Supplementary material

Key Determinants of Nucleotide-Activated G Protein-Coupled $P2Y_2$ Receptor Function Revealed by Chemical and Pharmacological Experiments, Mutagenesis and Homology Modeling

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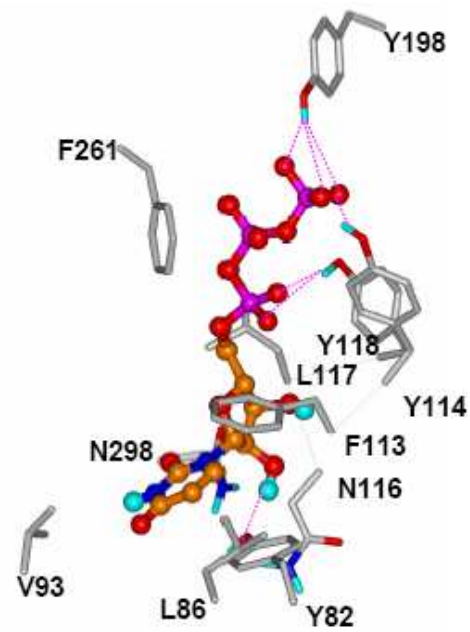
Table 1. Primers used for the site-directed mutagenesis of the human $P2Y_2$ receptor

mutant	primers (1) forward primer, 2) reverse primer)	annealing temperature
C106S	1) 5'CACGGTGCTCTCGAAGCTGGTGC 2) 5'GCACCAGCTTCGAGAGCACCGTG	60°C
Y114A	1) 5'CGCTTCCTCTTCGCGACCAACC 2) 5'GGTTGGTCGCGAAGAGGAAGCG	58°C
Y118A	1) 5'CACCAACCTTGCGTGCAGCATCC 2) 5'GGATGCTGCACGCAAGGTTGGTG	60°C
R177A	1) 5'CCGGGGGCAGAGTAACCTGCC 2) 5'GGCAGGTTACTCTGCCCCCGG	62°C
R180A	1) 5'GCGGGGGCGCCGTAACCTGCCAC 2) 5'GTGGCAGGTTACGGCGCCCCCGC	65.5°C
R177A_R180A	1) 5'CCACCAGCGCGGCCGGGGGCGCCGTAACCTGC 2) 5'GCAGGTTACGGCGCCCCCGGCCGCGCTGGTGG	55°C
Y198A	1) 5'CTTCGTGGCCGCTAGCTCAGTC 2) 5'GACTGAGCTAGCGGCCACGAAG	60°C
R272A	1) 5'CGCACCCTCTACTACTCCTTCGCTAGCCTGGAC CTCAGCTGCCACAC 2) 5'GTGTGGCAGCTGAGGTCCAGGCTAGCGAAGGAGTA GTAGAGGGTGCG	55°C
C278S	1) 5'CCTCAGCTCCCACACCCTC 2) 5'GAGGGTGTGGGAGCTGAGG	58°C
S296A	1) 5'CGCTGGCCGCTGCTAACAGTTG 2) 5'CAACTGTTAGCAGCGGCCAGCG	60°C

A



B

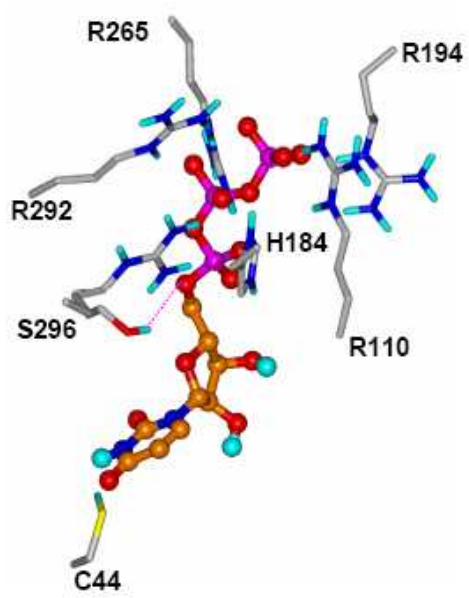
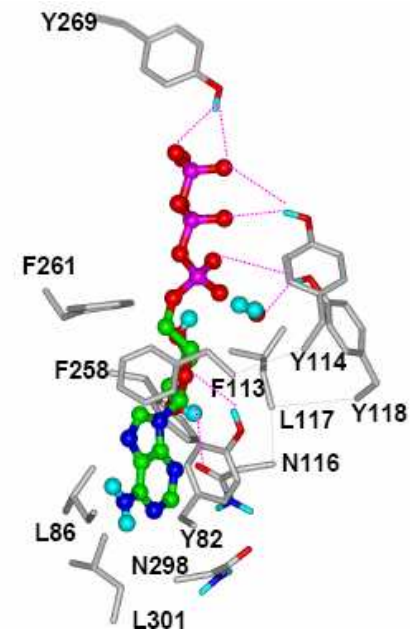


Figure 1. Interactions between UTP (**3**) and the $P2Y_2R$ after molecular dynamics (MD) simulation. **A.** Lipophilic interactions of UTP in the receptor binding pocket. **B.** Hydrogen bonding and ionic interactions of UTP binding to the $P2Y_2R$.

