Published in final edited form as:
Bioorg Med Chem Lett. 2007 February 1; 17(3): 761–766.

Molecular Dynamics Simulation of the $P2Y_{14}$ Receptor. Ligand Docking and Identification of a Putative Binding Site of the Distal Hexose Moiety

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Abstract

A rhodopsin-based homology model of the $P2Y_{14}$ receptor was inserted into a phospholipid bilayer and refined by molecular dynamics (MD) simulation. The binding modes of several known agonists, namely UDP-glucose and its analogues, were proposed using automatic molecular docking combined with Monte Carlo Multiple Minimum calculations. Compared to other P2Y receptors, the $P2Y_{14}$ receptor has an atypical binding mode of the nucleobase, ribose and phosphate moieties. The diphosphate moiety interacts with only one cationic residue, namely Lys171 of EL2, while in other P2Y receptor subtypes three Arg or Lys residues interact with the phosphate chain. Two other conserved cationic residues, namely Arg253 (6.55) and Lys277 (7.35) of the $P2Y_{14}$ receptor together with two anionic residues (Glu166 and Glu174 located in EL2), are likely involved in interactions with the distal hexose moiety.

Eight subtypes of P2Y receptors, namely $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$, have been cloned and characterized.[1] All of these are G protein-coupled receptors (GPCRs) belonging to the rhodopsin family. The $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ are G_q-coupled receptors and comprise the $P2Y_1$-like receptor family.[2] A second family referred to as the $P2Y_{12}$-like family consists of the $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors that couple via G_i proteins to the inhibition of adenylate cyclase.[2] P2Y receptors are widely distributed throughout the body and are involved in many physiological processes.[3] In particular, the $P2Y_{14}$ receptor is likely involved in regulation of neuroimmune functions and is highly expressed in placenta, stomach, and intestine, and with moderate expression in brain, heart, lung, and spleen.[4]

In contrast to other P2Y receptor subtypes, the $P2Y_{14}$ receptor is not activated by either nucleotide di- or triphosphates, such as ATP, UTP, or UDP, and dinucleotide.[5–7]

However, sugar-substituted analogues of UDP, namely UDP-glucose, UDP-galactose, and UDP-*N*-acetyl-glucosamine, are naturally occurring agonists of the $P2Y_{14}$ receptor.[4,8,9] Among these ligands, UDP-glucose is the most potent agonist.

Supporting information available: Calculation details including the MD simulation, automatic docking, and MCMM calculation parameters.

Recently, molecular models of all known subtypes of P2Y receptors, including the $P2Y_{14}$ receptor were published, and general configurations of the binding sites were proposed based on docking studies performed for the $P2Y_1$ and $P2Y_{12}$ receptors.[2] Several amino acid residues were suggested to be critical for ligand binding at both $P2Y_1$-like and $P2Y_{12}$-like receptor families of P2Y receptors.

Concerning the $P2Y_{12}$-like family three cationic residues, namely Arg3.29, Lys located in EL2, and Lys7.36 were proposed to be critical for coordination of the phosphate chain of a ligand. Also, Ser7.43, present in the $P2Y_1$-like family and in the $P2Y_{12}$ receptor, seems to be involved in ligand binding via H-bonding with the nucleobase ring. This position is Ala7.43 in the $P2Y_{13}$ and $P2Y_{14}$ receptors. Tyr1.39, which was found to be involved in ligand recognition in the $P2Y_1$ receptor, is conserved among all P2Y receptors, with the exception of the $P2Y_{11}$ subtype. Tyr2.53 can interact with the native ligands at $P2Y_{1, 2, 4, 6}$ subtypes, while in the $P2Y_{12, 13, 14}$ receptors the corresponding residue is Met2.53. The $P2Y_{12}$-like receptors, especially $P2Y_{13}$ and $P2Y_{14}$ receptors, likely have a different configuration of the binding site or different binding mode of its agonists in comparison to $P2Y_1$-like receptors.[2]

In this study, a molecular model of the human $P2Y_{14}$ receptor was refined by insertion into the phospholipid bilayer followed by 20 ns molecular dynamics simulation, and possible binding modes of four agonists of the $P2Y_{14}$ receptor were studied using automatic docking followed by conformational search analysis.

