Supporting Information

Molecular Modeling of the Human $P2Y_2$ Receptor and Design of a Selective Agonist, 2′-Amino-2′-deoxy-2-thio-UTP

Andrei A. Ivanov, Hyojin Ko, Liesbet Cosyn, Savitri Maddileti, Pedro Besada, Ingrid Fricks, Stefano Costanzi, T. Kendall Harden, Serge Van Calenbergh, Kenneth A. Jacobson[a]

Formal geometry of the $P2Y_2$ receptor model

The formal geometry of the $P2Y_2$ receptor model obtained after insertion of N- and C-terminal domains was tested with the ProTable command of Sybyl as well as the Procheck software.[1] Only 2% of the residues were located in disallowed regions of the Ramachandran plot; 78.2% and 16.9% of the residues were found in the most favored and in additional allowed regions, respectively, demonstrating the good quality of the molecular model.

Analysis of the Ramachandran plot performed for the $P2Y_2$ receptor model obtained after MD simulation indicated that the quality of the model significantly improved during the simulation. Thus, 83.5% of the residues were found to be in the most favorable region, 14.5% appeared in additional allowed regions, 1% were located in generously allowed regions, and 1% were found in disallowed regions. In particular, Y149 (4.41) and F171 (EL2), which are both located far from the putative ligand-binding site, were found in the disallowed region.

Selective Recognition for Nucleoside Tri- and Diphosphates at $P2Y_2$ and $P2Y_6$ Receptors

Although UDP is almost inactive at the $P2Y_2$ receptor,[2] it is the cognate agonist of the $P2Y_6$ receptor. To explain these differences, we compared residues of the $P2Y_2$ receptor directly involved in ligand interactions with the corresponding residues of the $P2Y_6$ receptor. Only three of these residues are not conserved in these two receptor subtypes. R6.55 of the $P2Y_2$ receptor corresponds to K6.55 of the $P2Y_6$ receptor, Y6.59 of the $P2Y_2$ corresponds to L6.55 of the $P2Y_6$ receptor, and H184 of the $P2Y_2$ receptor, located in EL2 directly after the conserved Cys, corresponds to Y178 of the $P2Y_6$ receptor. In the $P2Y_2$ receptor docking models obtained, both Y6.59 and H184 are located near the γ-phosphate group and are involved in H-bonding with this group. However, these ligand-receptor interactions were lost for UDP docked in the $P2Y_2$ receptor; all other interactions found for UTP were also observed in the $P2Y_2$-UDP complex (Figure 3).

Although some UTP analogues e.g., 5-bromo-UTP[3] and some dinucleoside triphosphates e.g., Up_3U[4] activate the $P2Y_6$ receptor, UTP itself is a weak or inactive agonist. Therefore, we built a chimeric $P2Y_2/P2Y_6$ receptor model in which the residues R6.55, Y6.59, and H184 in the model of the $P2Y_2$ receptor were replaced by corresponding residues of the $P2Y_6$ subtype: Lys, Leu, and Tyr, respectively. The molecular complex of UTP with this chimeric $P2Y_2/P2Y_6$ receptor (Figure 4) localized the methyl groups of L6.59 far from the ligand, indicating that these are not involved in any unfavorable interactions with UTP. In contrast, the UTP γ-phosphate group was localized between hydrophobic CH-groups of the tyrosine ring and CH_2-groups of K6.55, and no H-bonds were observed between the receptor and the γ-phosphate group.

These results allow us to propose that residues R/K6.55, Y/L6.59, and H184/Y178 of the $P2Y_2/P2Y_6$ receptors play a critical role in UTP versus UDP recognition. This hypothesis will be tested in future experiments with site-directed mutagenesis.

References

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2. Brunschweiger, A.; Müller, C.E. P2 receptors activated by uracil nucleotides--an update. *Curr. Med. Chem.* **2006**, *13*, 289-312.

3. El-Tayeb, A.; Qi, A.; Müller, C.E. Synthesis and structure-activity relationships of base-modified UDP and UTP analogues at the human $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors. *Purinergic Signalling* **2006**, *2*, 304-305.