A



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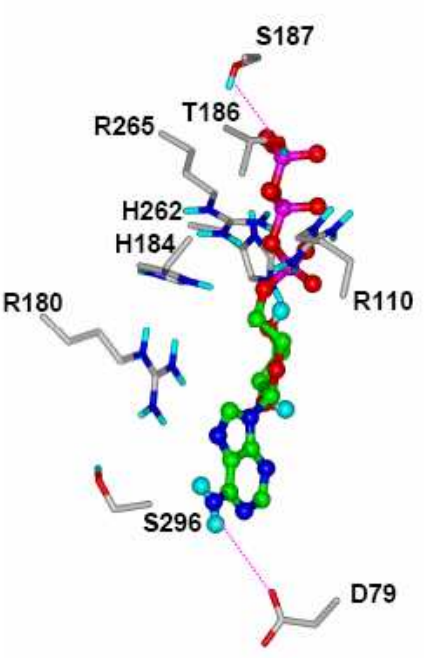
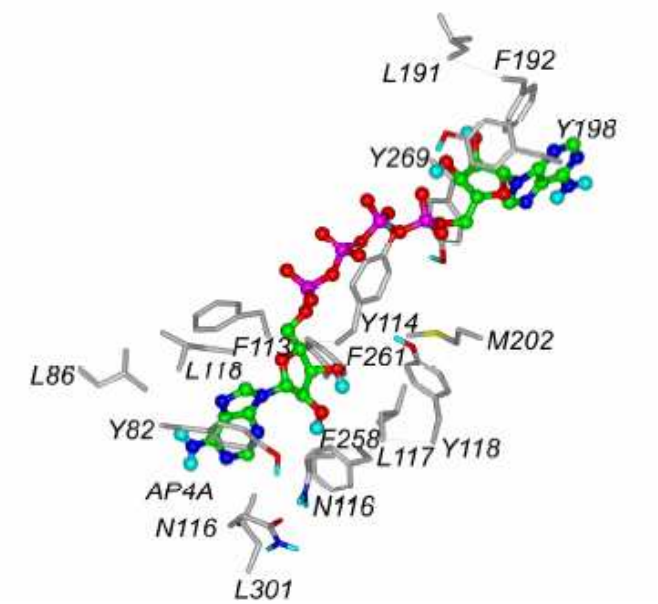


Figure 2. Interactions between ATP (**1**) and the $P2Y_2R$ after MD simulation. **A.** Lipophilic interactions of ATP in the receptor binding pocket. **B.** Hydrogen bonding and ionic interactions of ATP binding to the $P2Y_2R$.

A



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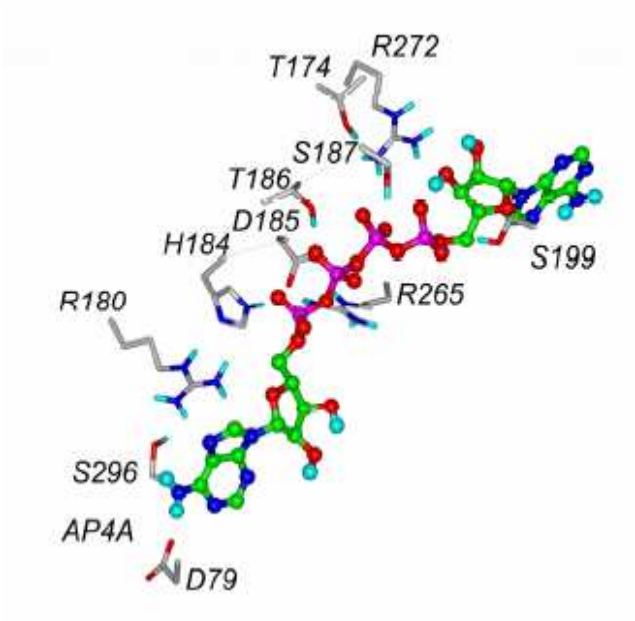
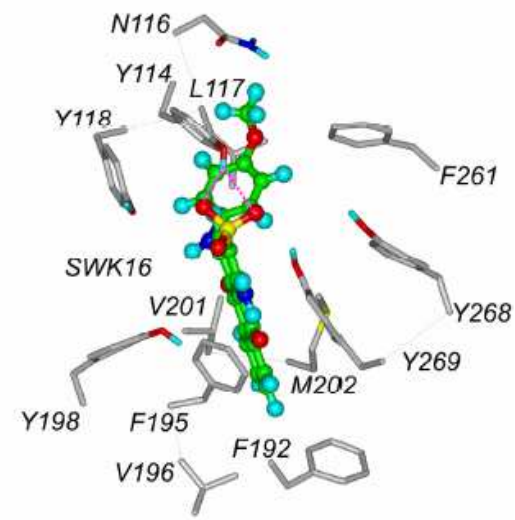