Homology modeling is one of the most effective methods to study the structure and ligand-receptor interactions of GPCRs. The models obtained using this approach can provide information about residue – residue interactions, configuration of the putative ligand binding site, and possible binding modes of the ligands. However, such models have very similar configurations of the transmembrane domains (TMs) in comparison to the corresponding TMs of the template, usually rhodopsin. Homology modeling often cannot provide an accurate prediction of the configuration of the loops and terminal domains, especially if these sequences are quite long. Moreover, in most cases homology modeling does not take into account the natural environment of the membrane-bound GPCRs, although this is an important factor for the general configuration of a receptor structure and orientation of its sidechains.[10]

In this study, a previously published rhodopsin-based molecular model of the human $P2Y_{14}$ receptor[2] was refined using 20 ns molecular dynamics (MD) simulation in the phospholipid bilayer.

The MD simulation was performed using the protocol developed by Woolf and Roux[11–14] and applied to MD simulation of the $P2Y_6$ receptor by Costanzi et al.[15] This simulation was run on the Biowulf cluster at the NIH (Bethesda, MD) using CHARMM 32a2[16] (details given in Supporting Information). As with the $P2Y_6$ receptor,[15] attempts to generate a MD trajectory without applying nuclear Overhauser enhancement (NOE) restraints led to a loss of the secondary structure of TM7. For this reason the first 5 ns of the MD were performed with the NOE restraints applied for the distances between the backbone carbonyl oxygen atom of the residue n and the backbone NH-group of the residue n+4 of TM7. That constraint preserved the helical structure of TM7, which remained stable after removing the NOE restraints.

The root mean square deviation (RMSD) of all atoms of the $P2Y_{14}$ receptor was calculated from the MD trajectory. As shown in Figure 1 the first plateau of the RMSD was reached after 2.5 ns of MD simulation.

After 5 ns (when the NOE restrains were removed) the value of the RMSD was slightly increased, and after approximately 8.5 ns of MD simulation the structure of the receptor became stable. The typical structure of the last 100 ps of the trajectory was considered as a final structure

of the MD simulation. The C_α-atoms of the transmembrane α-helices of this structure and an initial structure of the $P2Y_{14}$ receptor were aligned with RMSD of 3.1 Å (Figure 2).

It was found that in general both structures have very similar configurations of the TMs. However, the angles along the axes of TM6 and TM7 changed slightly in comparison to the initial structure. The calculated values of the RMS fluctuations (RMSF) allowed us to indicate the residues with positions that were most significantly changed during the MD simulation. Not surprisingly, the highest values of the RMSF corresponded to the residues located in the extracellular (EL) and intracellular (IL) hydrophilic loops and terminal domains, while the residues located in the TM domain had the lowest values of the RMSF (Figure 3). In particular, the greatest change occurred in EL1 and IL3. In addition, during the simulation EL2 was shifted slightly outward from its initial position. However, the configuration of this longest hydrophilic loop was not significantly altered during the simulation.

In addition to a conserved disulfide bridge and H bonds involved in formation of the β-sheet, several additional electrostatic bridges and H bonds were found to form among the residues located in EL2 (Figure 4).

It was found that additional H bonds can be formed between $Arg165_{(NH,\ sidechain)}$ − $Glu166_{(CO,\ backbone)}$, $Lys176_{(NH,\ sidechain)}$ − $Arg165_{(CO,\ backbone)}$, $Arg181_{(NH,\ sidechain)}$ − $Asn161_{(CO,\ backbone)}$. Also, Arg253 (6.55) can interact with Glu174 (EL2), Lys277 (7.35) interacted with Glu166 (EL2), and Glu12 located in the N-terminal domain appeared near Arg165 (EL2). These findings suggest that in the case of the $P2Y_{14}$ receptor, EL2 is a relatively rigid part of the receptor, and its conformation is constrained through a network of specific interactions.

