extended Data Figure 1 | Purification of the hGLP-1–rGLP-1R–Gs complex. Size-exclusion chromatography profile and corresponding SDS–PAGE gel of the purified hGLP-1–rGLP-1R–Gs complex (‘h’ indicates human and ‘r’ indicates rabbit homologue).
Extended Data Figure 2 | Cryo-EM micrograph and 2D class averages of the hGLP-1–rGLP-1R–G complex. a, Cryo-EM micrograph of the activated GLP-1R–G complex. Examples of particle projections are circled. Scale bar, 30 nm. b, Representative reference-free two-dimensional averages show distinct secondary structure features for G protein and GLP-1R embedded in MNG detergent micelle. The diameter of the circular windows is 17 nm.
Extended Data Figure 3 | Single-particle cryo-EM analysis of the hGLP-1–rGLP-1R–Gs complex. Flow chart of cryo-EM data processing of the hGLP-1–rGLP-1R–Gs complex, including particle projection selection, classification and 3D density map reconstruction, related to Fig. 1. Details are provided in the Methods section.
Extended Data Figure 4 | Resolution of cryo-EM map and validation of the hGLP-1–rGLP-1R–Gαs structure. a, Resolution estimation of the EM map. Gold standard Fourier shell correlation (FSC) curves, showing the overall nominal resolution at 4.1 Å (blue) and 3.9 Å (red) on the stable region including hGLP-1, transmembrane domain and the α5-helix the Gαs Ras-like domain. b, FSC curves of the final refined model versus the final cryo-EM map (full dataset, black), of the outcome of model refinement with a half map versus the same map (red), and of the outcome of model refinement with a half map versus the other half map (green). c, Final three-dimensional density map coloured according to local resolution.
**Extended Data Figure 5 | A near-atomic resolution model of the hGLP-1–rGLP-1R–Gαs complex.** EM density map and model are shown for all seven transmembrane α-helices and helix 8 of rGLP-1R, hGLP-1 peptide and the α5-helix of the Gαs, Ras-like domain. Bulky residues are indicated for each segment. The C-terminal half of TM6 exhibits relatively poor density, reflecting its intrinsic flexibility.
Extended Data Figure 6 | Features of cryo-EM map before density subtraction. a, GLP-1R–G<sub>β</sub> complex structure docked into cryo-EM density map before micelle density subtraction. Arrows indicate the density corresponding to the linker between the NTD and transmembrane bundle, and G<sub>βγ</sub> lipid moiety inserting into the detergent micelle. b, Close-up view in this map shows density connecting helix 8 and G<sub>β</sub> at the position of R419 of helix 8 and G310–H311 of G<sub>β</sub>. Model is coloured as in Fig. 1c.
Extended Data Figure 7 | Conformation of ECL2 in class B GPCRs.

a, Close-up view of R299 of ECL2 modelled into the density map at low threshold shows that the Arg side chain reaches into the GLP-1 binding pocket in close proximity to H7 and T11 of the peptide. 
b, Top-down view of structural overlay of the active GLP-1R transmembrane domain and the inactive CRF1R transmembrane domain (PDB code: 4K5Y) indicates the conformational similarity of ECL2 in the two structures. Detailed views of boxed regions show that W297 and R299 in the active GLP-1R structure adopt similar orientations compared to the equivalent residues in CRF1R. The model of the GLP-1R complex is coloured as in Fig. 1c. CRF1R is coloured purple.
Extended Data Figure 8 | Structures of class B GPCR ligands bound to NTDs. a, Close-up view of structural superposition of the cryo-EM structure of GLP-1–GLP-1R onto crystal structures of N-terminal domain of GLP-1R in complex with peptide exendin-4 (blue; a peptide approved for clinical use) and GLP-1 (purple), respectively. The model of hGLP-1–rGLP-1R–Gs is coloured as in Fig. 1. b, Structural superposition of the cryo-EM structure of GLP-1R NTD bound to GLP-1 to crystal structures of GIPR NTD bound to GIP (blue) and PTH1R NTD bound to PTH (cyan). a, b, Residues S14, S17, S18, F28 and W31 of GLP-1 and equivalent residues in the other peptides are shown in ball and stick (right panel only), highlighting that the corresponding side chains adopt a similar conformation in all available structures. c, Structure-based alignment of selected class B GPCR peptide ligand sequences.
Extended Data Figure 9 | Potential NTD–transmembrane bundle interaction, orthosteric agonist binding pocket in GLP-1R and β2AR.

a, Close-up view of the model docked into cryo-EM density map (grey) on the region of NTD–transmembrane bundle interaction at low threshold shows the potential hydrogen bond between Q213 of ECL1 and R40 of the NTD α1-helix. b, Overlay of GPCR transmembrane bundles in the activated GLP-1R complex and T4L-β2AR-Gs–Nb35 complex shown in light green and grey, respectively. Cut-through view showing that the GLP-1 peptide N-terminal H7 (orange ball and stick) reaches the same level as the orthosteric agonist BI-167107 (yellow).
Extended Data Figure 10 | Comparison of G protein trimer structures from activated GLP-1R–Gs–Nb35 complex and T4L-β2AR–Gs–Nb35 complex with alignment on Gαs Ras-like domain alone, related to Fig. 5. a, Views of superposition of G protein trimer structures from the activated GLP-1R–G,–Nb35 complex with alignment on Gαs Ras-like domain in gold, Gβ3 in light blue, Gγ3 in dark blue) and T4L-β2AR–Gs structure (all coloured in grey).

b–d, Schematic representation (b) of recognition between the C terminus of α5-helix (H387–L394) and active receptors of β2AR (c) and GLP-1R (d). The sequence of the C terminus of α5-helix (H387–L394) is shown in the middle in gold. Residues involved in the interaction with α5-helix (H387–L394) in the receptor of β2AR (green box) and GLP-1R (purple box) are shown above and below the schematic, respectively. Hydrophobic interactions are shown in blue and polar interactions in red. Ballesteros–Weinstein numbering in superscript is shown.

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