Agonist-bound structure of the human P2Y$_{12}$ receptor

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The P2Y$_{12}$ receptor (P2Y$_{12}$R), one of eight members of the P2Y family expressed in humans, is one of the most prominent clinical drug targets for inhibition of platelet aggregation. Although mutagenesis and modelling studies of the P2Y$_{12}$R provided useful insights into ligand binding, the agonist and antagonist recognition and function at the P2Y$_{12}$R remain poorly understood at the molecular level. Here we report the structures of the human P2Y$_{12}$R in complex with the full agonist 2-methylthio-adenosine-5’-diphosphate (2MeSATP, a close analogue of endogenous agonist ADP) at 2.5 Å resolution, and the corresponding ATP derivative 2-methylthio-adenosine-5’-triphosphate (2MeSATP) at 3.1 Å resolution. These structures, together with the structure of the P2Y$_{12}$R with antagonist ethyl 6-(4-((benzylsulfonyl)carbamoyl) Piperidin-1-yl)-5-cyano-2-methylcinotinate (AZD1283)5, reveal striking conformational changes between nucleotide and non-nucleotide ligand complexes in the extracellular regions. Further analysis of these changes provides insights into a distinct ligand binding landscape in the 6-group of class A G-protein-coupled receptors (GPCRs). Agonist- and non-nucleotide antagonist adopt different orientations in the P2Y$_{12}$R, with only partially overlapping binding pockets. The agonist-bound P2Y$_{12}$R structure answers long-standing questions surrounding P2Y$_{12}$R–agonist recognition, and reveals interactions with several residues that had not been involved in agonist binding. As a first example, to our knowledge, of a GPCR in which agonist access to the binding pocket requires large-scale rearrangements in the highly malleable extracellular region, the structural and docking studies will therefore provide invaluable insight into the pharmacology and mechanisms of action of agonists and different classes of antagonists for the P2Y$_{12}$R and potentially for other closely related P2YRs.

After sensing their endogenous extracellular ligands, GPCRs activate associated intracellular signal transduction pathways that subsequently lead to physiological responses. Structures of five GPCRs (rhodopsin, β$_1$-adrenergic receptors (β$_2$AR)11,12, A$_2A$ adenosine receptor (A$_2A$AR)13,14 and M2 muscarinic receptor15,16) have now been determined. The highly conserved C97 3.25–C175ECL2 disulphide bond, bridging the amino terminus (C17) with helix VII, is inherent to the P2Y$_{12}$R, as it is consistent in all three structures despite other residues at this region as compared with the AZD1283 complex. The highly conserved C97 3.25–C175ECL2 disulphide bond stabilizing the conformational change of ECL2 is clearly observed in the agonist-bound structure, although it was not resolved with antagonist bound. Formation of this disulphide bond in the P2Y$_{12}$R–2MeSADP complex requires an unwinding of the helical bulge structure in the extracellular portion of helix III that comprises ~4 residues instead of the ~3.6 residues in a regular 9-helical turn. As a result, there is a ~6 α-helix rotation of C97 3.25 along the helical path by over 60°, and a relocation of other residues at this region as compared with the AZD1283 complex.
P2Y12R structures the intracellular half of the 7TM domain has a very similar conformation to that observed in the PAR1 structure (Extended Data Fig. 3c). Only minor changes in helices VI and VII were observed in the intracellular part of P2Y12R–2MeSADP. They do not appear consistent, however, with large changes in helical positions observed in the intracellular region in active state agonist-bound A2AAR, or β2AR stabilized by G-protein12,14. It is therefore likely that the P2Y12R–2MeSADP structure represents an agonist-bound inactive state with respect to the intracellular region, similar to the one observed in agonist-bound β1AR and β2AR without G-protein or a G-protein mimic stabilizing their active state16,20.

The rearranged 2MeSADP-binding pocket consists of residues from helices III, IV, V, VI and VII as in the P2Y12R–AZD1283 structure4, but also extensively involves ECL2 and the N terminus. Both pockets described in the P2Y12R–AZD1283 structure are still present, although contracted, especially pocket 1, due to the inward movement of helices VI and VII (Extended Data Fig. 5). In particular, the inward shift of helix VI in the agonist-bound structure shrinks pocket 1 substantially so that it would preclude AZD1283 binding. As a result, although both 2MeSADP and AZD1283 bind to the same pocket, their orientations are completely different, with only partial overlap between them (Fig. 3).