The molecular model of the $P2Y_{14}$ receptor obtained after MD simulation was used for a molecular docking study of UDP-glucose **1**. During the first step, the ligand was docked automatically to the rigid putative binding site using the Autodock program[17] (using parameters given in Supporting Information). This approach allowed the automatic location of the most favorable position and conformation of a flexible ligand, while avoiding radical changes in the receptor structure. The results of the automatic docking were analyzed, and the 3 ligand-receptor complex with the most favorable energy scoring function and most in agreement with available published data,[2,15,18] was selected for further studies. To obtain a more accurate binding mode of UDP-glucose, the complex obtained after the automatic docking was used as a starting point for a Monte Carlo Multiple Minimum (MCMM) conformational search analysis of the ligand inside the putative binding site of the $P2Y_{14}$ receptor. The MCMM calculations were performed using MacroModel software.[19]

It should be noted that for four other subtypes of P2Y receptors the Northern (N) conformation of the ribose ring was proposed as the preferred conformation.[20] In the present study conformers of **1** having both (N)- and (S)-conformations of the ribose ring were examined. The binding modes of (N)- and (S)-UDP-glucose obtained after MCMM calculations demonstrated that in both cases the ether oxygen and the 3′-hydroxyl group of the ribose ring were not involved in H-bonding with the receptor. In contrast, the 2′-hydroxyl group of (N)-UDP-glucose was H-bonded with the backbone oxygen atom of Asn104 (3.35) (Figure 5), while the 2′-hydroxyl group of (S)-UDP-glucose formed a H bond with the sidechain amino group of Asn287 (7.45) and the backbone oxygen atom of Ser284 (7.42).

This means that in the case of (N)-UDP-glucose, the 2′-hydroxyl group could be a H bond donor, while in case of the (S)-conformation it is more likely to be both donor and acceptor.

The uracil ring of (N)- and (S)-UDP-glucose appeared near and oriented toward Tyr29 (1.39), which is a highly conserved residue among all P2Y receptors with the exception of Leu1.39

in the $P2Y_{11}$ receptor. A similar orientation of the uracil ring was previously proposed for other subtypes of P2Y receptors.[2,14,18] The carbonyl oxygen atom at position 4 of the uracil ring was located at a distance of 6.5Å from the Tyr29 hydroxyl group. The carbonyl oxygen atom at position 2 was found at a distance of 4.7Å from the Asn287 sidechain amino group, 6.0Å from its backbone NH-group, and 5.3Å from the backbone NH-group of Val288. The 3-NH group of **1** was oriented toward the sidechains of Val32 and Val288. However, the possible importance of these residues in ligand recognition is yet to be tested experimentally.

In the model, the α-phosphate group of **1** interacted with the hydroxyl groups of Thr280 (7.38) and Ser284 (7.42). Thr7.38 is conserved among all P2Y receptors with the exception of Met7.38 of the $P2Y_{11}$ receptor. Position 7.42 is Ala7.42 in the $P2Y_{1, 2, 4, 6}$ and $P2Y_{13}$ receptors, Met7.42 in the $P2Y_{11}$ receptor, and Thr7.42 in the $P2Y_{12}$ receptor.

It was proposed previously[2] that the ligand phosphate chain is located among three cationic residues, namely Arg3.29, Arg6.55, and Arg7.39 for $P2Y_{1, 2, 4, 6, 11}$ receptors, and Lys from the EL2, Arg6.55, and Lys7.35 for $P2Y_{12, 13, 14}$ receptors. The importance of these residues in $P2Y_1$ and $P2Y_2$ receptors was demonstrated experimentally.[2,21] The binding mode of **1** obtained in the present study demonstrated that in the case of the $P2Y_{14}$ receptor only Lys171 located in EL2 can interact with the phosphate chain of **1**. Arg6.55 and Lys7.35 appeared far from the phosphate groups and were located near the hexose moiety of the ligand. Moreover, the sidechain amino group of Lys7.35 was located between hydroxyl groups at the 5 and 3 positions of the glucose ring and formed H-bonds with both these groups. Also, the 3-hydroxyl group interacted with Glu166, while the 5-hydroxyl group seemed to be H-bonded with Glu174 located in EL2. Interestingly, Glu174 of the $P2Y_{14}$ receptor corresponds to Asp204 of the $P2Y_1$ receptor located in EL2 at a site two residues after the conserved Cys. It was demonstrated using molecular modeling[22,23] that Asp204 of the $P2Y_1$ receptor is located within 5Å from the triphosphate chain of ATP. Based on that model and mutagenesis data[24] the involvement of Asp204 in ligand recognition through a water molecule bridge was proposed. An important role of the corresponding Asp was also suggested in molecular modeling studies performed for the $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors.[15,18] Moreover, Glu767 of the human Ca^{2+} receptor[25] and Tyr181 of the THR-receptor[26] (both also located in EL2 at a site two residues after the conserved Cys) were shown to be crucial for receptor activation. In the model obtained, the ether oxygen atom and the 4- and 2-hydroxyl groups of the glucose moiety were not directly involved in H bonding with the receptor. As shown in Figure 5, the conformation of the hexose ring obtained automatically after the docking studies is different from the more stable alternate chair conformation of such rings. It could be proposed that the obtained conformation of the hexose moiety is stabilized inside the binding site by specific interactions with the receptor, namely by H-bonds with Arg253 (6.55), Lys277 (7.35), Glu166 (EL2), and Glu174 (EL2).