Figure 3: Interactions between Ap_4A (**2**) and the $P2Y_2R$ after MD simulation. **A.** Lipophilic interactions of Ap_4A in the receptor binding pocket. **B.** Hydrogen bonding and ionic interactions of Ap_4A binding to the $P2Y_2R$.

A



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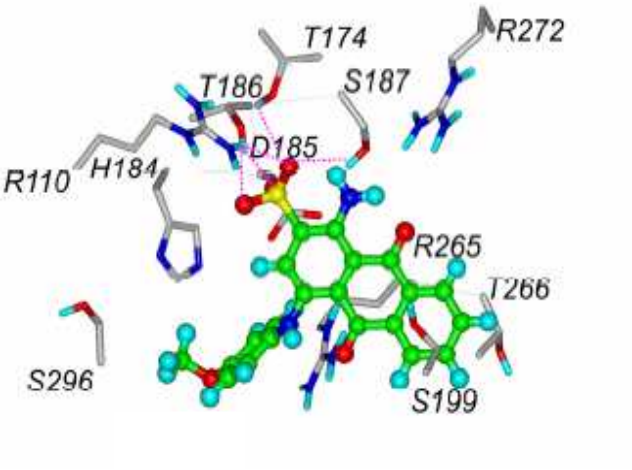
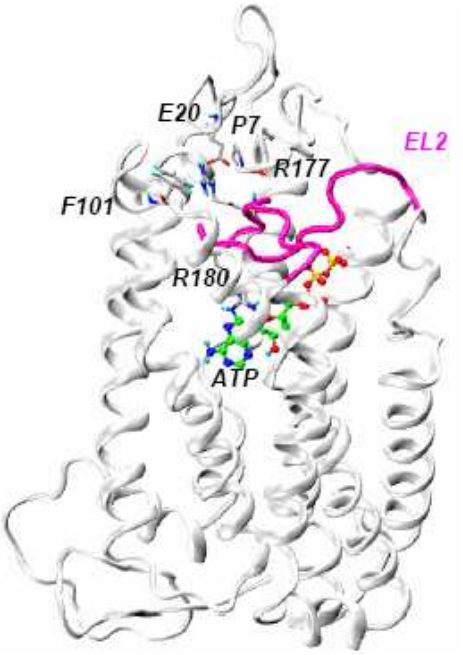
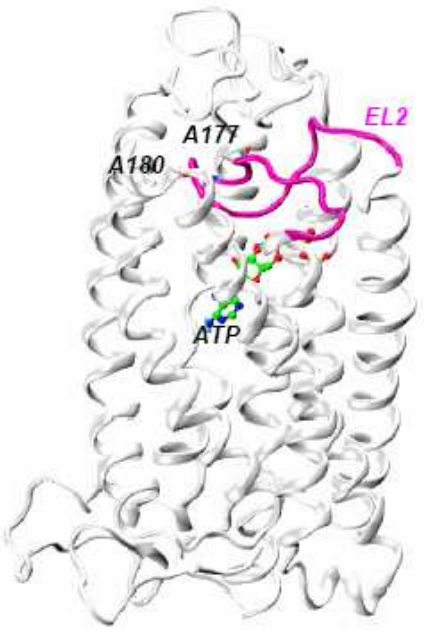


Figure 4. Computer-generated models of the $P2Y_2R$ after MD simulation.
A. Protein-ligand interactions of PSB-416 (**8**) with the $P2Y_2R$ after MD simulation studies: lipophilic interactions of PSB-416 in the receptor binding pocket.
B. Protein-ligand interactions of PSB-416 with the $P2Y_2R$ after MD simulation studies: hydrophilic interactions of PSB-416 in the receptor binding pocket.

