Structure of the active G\(_i\)-coupled human lysophosphatidic acid receptor 1 complexed with a potent agonist

Lysosphospholipids are simple phospholipids that activates GPCRs to evoke signals involved in a broad range of biological processes\(^1\). They are characterized by a single hydrocarbon chain and a polar head group, which can be divided into two subgroups; molecules containing the glycerol backbone (lysoglycerophospholipids) and the sphingoid base backbone (lysosphingolipids). The representative lysosphospholipids in each of the two groups are lysophosphatidic acid (LPA) and sphingosine-1-phosphate (SIP), which activate the LPA receptors (LPA\(_{1-3}\)) and the SIP receptors (SIP\(_{1-3}\)), respectively\(^2\). LPA\(_{1-3}\) and SIP\(_{1-3}\) belong to the endothelial differentiation gene (EDG) family, based on the amino acid sequence identity, and exhibit conserved structural features in the ligand-binding pocket. By contrast, LPA\(_{4-6}\) belong to the non-EDG family, which is more closely related to the purinergic P2Y receptor family\(^2,3\). Furthermore, phosphate-modified derivatives of LPA exist in vivo to mediate signaling through different GPCRs. For example, lysophosphatidylserine and lysophosphatidylinositol activate the lysophosphatidylserine receptors (LPS\(_{1-3}\)) and GPR55, respectively\(^4-6\). A dephosphorylated LPA derivative, 2-arachidonyl glycerol (2-AG), activates cannabinoid receptors (CB\(_{1,2}\)), which are most related to the EDG family at the phylogenetic level\(^7,8\). These diverse lipid-sensing GPCRs precisely discriminate between the chemical structures of lipid ligands\(^9\).

In 1996, LPA\(_1\) was the first identified LPA receptor\(^10\), and thus LPA-LPA\(_1\) signaling is the best-studied among the LPA receptors\(^1\). LPA\(_1\) couples to the G proteins such as G\(_{i}\), G\(_{q}\), and G\(_{12/13}\), and transduces various intracellular signals, e.g., increased Ca\(^{2+}\) concentration and actin reorganization by the Rho/ROCK pathway. LPA\(_1\) is widely expressed in several organs to control cell proliferation and survival, cell–cell contact, cell migration, and cytoskeletal morphological changes. The essential physiological functions of LPA\(_1\) are nervous-system tissue development and chondrocyte differentiation. LPA\(_1\) is associated with various diseases such as cancer, inflammation, and...
neuropathic pain, and thus is a pathologically important receptor that is an essential drug target. Because LPA₁ signaling promotes cancer progression in many tissues, LPA₁ antagonists have been well studied as anti-cancer drugs. Moreover, some preclinical studies suggested the potential therapeutic value of selective LPA₁ agonists for obesity and urinary incontinence. However, the metabolic instability of LPA and its resultant short half-life have complicated the functional characterization of supplemented LPA. Nonlipid LPA₁ agonists are poorly reported, and thus identifying new potent and more stable agonists would be useful to explore and consolidate the potential therapeutic benefits of LPA receptors agonistic drugs. To date, the agonist structures of the S1P receptors and CB receptors have been reported, revealing their lipid-ligand recognition mechanisms relevant for LPA₁. While the antagonist-bound LPA inactive structure was also reported, little is known about how LPA selectively activates the LPA receptors among the lipid-sensing GPCRs, limiting the design of drug-like LPA receptor agonists.

**Results**

**Overall structure**

For the structural study, we developed a chemically stable analog of LPA (Supplementary Method). The glycerol backbone of sn-2 LPA was partially replaced by an amide bond, and the cis-9 double bond in the acyl chain was replaced by an aromatic moiety (Fig. 1a). In a NanoBiT-G-protein dissociation assay, the resulting compound activates the LPA receptors among the lipid-sensing GPCRs, limiting the design of drug-like LPA receptor agonists.

Here we report the 3.5 Å-resolution cryo-electron microscopy (cryo-EM) structure of the human LPA₁-Gᵢ signaling complex bound to an LPA analog with more potent activity against LPA₁. Close examination of the LPA₁ structure reveals the mechanisms of ligand-lipid binding, receptor activation, and G-protein coupling.

**Fig. 1 Overall structure of the LPA₁-Gᵢβγ₂-ScFv₁₆ complex.**

(a) Chemical structures of LPA and ONO-0740556. B Ligand-induced Gᵢ activation by LPA₁-Gᵢ activation was measured by the NanoBiT-G-protein dissociation assay. Concentration–response curves are shown as means ± s.e.m. (standard error of the mean) from three independent experiments. Source data are provided as a Source Data file. c Sharpened cryo-EM maps and refined structures. d Densities around the agonist at different density levels. We observed three strong densities, and assigned the phosphate group, glycerol backbone, and aromatic group of ONO-0740556 to them, given the surrounding environment. Furthermore, we also observed a density above W2₁₀, so we extended the acyl chain to it.
ONO-0740556 showed agonist activity with an EC_{50} value of 0.26 nM for the human LPA_{1}, which is 30-fold higher than that of LPA (Fig. 1b, Supplementary Fig. 1, Supplementary Table 1, and Supplementary Methods). This result indicates that ONO-0740556 is more suitable for the structural study toward the design of a drug-like LPA receptor agonist.