The adenine group of 2MeSADP occupies the same aromatic binding site as the nicotinate group in AZD1283, forming a similar π–π interaction with the Y105ECL3 side chain. The 2-thioether inserts into a hydrophobic pocket formed by F106ECL3, L155ECL3, S156ECL3 and N159ECL3, and serves as an anchor to maintain the adenine core and the ribose ring in an optimal orientation (Extended Data Fig. 5a). Thus, 2MeSADP binds with greater complementarity, which explains the higher affinity of this ligand compared with ADP. The 2-thioether and amino groups of adenine overlay with the ethyl ester and cyano substituents on the nicotinate group (Fig. 3b). The orientation of the ribose moiety corresponds to that of the methyl group on the nicotinate moiety. The ribose 2‘ and 3‘ hydroxyl groups interact with K179ECL2 and H1875.36 and with T163ECL2 and K179ECL2, respectively.

The interactions between the receptor and the diphosphate of 2MeSADP involve numerous hydrophilic and positively charged residues (Fig. 3c). As predicted on the basis of the sequence analysis and confirmed through mutagenesis21–23, two essential cationic residues R256ECL2 and K280ECL2 (Extended Data Table 2) and an aromatic residue Y259ECL2 that is conserved...
in the P2Y12-R-like subfamily coordinate phosphate moieties. In addition to these residues, a residue not previously implicated in agonist binding, the third cationic residue R933.21, contacts the β-phosphate. Three water molecules also bridge the interaction between β-phosphate with a fourth cationic residue, R19 (N terminus). Some residues that are thought to participate in agonist binding, however, have no direct contact with the nucleotide ligands. The R265ECL3 side chain, which was previously implicated in activation of the receptor23, is positioned away from 2MeSADP. Interestingly, the conserved K174ECL2, which was previously predicted to participate in agonist binding, however, have no direct contact with the agonist, but forms a salt bridge to E2737.28 that apparently helps to stabilize the agonist-bound conformation.

Cysteine residues forming the conserved disulphide bond are also involved directly in the binding of 2MeSADP. The main chain carbonyl of C973.25 forms a hydrogen bond with the 3′ hydroxyl group of ribose, and the main chain NH group of C175ECL2 interacts with the β-phosphate group. Disruption of this labile disulphide bond, for example, as possible in the complex with antagonist AZD1283, would also prevent such a bidentate coordination system of the nucleoside. The active metabolites of the thienopyridine drugs are also predicted to destabilize interactions of the P2Y12-R with the nucleotide agonist by binding covalently to C973.25. Mutation of either of these cysteine residues to alanine greatly reduces agonist binding (Extended Data Table 2), although it is known that the P2Y12-R does not require this disulphide bond for overall structural integrity, that is, in the AZD1283 complex.

The action of nucleoside 5′-triphosphate derivatives at P2Y12-R, that is, agonism versus antagonism, has long been unclear. Although ATP and 2MeSATP were thought to be antagonists under physiological conditions25, the close conformational similarity between the 2MeSADP and 2MeSATP complexes suggests that ATP derivatives potentially qualify for similar signalling properties at P2Y12-R as ADP derivatives, which is also supported by recent pharmacological data1. Moreover, AR-C66096, a non-cleavable triphosphate mimic that could be docked into the P2Y12-R in an identical binding mode to 2MeSADP and 2MeSATP, displays characteristics of a partial agonist inhibiting cAMP production in CHO cells overexpressing P2Y12-R (Extended Data Fig. 6). Of course, we emphasize that partial agonist activity of ATP derivatives may be expression-level dependent26.

The 2MeSADP binding cavity appears to accommodate a number of other nucleotide ligands that mimic a triphosphate chain, including...