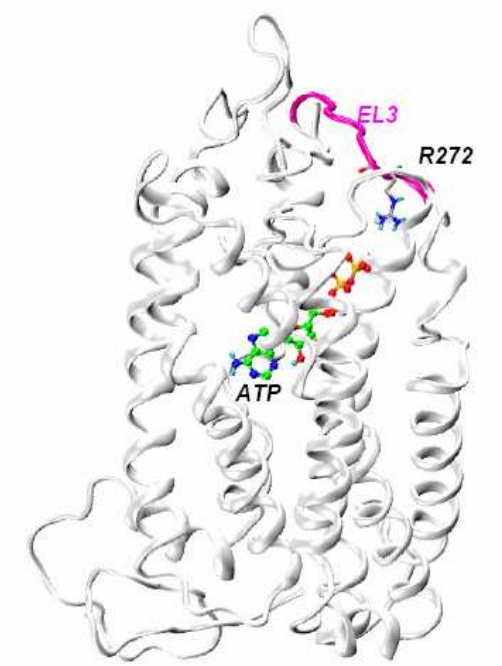
A


E20
P7
R177
EL2
F101
R180
ATP

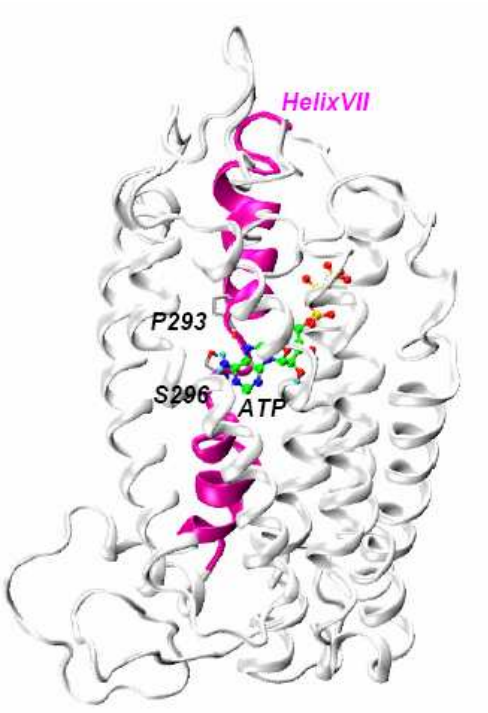
B


A177
EL2
A180
ATP

C


EL3
R272
ATP

D


HelixVII
P293
S296
ATP

E

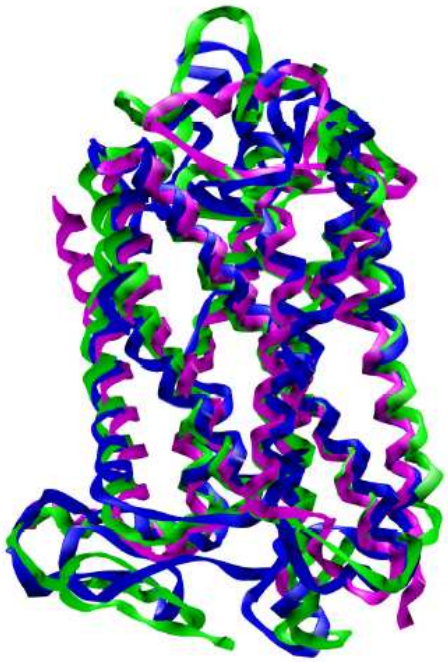


Figure 5. Models of the $P2Y_2R$, some mutant receptors, and comparison of the structures of rhodopsin and the β_2-adrenergic receptor.
A, B. Comparison of the EL2 structure of the ATP bound receptor protein (wild-type versus R177_R180A). In the wild-type receptor R180 interacts directly with ATP, R177 stabilizes the receptor structure. B: Upon mutation the EL2 changes its conformation and the interaction with ATP is reduced.
C. R272 (EL3) is positioned directly at the entry site of the $P2Y_2R$ and is responsible for initial recognition of ligands as well as binding of larger agonists like dinucleotides.
D. S296 (7.43) is essential for $P2Y_2R$ function. At the far down position of the binding pocket it forms H-bonds with the ligands. S296 also builds a hydrogen bond with P293 (7.40) in the same helix and therefore stabilizes the receptor structure.
E. Comparison of the crystallized bovine rhodopsin (green), the crystallized β_2 adrenergic receptor (purple) and the model of the $P2Y_2R$ (blue). Transmembrane domains show high similarity.

Figure 6

Figure 6. Alignment of the extracellular loop 3 (EL3) of selected GPCRs that are activated by negatively charged ligands. All receptors shown feature a basic amino acid residue at the beginning of EL3. Receptors: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, GPR17, cysteinyl leukotriene receptor1 (CSLTR1), cysteinyl leukotriene receptor 2 (CSLTR2), prostaglandin D2 receptor (PD2R). Amino acid positions in the receptor are given in brackets.

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