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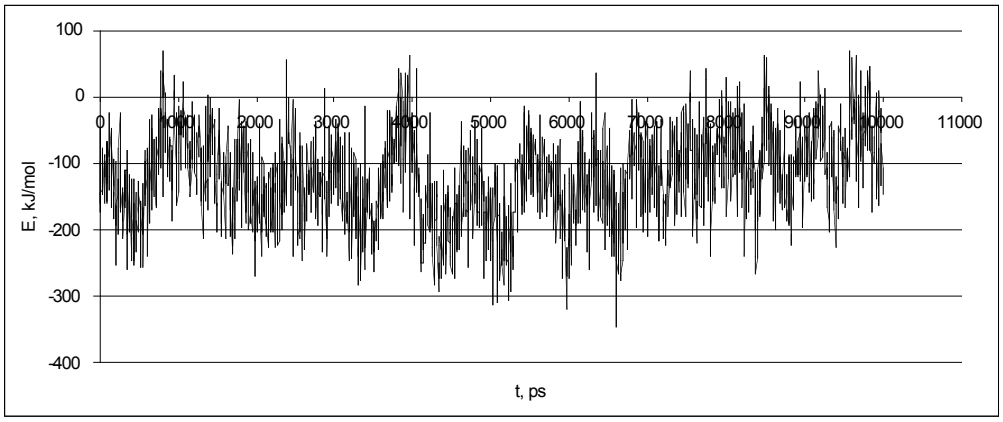
Contents:

a)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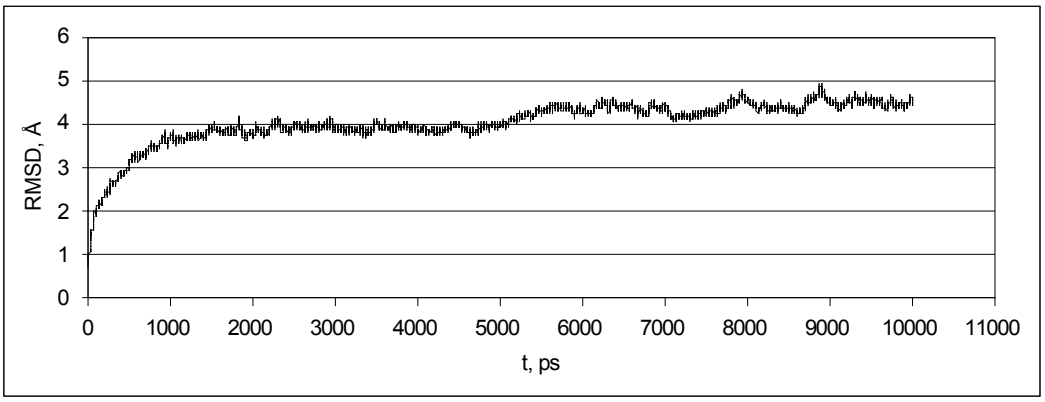
11000
t, ps

b)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RMSD, Å
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t, ps

Figure 1.

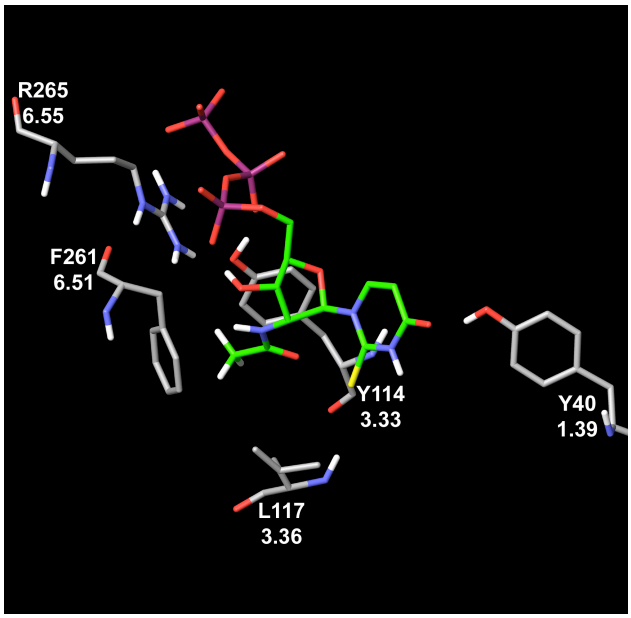


Figure 2.