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Figure 3 | P2Y12-R ligand binding pocket for 2MeSADP. a. The receptor is shown in cyan cartoon representation. The ligand 2MeSADP (orange carbons) and receptor residues (slate carbons) involved in ligand binding are shown in stick representation. Other elements are coloured as follows: oxygen, red; nitrogen, dark blue; sulphur, yellow; phosphorus, orange. The water molecules interacting with 2MeSADP are shown as red spheres. b. Comparison of the 2MeSADP and AZD1283 binding poses in the overlaid P2Y12-R complexes. Colour scheme as in Fig. 2. c. Summary of receptor interactions of 2MeSADP. Hydrogen bonds are displayed as blue dashed lines and the salt bridges as red dashed lines. The π-π interaction between 2MeSADP and Y1053.33 is indicated as green dashed lines. The NHα and COα indicate the main chain amine and carbonyl groups of the corresponding residue.

Figure 4 | Schematic illustration of conformational changes in P2Y12-R extracellular region. a. Unliganded (apo) state of P2Y12-R with open entrance to the pocket and partially disordered lid. A number of partially uncompensated positive electrostatic charges among the side chains in the pocket (R19Nterm, K807.35, R933.21, K173ECL2, K174ECL2, R2566.55 and K2807.33) disfavour formation of the stable closed state. b. The closed state is stabilized by binding of nucleotide agonist (for example, ADP, 2MeSADP). c. A similar conformation with ‘lid’ closure occurs in 2MeSATP structure and for various docked N6 unsubstituted nucleoside triphosphate and triphosphate-mimetic ligands. d. A helical reorganization is proposed for some N6 substituted nucleoside triphosphate and triphosphate mimetic ligands, especially with bulky N6 substituents. e. Binding of non-nucleotide antagonist AZD1283 blocks inward movement of helices VI and VII and prevents ‘lid’ closure.
AR-C67085 and cangrelor27 (Extended Data Figs 7 and 8). However, the nucleoside antagonist ticagrelor does not dock with a similar conformation to 2MeSADP in the P2Y12R because of the bulky N6 substituent, unless a helical rearrangement occurs, particularly in helix VI. This docking observation suggests that reduction of agonist efficacy of these ligands is likely facilitated by N6 substitutions in the nucleoside scaffold.28 Consistently, we have shown that non-nucleotides AZD1283 and ticagrelor behave as competitive P2Y12R antagonists. Thus, we predict that the ligand-bound receptor conformations and consequently pharmacology of nucleotide-mimetic and non-nucleotide antagonists will be divergent.

These structural and pharmacological findings provide unexpected insights into the mechanism of agonist and antagonist interactions with P2Y12R, as schematically illustrated in Fig. 4. As the agonist-bound pocket is sealed by a ‘lid’ formed by unusually cationic ECLs and the N terminus, agonist access to the binding pocket of apo P2Y12R would require plasticity of this extracellular region. Closing of the pocket in the absence of charged phosphates would also be disfavoured by electrostatic repulsion, with ~7 arginine and lysine side chains pointing towards the pocket. Binding of a nucleotide agonist like 2MeSADP involves stabilization of an inward position of helices VI and VII and formation of the lid, which is stabilized by numerous electrostatic interactions with the agonist phosphate groups. The additional phosphate group of 2MeSATP is also accommodated by a similar conformation of the lid, although distinct interactions may still affect the 2MeSATP binding and signalling profile. In stark contrast, an open extracellular side conformation is defined by binding of non-nucleotide antagonists like AZD1283, which keeps helices VI and VII away from the pocket and destabilizes the lid.

In conclusion, P2Y12R is the first receptor to demonstrate striking rearrangements in the extracellular regions, characterized by open and closed access to the ligand binding pocket. Whether such high plasticity of the binding pocket has evolved to enable optimal and specific recognition of negatively charged nucleotide ligands of the P2YR family, or is a more general feature of the δ subgroup of class A GPCRs is currently unknown. Additional structures will help us to understand similarities and differences in ligand binding and receptor activation of the δ-group receptors.