We independently expressed and purified LPA_{1}, G_{i}, trimer, and scFv16 in insect cells and mixed them, and then purified the complex by anti-Flag affinity chromatography and size exclusion chromatography. The structure of the purified complex was determined by single-particle cryo-EM analysis with an overall resolution of 3.5 Å (PDB 7YU3) (Fig. 1c, Supplementary Fig. 2, Supplementary Table 2, and “Methods”). In this analysis, we subtracted the minimal apparent density for the micelle and the α-helical domain of the G_{α} subunit to consider their flexibilities. The local resolution analysis demonstrated that the interaction site of G_{α}, β, scFv16 and the interface between the G_{α} subunit and the intracellular side of the receptor have higher resolutions. In contrast, the extracellular part of the receptor has a lower resolution (Supplementary Fig. 3). Thus, we performed a refinement with a mask on the receptor, and obtained the receptor structure with a nominal resolution of 3.7 Å (PDB 7YU4) (Supplementary Fig. 3 and Supplementary Table 2). Moreover, in this procedure, the density of ONO-0740556 ligand became more clearly observed within the orthosteric site (Fig. 1d). Based on this structure, we analyzed the modes of agonist binding and receptor activation.

**ONO-0740556 binding site**

ONO-0740556 provides an extensive interaction network with N-term, ECL1, 2, and TM6, 7, 8, 9, 10, and 11 of the receptor (Fig. 2a–d). The binding site consists of a polar recognition region on the extracellular side and a hydrophobic pocket within the transmembrane region (Fig. 2a–d). This binding manner configuration is also found in SIP receptors17–21. The head phosphate and glycerol moieties of ONO-0740556 are located in the polar recognition site (Fig. 2a–d). Two oxygen atoms of the head phosphate form salt bridges with K39N-term and R1243.28 (superscripts indicate Ballesteros–Weinstein numbers) (Fig. 2a–d). The phosphate group also forms a hydrogen bond with Y34N-term, and is tightly recognized by the positively charged residues K2947.36. Moreover, the nitrogen atom in the amide bond forms a hydrogen bond with E2937.36 (Fig. 2a–d). The agonist binding mode at the polar recognition region is consistent with the previous molecular dynamics simulation24–26, which revealed that Y34N-term and K39N-term bind the head phosphate group and the hydrophobic pocket region, which recognizes an acyl chain (Fig. 3c). The functional importance of W2105.43 for ONO-0740556 binding and receptor activation is different between LPA_{1} and CB_{1} (Fig. 3c, d). By contrast, the long acyl chain fits into the transmembrane pocket in a bent conformation and forms extensive hydrophobic interactions with the receptor (Fig. 2b–d). Notably, the aromatic moiety in the middle of the acyl chain is sandwiched between two leucines, L2786.48 and L2977.39. Among them, L2977.39 plays a critical role in ONO-0740556 binding. Furthermore, in the acyl chain, the C14 carbon forms a CH–π interaction with W2105.43 (Fig. 2b–d). The W2105.43 mutant completely lost the response elicited by ONO-0740556 (Fig. 2e).

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**Structural insight into LPA selectivity**

The lysophospholipids LPA and SIP and the dephosphorylated LPA derivative 2-AG can selectively activate the evolutionarily related LPA receptors, the SIP receptors, and CB receptors, respectively27–29. To elucidate the mechanism of their lipid preference, we compared the agonist-bound structures of LPA_{1}, SIP_{3}, and CB_{2}, 28. The transmembrane regions superimposed well (Fig. 3a), but the N-terminus of CB_{2} is different, with only partial structures observed (Fig. 3b). Focusing on the extracellular side of CB_{2}, F1772.44 is present at the position occupied by the head phosphates of the agonists in LPA_{1} and SIP_{3} (Fig. 3c–e). Thus, lipid ligands lacking phosphate groups selectively activate CB_{2}. A comparison of the phosphate sites in LPA_{1} and SIP_{3} revealed that the head phosphate group is in almost identical positions and forms a salt bridge with tyrosine (Y34N-term and K2947.36 in LPA_{1} and SIP_{3}, respectively) and arginine (R1243.28 and R1143.28 in LPA_{1} and SIP_{3}, respectively). Moreover, the phosphate group also forms a hydrogen bond with the tyrosine (Y34N-term and K27N-term in LPA_{1} and SIP_{3}, respectively) (Fig. 3f and Supplementary Table 4). Thus, SIP_{3} and LPA_{1} similarly recognize phosphate groups. Overall, the salt bridges near the tyrosine and arginine residues enhance the recognition of the head phosphate group in LPA_{1} and create selectivity for LPA over other lysophospholipid mediators that have modified phosphate groups with weak negative charges.