In order to explain experimentally observed differences in the activity of UDP-glucose **1**, UDP-galactose **2**, UDP-glucuronic acid **3**, and UDP-*N*-acetylglucosamine **4** (Table 1), the three latter ligands were also subjected to MCMM calculations.

For this purpose, the hexose ring of **1** was converted to a galactose or glucuronic acid moiety directly in the binding site. It allowed a ligand orientation inside the binding site similar to the orientation obtained for UDP-glucose. MCMM calculations were subsequently performed for **2** – **4** docked inside the $P2Y_{14}$ receptor.

The general position of the ligands inside the binding site was unchanged during the MCMM calculations. All ligand-receptor interactions found for the UDP moiety of **1** were also observed for analogues **2** – **4**. In the case of **3** one additional H-bond was found to form between Glu174 and the 4-hydroxyl group of the ligand, while in the case of **2** additional H-bonds between the hexose moiety and the receptor were not observed. However, the 4-hydroxyl group of **3** and a

hexose carbonyl oxygen atom of **2** were unfavorably located in proximity to the hydrophobic sidechain of Ile173 located in EL2.

When the least potent agonist **4** was docked in the $P2Y_{14}$ receptor, H-bonding was not observed between the receptor and the acetamide group of the ligand. In contrast, due to the short distance between the methyl groups of **4** and Met3.36, the sidechain of Met3.36 shifted away from the ligand. In addition, the methyl group of **4** was found close to the backbone oxygen atom of Phe3.32.

In conclusion, compared to other P2Y receptors, the $P2Y_{14}$ receptor has an atypical binding mode of the nucleobase, ribose and phosphate moieties. The ligand binding modes obtained in this study demonstrated that two key cationic residues, namely Arg6.55 and Lys7.35, together with two anionic residues (Glu166 and Glu174 located in EL2), can interact with the hexose moiety. A third conserved key cationic residue, namely Lys171, interacts with the α-phosphate group of the ligand. The lower activity of **4** and its receptor docking suggest limited steric tolerance at the 2 position of the hexose moiety.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Stefano Costanzi (NIDDK) and Addison Ault (Princeton University) for helpful discussions. This research was supported by the Intramural Research Program of the NIH, National Institute of Diabetes & Digestive & Kidney Diseases.

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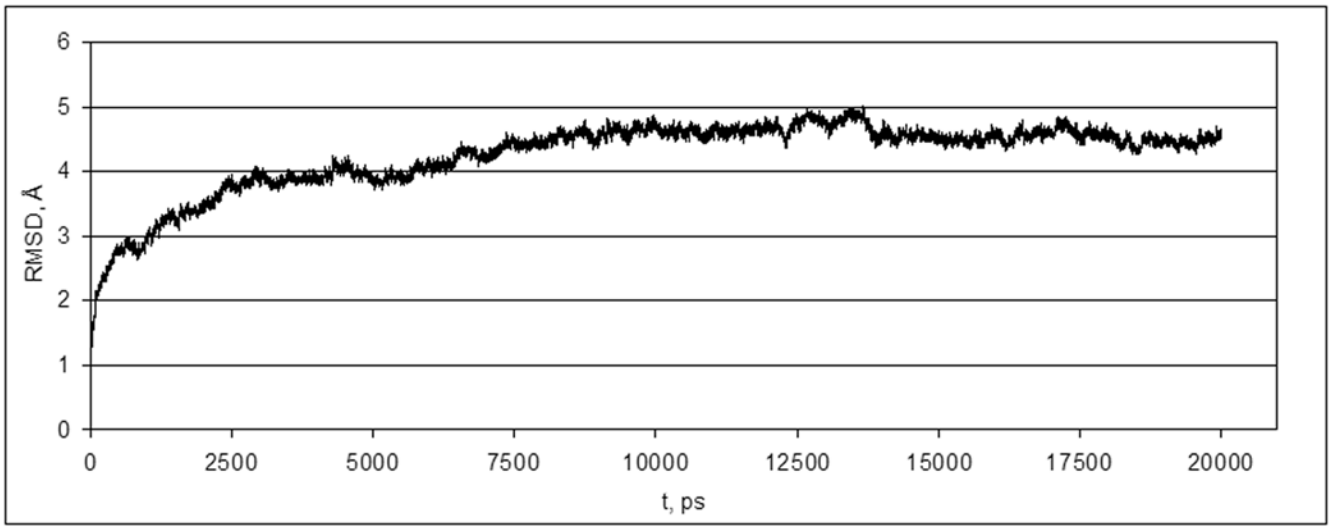