METHODS SUMMARY

The human P2Y12R–BRIL fusion protein was purified by metal affinity chromatography and crystallized in lipidic cubic phase (LCP). The diffraction data were collected from 17 crystals (P2Y12R–2MeSADP) and 6 crystals (P2Y12R–2MeSATP) on the BL41XU beamline at the Spring8, Japan, using a 10 μm minibeam. The structure was solved by molecular replacement and refined in Refmac5 and Buster. The P2Y12 antagonists, 2-methylthioadenosine 5′-monophosphate ticagrelor does not dock with a similar conformation to 2MeSADP in the P2Y12R because of the bulky N6 substituent, unless a helical rearrangement occurs, particularly in helix VI. This docking observation suggests that reduction of agonist efficacy of these ligands is likely facilitated by N6 substitutions in the nucleoside scaffold.28 Consistently, we have shown that non-nucleotides AZD1283 and ticagrelor behave as competitive P2Y12R antagonists. Thus, we predict that the ligand-bound receptor conformations and consequently pharmacology of nucleotide-mimetic and non-nucleotide antagonists will be divergent.

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Author Contributions

J.Z. optimized the construct, expressed and purified human P2Y12R–BRIL for crystallization, developed the purification procedure, performed crystallization trials and optimized crystallization conditions. K.Z. helped with construct and crystal optimization and collected diffraction data. Z.-G.G. designed, performed and analysed ligand binding and competition assays of wild-type and mutant P2Y12R, S.P. performed and analysed docking assays. D.Z. helped in expression and purification. G.W.H. solved and refined the structure. T.L. helped the expression for initial expression and purification protocol for P2Y12R. C.E.M. helped to design and optimize LCP crystallization trials and processed crystallographic data and wrote the manuscript. V.C., V.K., R.C.S.; Target GPCR-87, the National Science Foundation of China grants 31370729 and National Science and Technology Major Project 2013ZX09507001 and 2013ZX09501001 (B.W., Q.Z.), National Institutes of Health NIDDK Intramural Research Program (K.A.J.) and the National Natural Science Foundation of China 91313000 (H.J.). The authors thank S. Nylander, E. Kiselev and S. Moss for scientific feedback on the manuscript, A. Walker for assistance with manuscript preparation and P.K. Kudinovskaya for help with figure preparation. The synchrotron radiation experiments were performed at the BL41XU of SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (proposal no. 2013B1049). We thank the beamline staff members of the BL41XU for help with X-ray data collection.

Author Information

Atomic coordinates and structure factors for the P2Y12R–2MeSADP and P2Y12R–2MeSATP structures have been deposited in the Protein Data Bank with identification codes 4PK2 and 4PY0, respectively. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to B.W. (biuliu@siim.ac.cn) or Q.Z. (zhaoq@simm.ac.cn).
METHODS

Purification of P2Y12R–BRIL protein and crystallization in lipidic cubic phase. P2Y12R–BRIL construction, expression and membrane preparation were performed using the same procedure as described in the companion manuscript. In brief, the human P2Y12R was subcloned into a modified pFastBac1 vector, with thermostabilized BRIL (Protein Data Bank accession code 1M6T) inserted at ICL3 (T223–R224) and a D294N mutation. The fusion was expressed using Bac-to-Bac Baculovirus Expression System (Invitrogen) in Spodoptera frugiperda (99) cells for 48 h and membrane was washed repeatedly using hypertonic buffer with low and high salt.

Before solubilization, purified membranes were incubated with 20 μM corresponding ligand (2MeSADP or 2MeSATP obtained from Tocris) in the presence of 2 mg ml−1 1-iodoacetamide, and EDTA-free protease inhibitor cocktail (Roche) for 30 min. P2Y12R–BRIL was extracted from the membrane by adding n-dodecyl-β-D-maltopyranoside (DDM, Affymetrix) and cholesterol hemisuccinate (CHS, Sigma) to the membrane solution to a final concentration of 0.5% (v/v) and 0.1% (v/v), respectively, and stirring was continued at 4°C for 2.5 h. The supernatant was isolated by centrifugation at 160,000g for 30 min, followed by incubation in TALON IMAC resin (Clontech) at 4°C overnight. The resin was washed with twenty column volumes of 50 mM HEPES, pH 7.5, 1 mM NaCl, 10% (v/v) glycerol, 0.05% (w/v) DDM, 0.01% (v/v) CHS, and 30 mM imidazole. The protein was eluted with 5 column volumes of 50 mM HEPES, pH 7.5, 1 mM NaCl, 10% (v/v) glycerol, 0.05% (w/v) DDM, 0.01% (v/v) CHS, 270 mM imidazole and 50 μM corresponding ligand. After removing imidazole by using a PD MiniTrap G-25 column (GE Healthcare), ligand concentration was increased to 2 mM. The protein was then treated overnight with His-tagged PreScission protease (20 μg per 500 μl of expressed material) and His-tagged PNGase F (20 μg per 500 μl of expressed material) to remove the carboxy-terminal His-tag and deglycosylate the receptor. PreScission protease, PNGase F and the tagged PNGase F (20 μg per 500 μl of expressed material) were subjected to visual inspection and analysis of protein–ligand interactions to select the final binding conformations in agreement with the experimental data.