We next focused on the hydrophobic pockets accommodating the acyl chain. At the position 5.43, a tryptophan residue creates the bottom of the pocket in LPA_{1} and CB_{2} (Fig. 3c, e). The presence of tryptophan in this position only occurs in 1% of all class A GPCRs and is unique to the LPA and CB receptors30, and it is involved in the agonist binding in both receptors (Fig. 3c, e). The corresponding residue in SIP_{3} is C2005.43, with a smaller side chain (Fig. 3d). This amino-acid difference allows to create a deeper pocket in SIP_{3} as compared to LPA_{1} and CB_{2} (Fig. 3c–e, g). Furthermore, F1703.38, L189C2.12, L259H9.31, and F263H9.35 in SIP_{3} are replaced by D129G9.32, A199G12.22 G7254.31, and L278W6.55 in LPA_{1}, respectively. As a result, they create a bulge of the hydrophobic pocket toward TM 5–7 in LPA_{1} (Fig. 3g). These amino-acid replacements allow the hydrophobic pockets of LPA_{1} and CB_{2} to be spherical (Fig. 3c–e, g) and thus they can accommodate long and bent unsaturated acyl chains (Fig. 3c, e), accounting for the fact that LPA_{1} prefers unsaturated LPA species with a cis-9 double bond in bent shapes (oleic (I3:1), linoleic (I3:2), and linolenic (I3:3))27. By contrast, the SIP in the human body has only I3:1, with the trans-4 double bond in a linear configuration. Thus, LPA can activate SIP receptors with a deep, linear pocket, in contrast to LPA_{1} with a shallow, wide pocket. Together, the polar recognition site, which strongly recognizes phosphate groups, and the hydrophobic pocket region, which recognizes an unsaturated acyl chain, contribute to the LPA selectivity by LPA_{1}.

**Receptor activation**

To examine the activation mechanism of LPA_{1}, we compared the LPA_{1} structures in the present agonist-bound active state and the previously-reported antagonist-bound inactive states27. On the intracellular side, TM6 is displaced outward by about 8.2 Å, and TM7 is shifted inward by about 4.1 Å. Such structural changes are typical of class A GPCRs and allow G-protein coupling and activation32 (Fig. 4a, b). At the ligand-binding site, the positively charged residues K39N-term and R1243.28 similarly recognize the negative charges in both antagonists and agonists (Supplementary Fig. 5a, b). The antagonist is close to TM7, and the methoxycarbonyl group sterically prevents K2947.36 from accessing the polar head (Fig. 4c and Supplementary Fig. 5a, b). By contrast, the agonist is closer to TM7 than the antagonist. Since the agonist closely interacts with TM7, the extracellular side of TM7 is shifted inwardly by 1.3 Å (Fig. 4c–e). Accompanied by the shift of TM7, A3003.43, and N303H9.44 move toward TM6 and push the W271H6.48 rotamers inwardly (Fig. 4d, f). W271H6.48 is a part of the C44-W44-X45 motif, an essential mechanical activation switch conserved in class A GPCRs30.
These observations suggest that the agonist interaction with TM7 affects the essential residue W271, leading to the receptor activation on the intracellular side, as discussed later. The bottom of the pocket also affects the rearrangement of the C6.47-W6.48x-P6.50 motif. In the antagonist-bound structure, L132 and W210 constitute the bottom of the pocket, forming extensive hydrophobic interactions with the antagonist. Notably, L132 forms a CH–π interaction with W271, stabilizing the inactive conformation, while in the agonist-bound structure, C14 in the acyl chain of the agonist forms CH–π interactions with W210 and induces its side chain flipping. The rotamer change of W210 leads L132 to point towards the ligand. These structural changes weaken the interaction between L132 and W271 and allow their synergistic conformational changes (Fig. 4f and Supplementary Fig. 5c). A similar structural rearrangement is observed in CB1, in which the homologous residues F200 and W356 are flipped.
upon agonist binding (referred to as a twin toggle switch)\textsuperscript{18,19,28} (Supplementary Fig. 5d). The density corresponding to C14 in the agonist is relatively well-observed (Fig. 1d), and the W210\textsubscript{5.43} mutant showed no G\textsubscript{i} dissociation signal (Fig. 2e), indicating the strength and importance of the interaction with W210\textsubscript{5.43}. These observations suggest that the inward movement of TM7 and the acyl chain interaction with W210\textsubscript{5.43} cooperatively induce the toggle switch activation of W271\textsubscript{6.48} (Fig. 4f).

The movement of the C\textsuperscript{5.47}W\textsuperscript{5.48}xP\textsuperscript{6.50} motif upon agonist binding causes a structural rearrangement in the P\textsuperscript{3.33}F\textsuperscript{3.34}E\textsuperscript{3.35} motif, which is also essential for receptor activation\textsuperscript{30–32}. The inward rotations of the W271\textsubscript{6.48} rotamer and N303\textsuperscript{7.45} allow the F267\textsuperscript{6.44} flipping toward TM5 (Fig. 4f), followed by the significant displacement of F218\textsuperscript{5.51} proximal to the motif (Fig. 4g). The movement of the P\textsuperscript{3.33}F\textsuperscript{3.34}E\textsuperscript{3.35} motif is responsible for the large outward movement of the intracellular portion of TM6. Accompanying the movement, structural rearrangements are observed in the N\textsuperscript{7.49}P\textsuperscript{7.50}xxY\textsuperscript{7.53} and D\textsuperscript{3.49}R\textsuperscript{3.50}Y\textsuperscript{3.51} motifs conserved in most class A GPCRs\textsuperscript{30,31}. In the N\textsuperscript{7.49}P\textsuperscript{7.50}xxY\textsuperscript{7.53} motif, Y311\textsuperscript{7.53} shows a significant displacement toward the intracellular core and contacts L139\textsuperscript{3.43}, I\textsuperscript{142}3.46, and R146\textsuperscript{3.50}, leading to the inward movement of TM7 (Fig. 4h). In the D\textsuperscript{3.49}R\textsuperscript{3.50}Y\textsuperscript{3.51} motif, R146\textsuperscript{3.50} forms a hydrogen bond with the backbone carbonyl of C351G.H5.23 (superscript indicates the common G\textalpha\number system\textsuperscript{33}), which is typically observed in other GPCR-G\textalpha\ complexes\textsuperscript{34,35}. Additional hydrogen-bonding interactions are observed between the a5-helix and ICL2 (Fig. 5a). In addition to these polar contacts, there are extensive hydrophobic contacts
between the receptor and Gi (Supplementary Fig. 6). These interactions allow the receptor to couple with Gi.