Figure 1.
Changes in RMSD of atoms of the $P2Y_{14}$ receptor during the MD simulation.

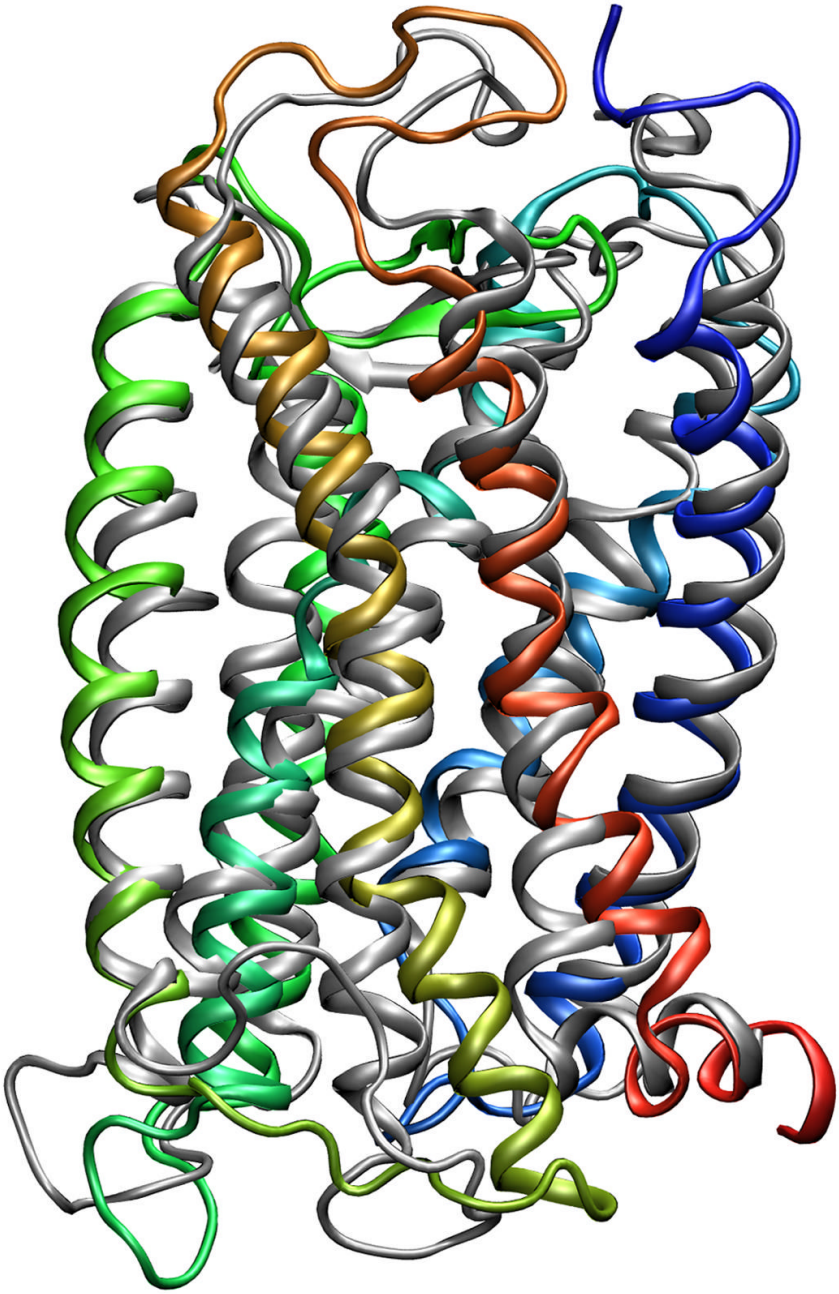


Figure 2.
The alignment of the initial structure of the $P2Y_{14}$ receptor (grey) and the structure obtained after MD simulation (colored). TMI – blue; TMII – light blue; TMIII – cyan; TMIV – lime green; TMV – green; TMVI – orange; TMVII – red.