DNA sequence of the crystallization construct. The sequence was: ATGAAAGCC ATCATCGCCCCCTGACATCTCATTCTGCGTGTGTTGCGCCACTAAGG AGATGAGTGGGCGGGGCGGCGGACGTCGTCGTCGTCGTCGGTGTAACCC 3′ (ligand diameter midpoint box) of 14 Å and an outer box that extended 10 Å in each direction from the inner one (so that ligands up to 20 Å could be docked). Docking of ligands was performed in the rigid binding site using the XP (extra precision) procedure. The top scoring docking conformations for each ligand were subjected to visual inspection and analysis of protein–ligand interactions to select the final binding conformations in agreement with the experimental data.

Data collection, structure solution and refinement. X-ray diffraction data were collected at the SPring-8 beam line 41XU, Hyogo, Japan, using a Rayonix MX225HE detector (X-ray wavelength 1.000 Å). The crystals were exposed with a 10 μm mini-beam for 1 s and 1° oscillation per frame, and a rastering system was used to find the best diffracting parts of single crystals. Most crystals of P2Y12R in complex with 2MeSADP or 2MeSATP and BRIL reached their full size (80 × 80 × 5 μm3) within 10 days. Crystals were collected directly from LCP using 50–150 μm micromotors (M2-L19-50/150, MiTeGen) and flash frozen in liquid nitrogen.

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Extended Data Figure 1 | Crystals and electron density of nucleotides for P2Y12R–2MeSADP and P2Y12R–2MeSATP complexes. a, Crystals of the P2Y12R–2MeSADP complex. The size of the crystals is roughly 80 × 50 × 5 μm. b, Crystals of the P2Y12R–2MeSATP complex. The size of the crystals is roughly 30 × 30 × 5 μm. c, The 2mFo–DFc map for the 2MeSADP contoured at 1σ. d, The 2mFo–DFc map for the P2Y12R–2MeSATP contoured at 1σ. The relatively high B-factor of the γ-phosphate group (98 Å²) compared with β-phosphate and surrounding protein atoms (∼75 Å²), and the propensity of 2MeSATP to hydrolyse to 2MeSADP suggest partial occupancy for the γ-phosphate group. However, given the differences in crystal forms and packing, as well as the clear density of the γ-phosphate group, the P2Y12R–2MeSATP complex structure should provide relevant information about 2MeSATP binding.
Extended Data Figure 2 | Crystal packing of P2Y12R–2MeSADP, P2Y12R–2MeSATP and P2Y12R–AZD1283 complexes. a, Overall structure of the P2Y12R–2MeSADP complex, P2Y12R and BRIL are shown in cyan and blue, respectively. b, c, Crystal packing of P2Y12R–2MeSADP complex shown in two different views. d, Overall structure of the P2Y12R–2MeSATP complex, P2Y12R is shown in pink. e, f, Crystal packing of P2Y12R–2MeSATP complex in two different views. g, Overall structure of the P2Y12R–AZD1283 complex, P2Y12R is shown in orange. h, i, Crystal packing of P2Y12R–AZD1283 complex shown in two different views.
Extended Data Figure 3 | Comparison of antagonist- (orange) and agonist- (green/cyan) bound P2Y₁₂R structures with the PAR1 structure (yellow).