Structural polymorphism at the receptor-Gi interface

Previous structural studies showed that the G\(\alpha_i\) binding manner is variable, with different G\(\alpha_i\) rotations relative to the receptor\(^3\). Moreover, canonical (C) and non-canonical (NC) states were observed in the NTSRI-G\(\alpha_i\) complex, with a 45° rotation of the G-protein relative to the receptor\(^3\). Compared with the C and NC states, the G\(\alpha_i\) protein in the LPA\(_1\) structure resides in their intermediate positions (Fig. 5b, c). This difference seems to be derived from the receptor-Gi interaction at ICL2. In most class A GPCRs, ICL2 adopts a short \(\alpha\)-helix in the active state\(^18,19,37–39\). Position F174 in the NTSRI C state binds within the hydrophobic pocket formed by L194\(^\text{35.05}\), F336\(^\text{G9.08}\), and T340\(^\text{G9.12}\).

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**Fig. 4 | Comparison of the agonist- and antagonist-bound LPA\(_1\) structures.**

Superimposition of the agonist- and antagonist-bound LPA\(_1\) structures, colored cyan and gray (PDB 4Z34), respectively, viewed from the membrane plane (a), the intracellular side (b), and the extracellular side (c). D251 in TM6 and Y311 in TM7 are shifted by about 8.2 and 4.1 Å, respectively. d–f Structural changes of the intramolecular interactions induced by agonist binding (d). Panels (e) and (f) are focused on the extracellular side of TM7 and the receptor core, respectively. Rearrangement of the PIF (g), NPxxY (h), and DRY (i) motifs upon receptor activation. Hydrogen bonds are indicated by black dashed lines.
and I343G.H5.18 in Goi (Fig. 5d) and plays an essential role in G-protein activation. In the NTSR1 NC state, F174L34.52 is located away from the hydrophobic pocket of the NTSR1 C state, F174L34.52, in a dynamic equilibrium of the receptor-Gi interface. These movements are responsible for the different positions of the Gi protein in the NTSR1 C and NC states. The disordered ICL2 and the G-protein position in the LPA1-Gi complex are similar to those in other S1P-Gi complexes (Fig. 5b, c), illuminating the conserved structural feature for Gi coupling in EDG family members.

To determine whether the conformational transition of the Gi coupling is observed, in NTSR1, we performed 3D classifications focusing on the alignment of LPA1 and Gi protein. Accordingly, we obtained cryo-EM maps for four classes (S1–S4) with nominal resolutions of 3.7, 3.9, 4.5, and 5.6 Å (PDB 7YU5, 7YU6, 7YU7, and 7YU8) (Fig. 6a and Supplementary Table 2). The maps of S1 and S2 enabled model building and refinement. Moreover, those of S3 and S4 enabled them with accuracy of the Cα atoms (Supplementary Fig. 7). Thus, we discuss the conformational changes in the main chains.

To visualize the G-protein movement, we superimposed the 3.5 Å-resolution structure described above (stable state) and S1–S4 at the receptor. S1 and S2 superimposed well on the stable state, with limited in-plane rotations within 3–4° of the G-protein (Fig. 6b), suggesting that this is the most stable position of the G-protein in the nucleotide-free state. By contrast, S3 and S4 both moved from the stable state in opposite directions from each other (Fig. 6c). As compared to S3, the entire Goi in S4 is shifted downward by about 4 Å, followed by the lateral movement of Gβγ by 5.3 Å. Focusing on the α5-helix, it moves 3 Å away from the receptor with the structural changes in the C-terminal residues (Fig. 6d). ICL2 follows the movement of the α5-helix to maintain the interaction with it. When aligned the S1–S4 and the stable state at the Goi protein, the orientations of the C-terminal 2-turn helix are variable (Fig. 6e). This region does not adopt the α-helix in the GDP-bound inactive Gi heterotrimer40, and receptor interaction induces its helix formation. This notion suggests the innate structural flexibility in the C-terminal residues of the α5-helix, which is responsible for the structural polymorphism observed in this study, reflecting the dynamic equilibrium of the receptor-Gi interface. These movements are totally distinct from the rotational movements observed in NTSR1 (Fig. 5b), which reflect the activation pathway of G protein41. Since the downward movement of Goi weakens the receptor–Goi interactions (Fig. 6d), S4 might represent the dissociation process of the receptor and G protein upon GTP binding.