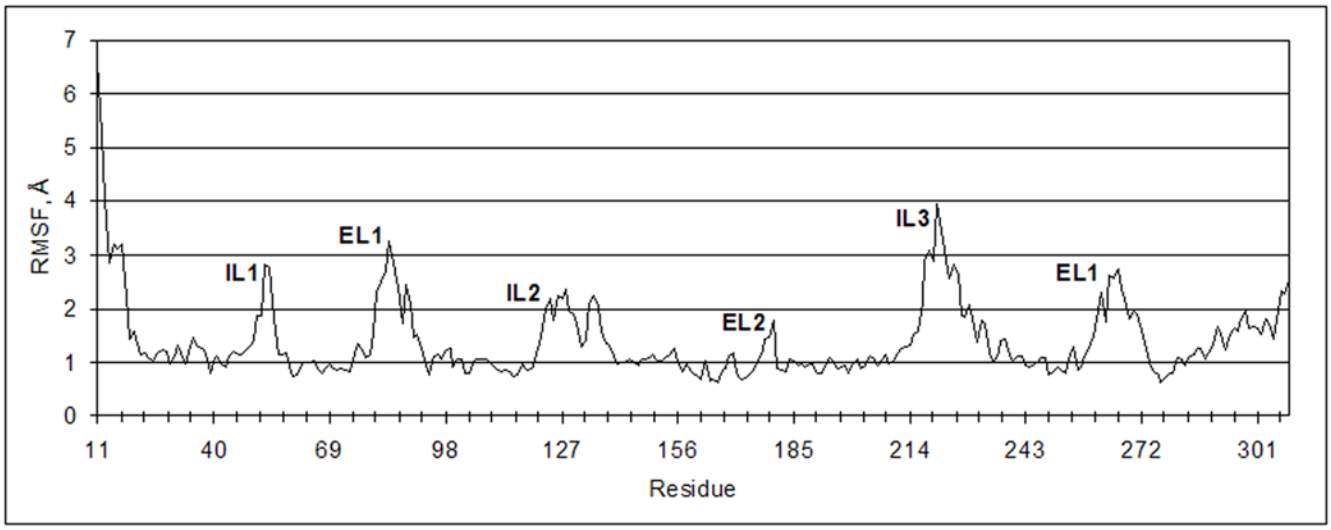


Figure 3.
RMSF of the C_α-atoms of the $P2Y_{14}$ receptor.