a, Side view of the three structures. The receptor structures are shown as cylindrical helices and AZD1283 and 2MeSADP are shown as sticks with green carbons and wheat carbons, respectively. b, Comparison view from the extracellular side. c, Comparison view from the intracellular side.
Extended Data Figure 4 | The distortion of helix III by the disulphide bond. a, Comparison of P2Y12R–AZD1283 (orange) and P2Y12R–2MeSADP (green/cyan). b, Corresponding positions of residues around C973.25 in P2Y12R–AZD1283 (orange) and P2Y12R–2MeSADP (green/cyan).
Extended Data Figure 5 | Comparison of pocket 1 (a–c) and pocket 2 (d–f) of P2Y_{12}R structures with different ligands. 

- **a**, **d**, The P2Y_{12}R–2MeSADP structure.
- **b**, **e**, The P2Y_{12}R–2MeSATP structure.
- **c**, **f**, The P2Y_{12}R–AZD1283 structure. The 2MeSADP, 2MeSATP and AZD1283 ligands are shown in sticks with wheat, grey and green carbons, respectively.
Extended Data Figure 6 | Functional properties of different ligands at P2Y12R. Data (mean ± s.e.m.) were determined in triplicate. a, Parallel right shifts induced by antagonist AZD1283 (AZD) of the activation curves by agonist 2MeSADP in inhibition of cAMP production in P2Y12R expressing CHO cells. b, Parallel right shifts induced by antagonist ticagrelor (Tica) of the activation curves by agonist 2MeSADP in inhibition of cAMP production in P2Y12R expressing CHO cells. The pK\textsubscript{b} values of AZD and Tica are 8.17 ± 0.45 and 7.70 ± 0.18, respectively. c, Partial agonist effects of AR-C66096 (ARC) in inhibition of cAMP production in P2Y12R expressing CHO cells. The half-maximum effective concentration (EC\textsubscript{50}) value of AR-C66096 was determined to be 34.9 ± 2.9 nM, and its E\textsubscript{max} 41.9 ± 3.6% compared with 2MeSADP as 100%. A final concentration of 10 μM forskolin was used in the experiment. DMSO was used as a solvent for the stock solution of forskolin, AZD1283 and ticagrelor. The stock solution of AR-C66096 was made with water.
Extended Data Figure 7 | Docking models of different nucleotide analogues to the P2Y<sub>12</sub>R structure. a, The crystal structure of P2Y<sub>12</sub>R–2MeSADP complex. b, Docking of 2MeSADP to the P2Y<sub>12</sub>R structure. c, Docking of ADP to the P2Y<sub>12</sub>R structure. d, The crystal structure of P2Y<sub>12</sub>R–2MeSATP complex. e, Docking of 2MeSATP to the P2Y<sub>12</sub>R structure. f, Docking of ATP to the P2Y<sub>12</sub>R structure. g, Docking of AR-C66096 to the P2Y<sub>12</sub>R structure. h, Docking of AR-C67085 to the P2Y<sub>12</sub>R structure. i, Docking of AR-C69931MX (cangrelor) to the P2Y<sub>12</sub>R structure. 2MeSADP and 2MeSATP poses from corresponding crystal structures are shown in stick with orange and grey carbons, respectively. Docking was performed to the conformation of P2Y<sub>12</sub>R found in the 2MeSADP-bound structure, and the docked ligands are shown in sticks with purple carbons. AR-C66096 and AR-C67085 show the same interactions observed in the 2MeSADP complex. In addition, the C<sub>2</sub>-propylthio substituent of AR-C66096 and AR-C67085 is located in a hydrophobic pocket in proximity to helix IV surrounded by F106<sup>3.34</sup>, Y109<sup>3.37</sup>, M152<sup>4.53</sup> and L155<sup>4.56</sup>. The γ-phosphonate group is directed towards helix III and interacts with K80<sup>2.60</sup> and R93<sup>3.21</sup>. The C<sub>2</sub> substituent and the γ-phosphonate group of AR-C69931MX show similar orientation as observed in the docking pose of AR-C66096 and AR-C67085. The N<sub>6</sub> substituent is directed towards helix VI in proximity to Y109<sup>3.37</sup>, Q195<sup>5.44</sup>, F252<sup>6.51</sup>, H253<sup>6.52</sup> and R256<sup>6.55</sup>.
Extended Data Figure 8 | Ligands used in the docking studies. The chemical structures of parts of ligands that are discussed and used in the docking studies are shown. Ticagrelor and AR-C78511 could not be docked in a conformation similar to 2MeSADP because the presence of their bulky N° substituents would cause a steric clash with helices V and VI. AR-C78511 was previously shown to lack partial agonist properties.
## Extended Data Table 1 | Data collection and refinement statistics