**Discussion**

We determined the structure of the LPA1-Gi complex bound to the LPA analog ONO-0740556, which revealed the tight recognition of the head phosphate and the accommodation of the bent acyl chain in the spherical pocket. Close examination of the active and inactive LPA1 structures elucidated that two factors cooperatively play key roles in receptor activation. One is the recognition of the phosphate groups and glycerol backbone by TM7, and the other is the hydrophobic interactions with a long acyl chain by the residues at the bottom of the pocket. The ligand recognition by TM7 agrees with the properties of the binding module, in which the ligand is closer to TM7 and the hydrophobic pocket is more expanded to TM7 in LPA1 than in S1P3. Moreover, at the bottom of the pocket, the position of the dimethoxyphenyl clash.

![Figure 5](https://example.com/fig5.png)

**Fig. 5** | **Binding modes of Gαi**. (a) Main hydrogen-bonding interactions between the receptor and the α5 helix of Gαi. (b, c) Structural comparisons of LPA1-Gi with other GPCR-G-protein complexes at the interface, viewed from the cytoplasmic side (b) and membrane plane (c). Structural comparisons of the interactions between ICL2 and Gα in the NTSR1 C state (d), NTSR1 NC state (e), LPA1 (f), and S1P1 (g). Residues are shown as stick models. Hydrogen bonds are indicated by black dashed lines.

**Nature Communications** | (2022) 13:5417 | https://doi.org/10.1038/s41467-022-33121-2
performed a structural comparison of the LPA<sub>1</sub> bound to LPA with our structure bound to ONO-740556. The two structures of LPA<sub>1</sub> superimposed well with a root mean square deviation of Ca atoms of 0.583 Å (Fig. 7a), and there are no significant differences in the recognition of polar regions on the extracellular side, and in the interaction of the ligand with W210<sup>43</sup> and L297<sup>39</sup> in the hydrophobic pocket (Fig. 7b, c). Given that these interactions mediate the receptor activation, LPA and ONO-740556 activate the receptor in similar manners. However, interestingly, the route of the acyl chain is different between our new compound and LPA. The acyl chain of LPA folds on the TMS side and extends toward TM7, but ONO-740556 goes from TM7 to TM5 (Fig. 7c). This fact suggests that LPA<sub>1</sub> permits the acceptance of various forms of acyl chains within the spherical hydrophobic pocket. The interaction of L297<sup>39</sup> with the hydrocarbon chain of LPA is weaker than the CH-π interaction with the aromatic moiety of ONO-740556. This difference would be one of the factors causing the distinct affinities of the agonists (Fig. 1b and Supplementary Table 1). Our study clarifies the detailed structure-activity relationship of LPA<sub>1</sub> and will facilitate the design of novel LPA-mimetic agonists to explore the therapeutic potential of LPA<sub>1</sub>.

G<sub>i</sub> movement was observed in the LPA-bound LPA<sub>1</sub>-G<sub>i</sub> complex, as in our study. The 3D variability analysis (3DVA) of the LPA-bound complex identified two states (Fig. 7d) distinguished by the relative rotation of Gα<sub>i1</sub> about LPA<sub>1</sub> in the plane of the membrane, -5° in both directions away from the consensus structure<sup>41</sup>. By contrast, in the ONO-740556 bound complex, the entire Gα<sub>i</sub> in S4 is shifted downward by about 4 Å (Fig. 7e), indicating a weakening of the receptor-Gα<sub>i</sub> interactions. However, there are significant differences in the experimental conditions between our study and previous studies (e.g., ligands, detergents, analysis methods, etc.), and thus we cannot ignore their influence on the G<sub>i</sub> movements. Moreover, the 3DVA analysis of LPA<sub>1</sub> and S1P<sub>1</sub> elucidated the rocking, twisting, and flexing motions of the receptor about the G protein<sup>11</sup>. These structural polymorphisms indicated the flexible coupling between GPCR and G protein, which may be observed in other GPCR-G-protein complexes by more careful analysis. The G-protein movement upon dissociation is also observed in the recently reported PTH1R-G<sub>α</sub> complex<sup>42</sup>. Future studies will shed light on whether the observed structural polymorphism reflects the structural flexibility in the purified condition, or the process of G-protein activation and dissociation by GPCRs.