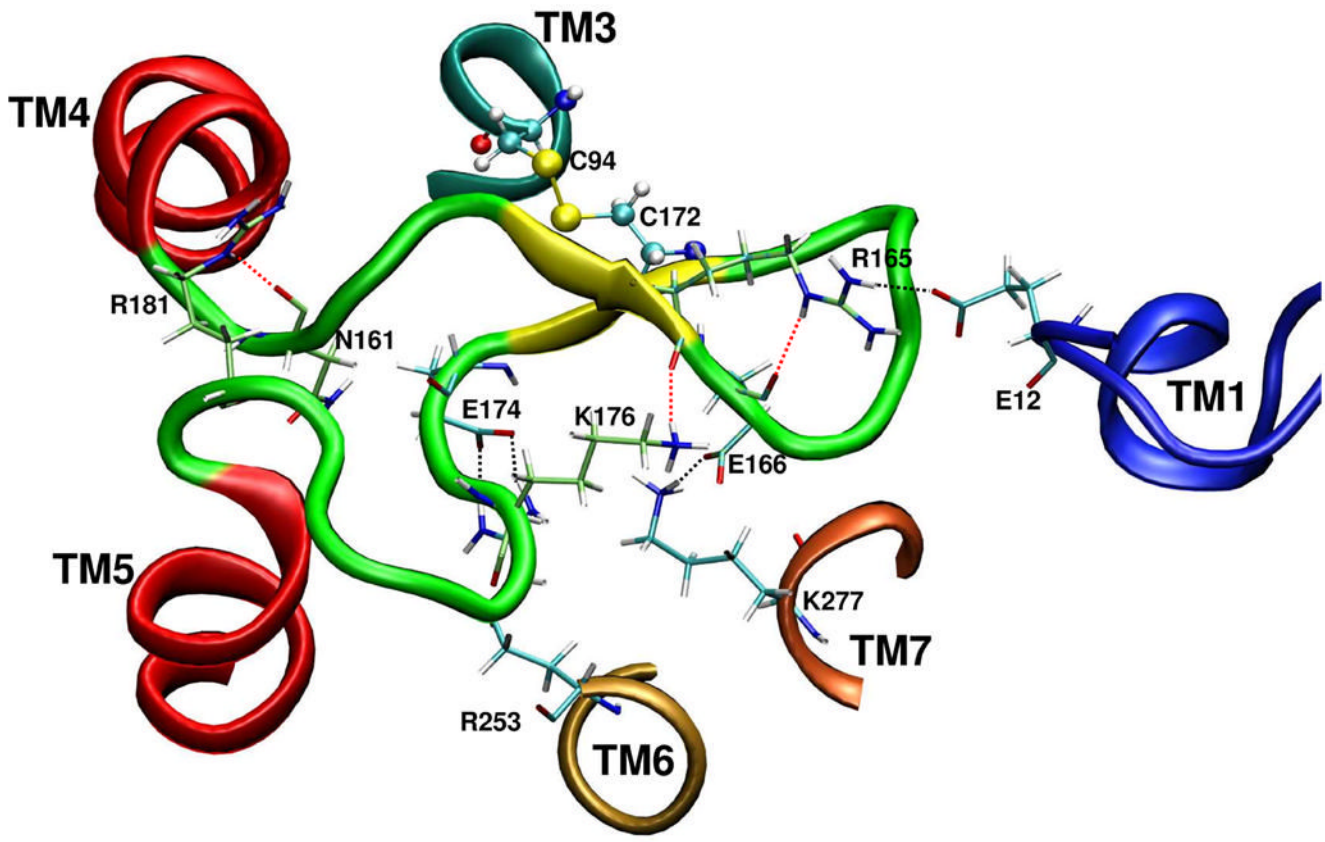


Figure 4.
The residue – residue bridges served to constrain the configuration of the EL2.