|                      | P2Y12R-2MeSADP | P2Y12R-2MeSATP |
|----------------------|----------------|----------------|
| **Data Collection**  |                |                |
| Number of Crystals used | 17             | 6              |
| Space group          | C222₁          | C2             |
| Cell dimensions      |                |                |
| a, b, c (Å)          | 65.1, 104.2, 169.4 | 75.7, 65.1, 100.7 |
| α, β, γ (°)          | 90.0, 90.0, 90.0       | 90.0, 90.5, 90.0 |
| Number of reflections processed | 336,625         | 26,125         |
| Number of unique reflections | 20,345          | 8,273          |
| Resolution (Å)       | 50.0-2.50 (2.63-2.50) † | 30.0-3.10 (3.27-3.10) † |
| Rmerge (%)           | 19.4 (99.8)     | 22.2 (92.2)    |
| CC1/2                | 0.996 (0.587)   | 0.968 (0.413)  |
| Mean I/σ(I)          | 12.4 (2.3)      | 5.6 (2.2)      |
| Completeness (%)     | 100.0 (100.0)   | 92.2 (91.2)    |
| Redundancy           | 16.5 (8.1)      | 3.2 (2.9)      |
| **Refinement**       |                |                |
| Resolution (Å)       | 50.0-2.50       | 50.0-3.10      |
| Number of reflections (test set) | 20,345 (1,041) | 8,273 (422)   |
| Rwork / Rfree (%)    | 20.0 / 23.0    | 22.2 / 26.5    |
| Number of atoms      |                |                |
| Protein              | 3,040          | 3,053          |
| Ligand               | 29             | 33             |
| Cholesterol          | 28             | 0              |
| Lipids, PEG and waters | 105            | 33             |
| **Overall B values (Å²)** |                |                |
| P2Y12R                | 65.0           | 69.1           |
| BRIL                  | 59.3           | 146.4          |
| Ligand                | 44.0           | 69.0           |
| Cholesterol           | 87.8           | n/a            |
| Lipids and waters     | 70.2           | 84.9           |
| **RMSD**              |                |                |
| Bond lengths (Å)      | 0.010          | 0.010          |
| Bond angles (°)       | 1.03           | 0.87           |
| Ramachandran plot statistics (%) ‡ |                |                |
| Favored regions       | 98.7           | 97.1           |
| Allowed regions       | 1.3            | 2.9            |
| Disallowed regions    | 0.0            | 0.0            |

The highest resolution shell is shown in parentheses.
† Values in parentheses are for highest-resolution shell.
‡ As defined in MolProbity.
## Extended Data Table 2 | Binding affinities for different P2Y12R constructs

### a

| Constructs | $[^{3}H]$2MeSADP ($K_d$ nM) |
|------------|-----------------------------|
| WT         | 4.9±1.3                     |
| S83A       | 5.6±1.4                     |
| C97A       | N.S.                        |
| R256A      | 16.1±6.2                    |
| C175A      | N.S.                        |
| K280A      | N.S.                        |

### b

| Constructs | AZD1283 ($K_i$, nM) |
|------------|---------------------|
| WT         | 41.8±16.3           |
| S83A       | 36.5±8.8            |
| R256A      | 140±39              |

Affinity values of the agonist $[^{3}H]$2MeSADP determined in saturation binding to WT and mutant P2Y12Rs expressed transiently in COS7 cells (a) and inhibition by antagonist AZD1283 (b). Results are expressed as mean ± s.e.m. from 3–6 independent experiments performed in duplicate by methods described in ref. 5. N.S., not saturable or negligible specific binding within the radioligand concentrations used (0.4–46 nM).