**Methods**

**NanoBiT-G-protein dissociation assay**

LPA<sub>1</sub>-induced G<sub>i</sub> activation was measured by a NanoBiT-G-protein dissociation assay<sup>32</sup>, in which the LPA<sub>1</sub>-induced dissociation of a G<sub>α</sub> subunit from a G<sub>βγ</sub> subunit was monitored by a NanoBiT system (Promega). Specifically, a NanoBiT-G<sub>α</sub> protein consisting of a large fragment (LgBiT)-containing Gα<sub>i1</sub> subunit and a small fragment (SmBiT)-fused Gγ<sub>1</sub> subunit with the C68S mutation, along with the untagged G<sub>β</sub> subunit, was expressed with a test LPA<sub>1</sub> construct, and the ligand-induced change in the luminescent signal was measured. We used the N-terminal FLAG (DYKDDDDK)-tagged constructs of human LPA<sub>1</sub> (HEK293T cells were seeded in a six-well culture plate at a concentration of 2 × 10<sup>5</sup> cells ml<sup>-1</sup> (2 ml per well in DMEM supplemented with 10% fetal bovine serum), 1 d before transfection. The transfection solution was prepared by combining 2.5 µl (per well hereafter) of Lipofectamine 2000 (ThermoFisher Scientific) and a plasmid mixture consisting of 100 ng LgbBiT-containing Gα<sub>i1</sub> subunit, 500 ng Gβ<sub>c</sub>, 500 ng SmBiT-fused Gγ<sub>2</sub> with the C68S mutation, and 200 ng LPA<sub>1</sub> in 500 µl of Opti-MEM (ThermoFisher Scientific). After an incubation for 1 d, the transfected cells were harvested with 0.5 mM EDTA-containing PBS, centrifuged, and suspended in 2 ml of HBSS containing 0.01% bovine serum albumin (BSA fatty acid-free grade, SERVA) (assay buffer). The cell suspension was dispensed into a white 96-well plate at a volume of 80 µl per well, and loaded with 20 µl of 50 µM coelenterazine diluted in

![Fig. 6 | Structural polymorphism of the LPA<sub>1</sub>-G<sub>i</sub> interface. a Density maps of the 3.5 Å resolution stable state and S1–4. b Comparison of the stable state, S1, and S2. c Comparison of the stable state, S3, and S4. d Superimposition of the stable state and S1–4, aligned at the receptor. TMS is omitted. e Superimposition of the GDP subunits in the stable state, S1–4, and the GDP-bound inactive G<sub>α</sub> heterotrimer (PDB 1GG2).](image)
were dispensed into the tubes. A 50 µL of 6× test compound, diluted in the assay buffer, was manually added. After an incubation for 8–10 min at room temperature, the plate was read for the second measurement. The second luminescence counts were normalized to the initial counts, at room temperature, the plate was read for the second measurement.

**Measurement of receptor cell-surface expression by ELISA**

To measure the cell surface expression level of wild-type LPA1 and its mutants, HEK293T cells were transiently transfected in 12-well plates and incubated for 1 d. Transfection was performed by following the same procedure as described in the NanoBiT-G-protein dissociation assay section, with a downscaled volume (250 µL transfection solution). The transfected cells were harvested with 0.5 mM EDTA-containing PBS, centrifuged, and blocked with 5% (w/v) BSA at room temperature, in buffer containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 10% glycerol. The membrane fraction was collected by ultracentrifugation at 180,000 × g for 1 h, in buffer containing 20 mM Tris (pH 8.0), 150 mM NaCl, and 10% glycerol. The crude membrane fraction was solubilized in buffer, containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% n-dodecyl-beta-D-maltopyranoside (DDM) (Calbiochem), 0.2% CHS, 10% glycerol, and 2 µM ONO-0740556 for 1 h at 4 °C. The membrane fraction was solubilized in buffer, containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% n-dodecyl-beta-D-maltopyranoside (DDM) (Calbiochem), 0.2% CHS, 10% glycerol, and 2 µM ONO-0740556 for 1 h at 4 °C. The membrane fraction was solubilized in buffer, containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 10% glycerol. The crude membrane fraction was collected by ultracentrifugation at 180,000 × g for 30 min, and incubated with TALON resin (Clontech) for 30 min. The resin was washed with ten column volumes of buffer, containing 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 0.05% glyco-diosgenin (GDN) (Anatrace), 0.1 µM ONO-0740556, and 15 mM imidazole. The receptor was eluted in buffer, containing 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 0.01% GDN, 0.1 µM ONO-0740556, and 200 mM imidazole. The receptor was concentrated and loaded onto a Superdex200 10/300 Increase size-exclusion column, equilibrated in buffer containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% GDN, and 0.1 µM ONO-0740556. Peak fractions were pooled and frozen in liquid nitrogen.