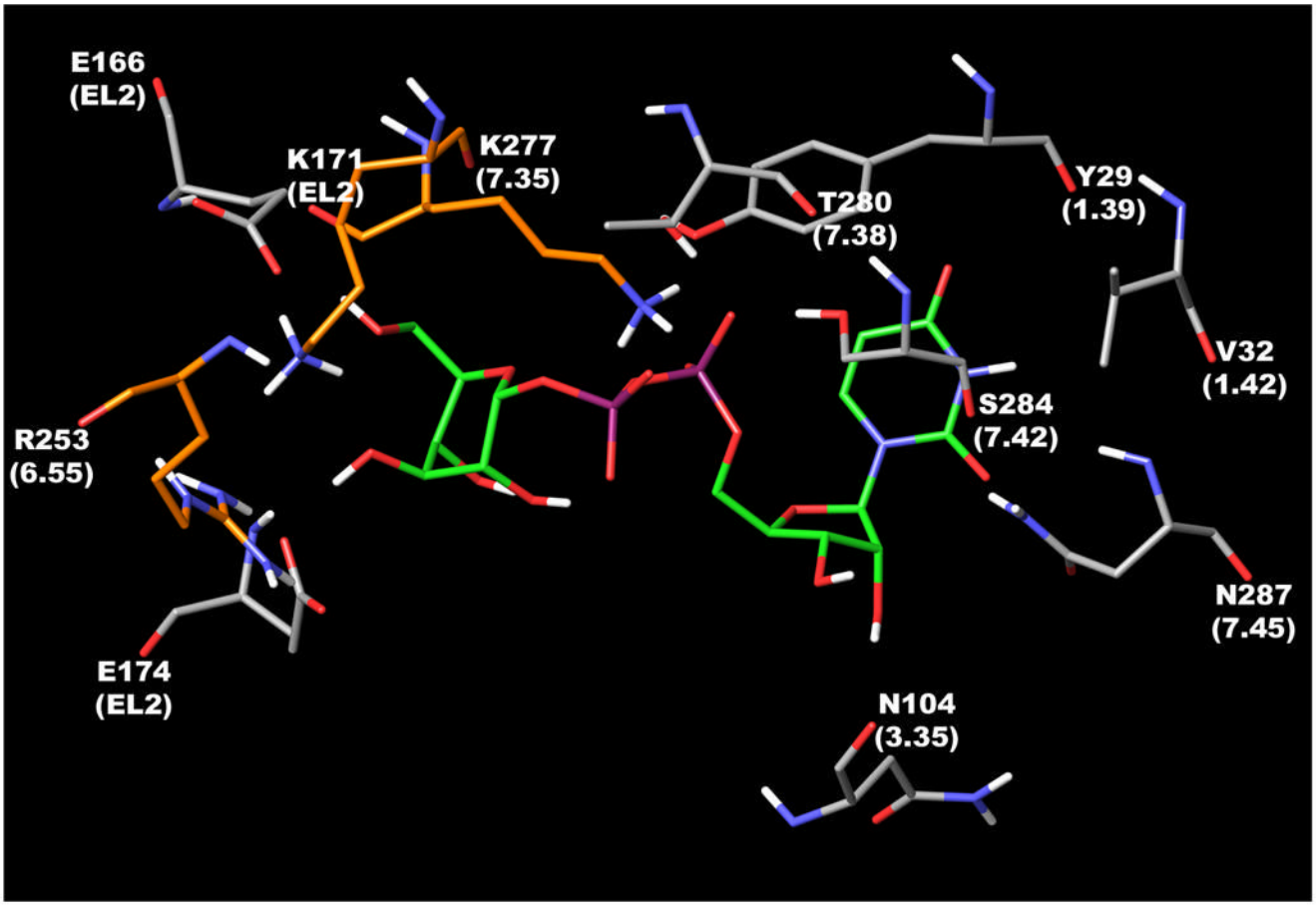
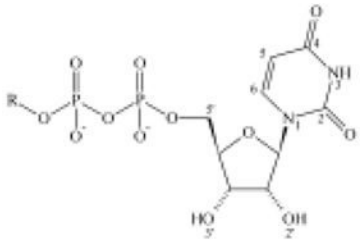
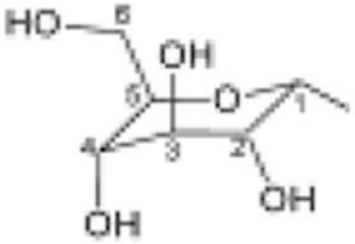
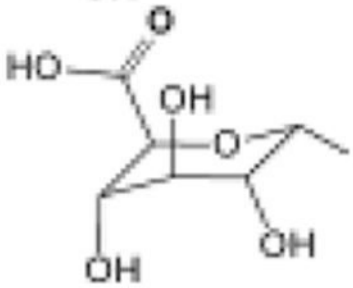
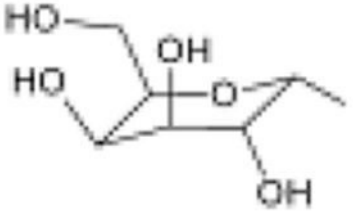
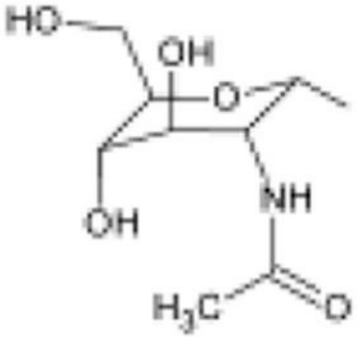


Figure 5.
The binding mode of the UDP-glucose with the $P2Y_{14}$ receptor obtained after MCMM calculations. Three key cationic residues, namely Lys171, Arg253, and Lys277 are colored in orange.

Table 1

Agonist potencies at the human $P2Y_{14}$ receptor. The experimental values of EC_{50} for stimulation of PLC (n = 6) were determined in COS-7 cells expressing the receptor (and an engineered G protein for coupling to PLCβ) were obtained as previously described.[8]



Ligand	R	EC_{50}, nM
1, UDP-glucose		354±115
2, UDP-glucuronic acid		370±69
3, UDP-galactose		671±90
4, UDP-*N*-acetyl-glucosamine		4380±1050