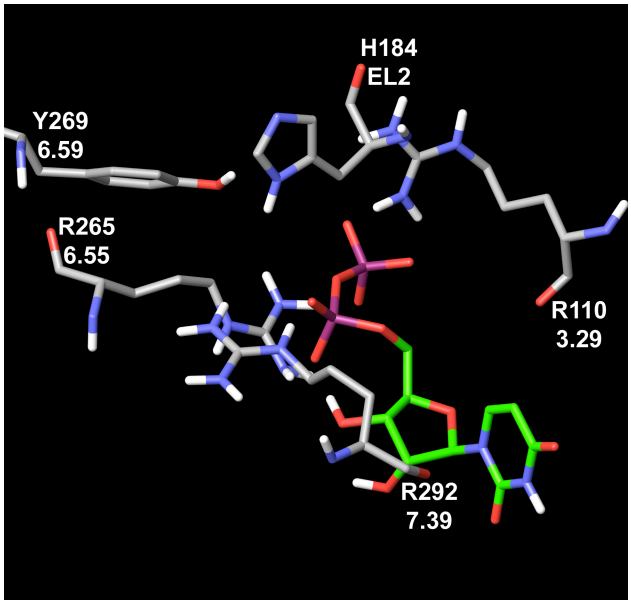


Figure 3.