**Expression and purification of the G<sub>i</sub> heterotrimer**

The G<sub>i</sub> heterotrimer was expressed and purified using the Bac-to-Bac baculovirus expression system, according to the method reported previously<sup>13</sup>. Sf9 insect cells were infected at a density of 3–4 × 10<sup>6</sup> cells ml<sup>-1</sup> with a one 100th volume of two viruses, one encoding the WT human G<sub>a</sub><sub>q</sub> subunit and the other encoding the WT bovine G<sub>Y2</sub> subunit and the WT rat G<sub>i</sub> subunit containing a His<sub>n</sub> tag followed by an N-terminal TEV protease cleavage site. The infected Sf9 cells were incubated in SF900II medium at 27 °C for 48 h. The Sf9 cells were collected by centrifugation at 6200 × g for 10 min. The collected cells were lysed in buffer containing 20 mM Tris, pH 8.0, 150 mM NaCl, and 10% glycerol. The G<sub>a</sub><sub>q</sub><sub>β1γ2</sub> heterotrimer was solubilized at 4 °C for 1 h, in buffer containing 20 mM Tris (pH 8.0), 150 mM NaCl, 10% glycerol, 1% (w/v) n-dodecyl-beta-D-maltopyranoside (DDM) (Anatrace), 50 µM GDP (Roche), and 10 mM imidazole. The soluble...
fraction containing G_{i} heterotrimers was isolated by ultracentrifugation (186,000 × g for 20 min) and the supernatant was mixed with Ni-NTA Superflow resin (Qiagen) and stirred at 4 °C for 1 h. The resin was washed with 10 column volumes of buffer, containing 20 mM Tris, pH 8.0, 150 mM NaCl, 0.02% DDM, 10% glycerol, 10 μM GDP, and 30 mM imidazole. Next, the G_{i} heterotrimers were eluted with two column volumes of buffer containing 20 mM Tris, pH 8.0, 150 mM NaCl, 0.02% (w/v) DDM, 10% (v/v) glycerol, 10 μM GDP and 300 mM imidazole. The eluted fraction was dialyzed overnight at 4 °C against 20 mM Tris, pH 8.0, 50 mM NaCl, 0.02% DDM, 10% glycerol, and 10 μM GDP. To cleave the histidine tag, TEV protease was added during the dialysis. The dialyzed fraction was incubated with Ni-NTA Superflow resin at 4 °C for 1 h. The flow-through was collected and purified by ion-exchange chromatography on an HiTrapQ HP column (GE), using buffer I1 (20 mM Tris, pH 8.0, 50 mM NaCl, 0.02% DDM, 10% glycerol, and 1 μM GDP). The eluted fraction containing G_{i} heterotrimers was mixed with phenyl-Sepharose CL-4B (Amersham) and stirred at 4 °C for 1 h. The protein was eluted with 20 mM Tris (pH 8.0), 500 mM NaCl and 20 mM imidazole, and further washed with 10 column volumes of buffer containing 20 mM HEPES (pH 8.0), 500 mM NaCl and 20 mM imidazole. Next, the protein was eluted with 20 mM Tris (pH 8.0), 500 mM NaCl and 400 mM imidazole. The eluted fraction was concentrated and loaded onto a Superdex200 10/300 Increase size-exclusion column, equilibrated in buffer containing 20 mM Tris (pH 8.0) and 150 mM NaCl. Peak fractions were pooled, concentrated to 5 mg ml⁻¹ using a centrifugal filter device (Millipore 10 kDa MW cutoff), and frozen in liquid nitrogen.

**Expression and purification of scFv16**
The gene encoding scFv16 was synthesized (GeneArt) and subcloned into a modified pFastBac vector, with the resulting construct encoding the GP67 secretion signal sequence at the N terminus, and a His-tag followed by a TEV cleavage site at the C terminus. The His-tagged scFv16 was expressed and secreted by Sf9 insect cells, as previously reported. The Sf9 cells were removed by centrifugation at 5000 × g for 10 min, and the supernatant was thawed and frozen in liquid ethane by using a Vitrobot Mark IV. Data collections were performed at 1.3 m, using a cryo electron microscope (Cryo-EM) (JEM-2100, JEOL) at 120 kV, on a Quantifoil holey carbon grid (R1.2/1.3, Au, 300 mesh), and plunge-frozen in liquid ethane. The cryo-EM density map and the α-helical domain of the G_{i} subunit of the 363,784 particles were subjected to a higher signal-to-noise ratio. The quality of the micelle-subtracted density map was sufficient to build a model manually in COOT. The model building was facilitated by the predicted LPA_{1} model in the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/) and the cryo-EM structure of the LPA_{1}-G_{i} and µOR-G_{i} complex (PDB 7TD0 and 6DDE, respectively). We manually modeled LPA_{1}, the G_{i} heterotrimer and scFv16 into the map by jiggle fit using COOT. We then manually readjusted the model into the density map using COOT and refined it using phenix.real_space_refine with the secondary-structure restraints using phenix secondary_structure_restraints. Finally, we refined the model using servalc. The quality of the micelle-subtracted density map was sufficient to build a model manually in COOT. The model building was facilitated by the predicted LPA_{1} model in AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/) and the cryo-EM structure of the LPA_{1}-G_{i} and µOR-G_{i} complex (PDB 7TD0 and 6DDE, respectively). We manually modeled LPA_{1}, the G_{i} heterotrimer and scFv16 into the map by jiggle fit using COOT. We then manually readjusted the model into the density map using COOT and refined it using phenix.real_space_refine with the secondary-structure restraints using phenix secondary_structure_restraints. Finally, we refined the model using servalc.

**Formation and purification of the LPA_{1}-G_{i} complex**
Purified LPA_{1}-G_{i}-FP was mixed with a 1:2 molar excess of G_{i} heterotrimer, scFv16, and TEV protease. After the addition of apyrase to catalyze hydrolysis of unbound GDP, and ONO-074056 (final 10 μM) the coupling reaction was performed at 4 °C for overnight. To remove excess G_{i} protein, the complexing mixture was purified by M1 anti-Flag affinity chromatography. Bound complex was washed in buffer, containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% GDN, 10 μM ONO-074056, 10% Glycerol, and 5 mM CaCl_{2}. The complex was then eluted in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% GDN, 10 μM ONO-074056, 10% Glycerol, 5 mM EDTA, and Flag peptide. The LPA_{1}-G_{i}-scFv16 complex was purified by size exclusion chromatography on a Superdex200 10/300 column in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% GDN, and 1 μM ONO-074056. Peak fractions were concentrated to -12 mg/ml for electron microscopy studies.