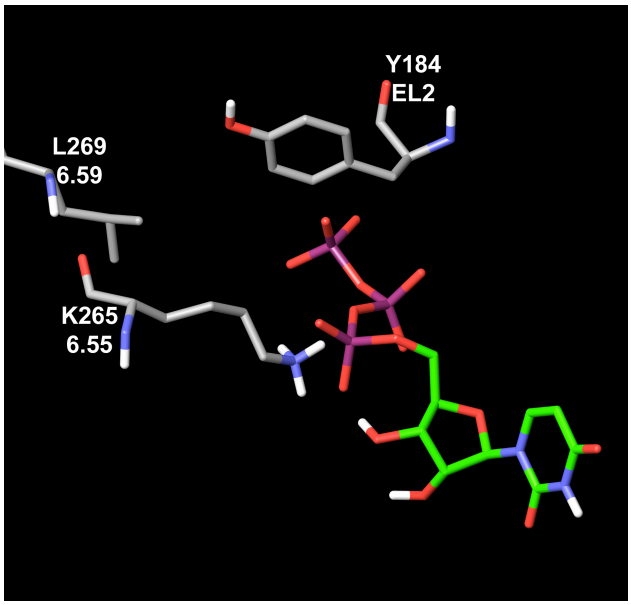
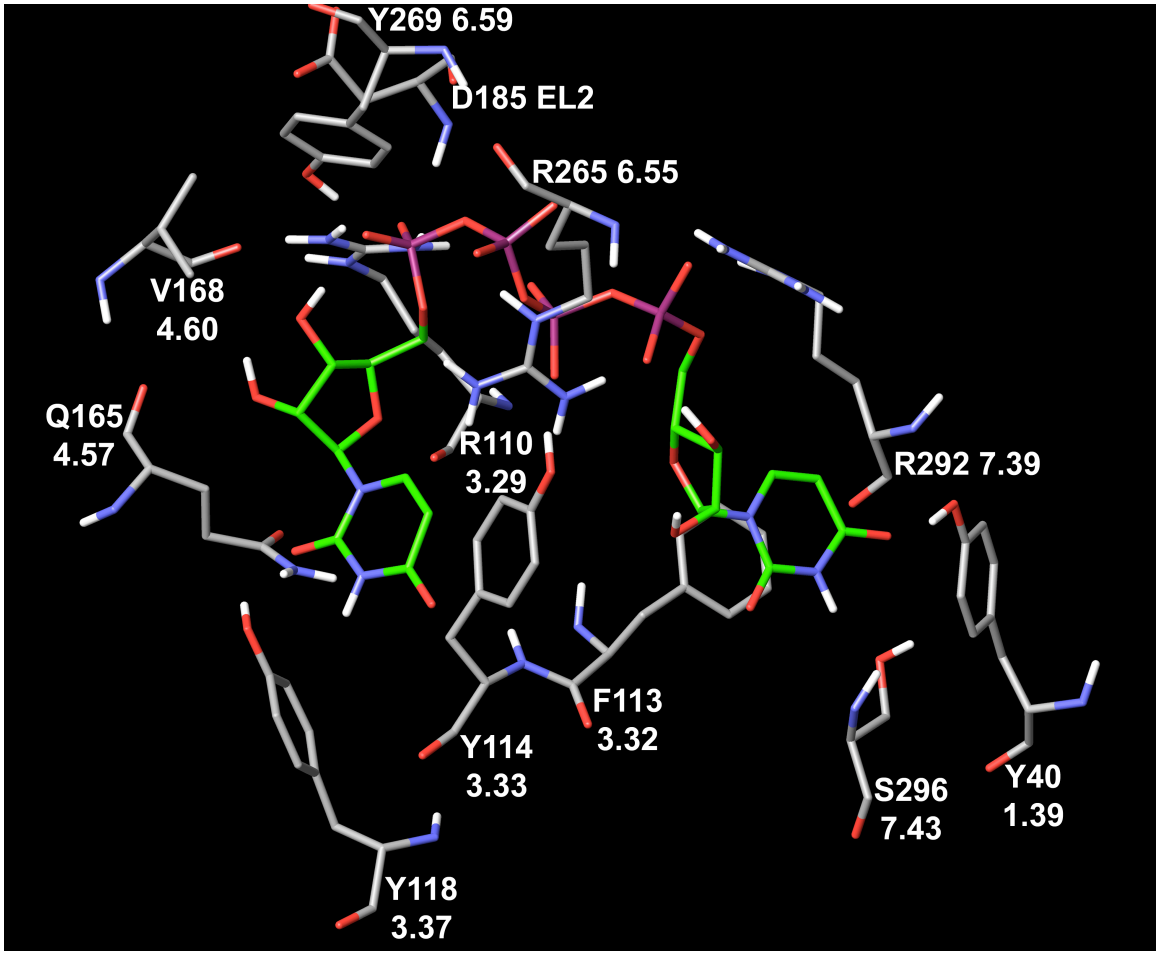


Figure 4.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D185 EL2
R265 6.55
V168
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Q165
4.57
R110
3.29
R292 7.39
F113
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Y114
3.33
S296
7.43
Y40
1.39
Y118
3.37

Figure 5

Table 1. Purity of target compounds measured by HPLC analysis.[a]

Compound No.	Purity (%)	Retention Time	Solvent System[b]
8	> 98%	15.2 min	A
	> 98%	6.6 min	B
9	> 98%	18.3 min	A
	> 98%	7.7 min	B
10	> 98%	17.2 min	A
	> 98%	6.7 min	B
4a	> 98%	20.5 min	A
	> 98%	7.7 min	C
5	> 99%	18.6 min	A
	> 99%	7.1 min	C

[a] Hewlett–Packard 1100 HPLC equipped with a Zorbax Eclipse 5 μm XDB-C18 analytical column (250 × 4.6 mm; Agilent Technologies Inc., Palo Alto, CA).

[b] System A: 5 mM TBAP (tetrabutylammonium dihydrogenphosphate)-CH_3CN from 80:20 to 40:60 in 20 min; flow rate 1 mL/min. System B: 10 mM TEAA (triethylammonium acetate)-CH_3CN from 80:20 to 60:40 in 20 min; flow rate 1 mL/min. System C: 10 mM TEAA (triethylammonium acetate)-CH_3CN from 100:0 to 80:20 in 20 min; flow rate 1 mL/min.