**Sample vitrification and cryo-EM data acquisition**
The purified complex was applied onto a freshly glow-discharged Quantifoil holey carbon grid (Rio.2/L, Au, 300 mesh), and plunge-frozen in liquid ethane by using a Vitrobot Mark IV. Data collections were performed on a 300 kV Titan Krios G3i microscope (Thermo Fisher Scientific) and equipped with a BioQuantum K3 imaging filter and a K3 direct electron detector (Gatan). In total, 6,227 movies were acquired with a calibrated pixel size of 0.83 Å pix⁻¹ and with a defocus range of −0.8 to −1.6 μm, using the SerialEM software. Each movie was acquired for 2.57 s and split into 48 frames, resulting in an accumulated exposure of about 49.530 e⁻ Å⁻² at the grid.

**Image processing**
All acquired movies were dose-fractionated and subjected to beam-induced motion correction implemented in RELION 3.1. The contrast transfer function (CTF) parameters were estimated using CTFIND 4.0 (Rohou & Glauser, 2015). A total of 3,021,676 particles were extracted. The initial model was generated in RELION 3.1. The particles were subjected to several rounds of 2D and 3D classifications, resulting in the optimal classes of particles, which contained 363,784 particles. Next, the particles were subjected to 3D refinement, CTF refinement, and Bayesian polishing (Zivanov et al., 2018). The GDN detergent micelles and the α-helical domain of the G_{i} subunit of the 363,784 particles were subjected to a higher signal-to-noise ratio. The quality of the micelle-subtracted density map was sufficient to build a model manually in COOT. The model building was facilitated by the predicted LPA_{1} model in AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/entry/Q92633) and the cryo-EM structure of the LPA_{1}-G_{i} and µOR-G_{i} complex (PDB 7TD0 and 6DDE, respectively). We manually modeled LPA_{1}, the G_{i} heterotrimer and scFv16 into the map by jiggle fit using COOT. We then manually readjusted the model into the density map using COOT and refined it using phenix.real_space_refine with the secondary-structure restraints using phenix secondary_structure_restraints. Finally, we refined the model using servalc.

**Model building and refinement**
The quality of the micelle-subtracted density map was sufficient to build a model manually in COOT. The model building was facilitated by the predicted LPA_{1} model in AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/entry/Q92633) and the cryo-EM structure of the LPA_{1}-G_{i} and µOR-G_{i} complex (PDB 7TD0 and 6DDE, respectively). We manually modeled LPA_{1}, the G_{i} heterotrimer and scFv16 into the map by jiggle fit using COOT. We then manually readjusted the model into the density map using COOT and refined it using phenix.real_space_refine with the secondary-structure restraints using phenix secondary_structure_restraints. Finally, we refined the model using servalc.

**Reporting summary**
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The data that support this study are available from the corresponding authors upon reasonable request. The cryo-EM density map and atomic coordinates for the LPA_{1}-G_{i} complex have been deposited in the Electron Microscopy Data Bank and the PDB, under accession codes: EMD-34097 (LPA_{1}-G_{i} stable state), EMD-34098 (focused on LPA_{1}), EMD-34099 (LPA_{1}-G_{i} state 1), EMD-34100 (LPA_{1}-G_{i} state 2), EMD-34101 (LPA_{1}-G_{i} state 3), EMD-34102 (LPA_{1}-G_{i} state 4), and PDB 7YU3 (LPA_{1}-G_{i} stable state), 7YU4 (focused on LPA_{1}), 7YU5 (LPA_{1}-G_{i} state 1), 7YU6 (LPA_{1}-G_{i} state 2), 7YU7 (LPA_{1}-G_{i} state 3), 7YU8 (LPA_{1}-G_{i} state 4). Source data are provided with this paper.

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Acknowledgements

We thank K. Ogomori and C. Harada for technical assistance, Inoue, A for the advice on the assay experiment and Ono Pharmaceutical Co., Ltd. for the synthesis and characterization of ONO-0740556. This work was supported by grants from the Platform for Drug Discovery, Informatics and Structural Life Science by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and JSPS KAKENHI grants 21H05037 (O.N.), 22K19371 and 22H02751 (W.S.), and 21J20692 (T.T.); ONO Medical Research Foundation (W.S.); The Kao Foundation for Arts and Sciences (W.S.); The Takeda Science Foundation (W.S.); The Uehara Memorial Foundation (W.S.); the Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)) from AMED, under grant numbers JP19am0101115 (support no. 1109, O.N.).

Author contributions

H.A. performed all of the experiments. T.T. assisted with the grid preparation, the cryo-EM data collection, and the single particle analysis. F.S. assisted with the single particle analysis. Y.M. assisted with the NanoBiT-G-protein dissociation assay. W.S. performed the initial screening of the LPA1 expression. The manuscript was mainly prepared by H.A. and W.S., with assistance from O.N.

Competing interests

O.N. is a co-founder and scientific advisor for Curreio. All other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-33121-2.

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Peer review information Nature Communications thanks Arun Shukla, Abby Parill-Baker, and Haitao Zhang for their contribution to the peer review of this work. Peer review reports are available.

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