REVIEW

ATP binding and channel activation in P2X receptors

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Purinergic P2X receptors are a family of membrane channels activated by extracellular adenosine 5′-triphosphate. They are valuable therapeutic targets primarily for their critical role in neuropathic pain and inflammation. ATP binds allow Na^+ and Ca^{2+} to pass through the open pore, and leads membrane depolarization and an increase of the cytosolic Ca^{2+} concentration that initiating Ca^{2+}-dependent signaling transduction. A concerted effort by investigators over the last two decades has culminated in significant advances in our understanding of where ATP binds and how channels gating. The recent publication of the crystal structures of the zebrafish P2X4 receptor adds something new on how P2X receptors work. In this review, we will attempt to present the existing functional data regarding ATP binding with the available crystal structure data and different experimental approaches that have been used to explore the ATP-binding sites.

Keywords: P2X receptors; ATP binding sites; mutagenesis; ectodomain movements

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Purinergic P2X receptors (P2XRs) are a family of membrane channels activated by extracellular adenosine 5′-triphosphate (ATP) [1]. In the absence of ATP, the channels are close. When they are exposed to ATP, the channels undergo a series of conformational changes and are active, which allows the entry of Ca^{2+} and Na^+. The P2XR has seven subtypes, which expressed in numerous types of cells [2]. Preclinical research programs are studying the P2XR currently, which has been shown to play key roles in pathological conditions, including neurological disorders [3], cancer [4] and inflammatory reactions [5]. And several pharmaceutical companies are now pursuing P2XRs as new drug targets for neuropathic pain or pain caused by inflammation [6].

First isolated in 1994 [7], P2XRs showed different pharmacological characteristics between different subtypes. The last two decades after the cloning era of purinergic receptors has seen an explosion of new information about the channel structure and function. Molecular biological techniques and patch clamp electrophysiology studies had previously identified P2XRs are form as trimers of homomers or heteromers [8] with each subunit composed of two transmembrane α-helices that are separated by a large extracellular loop [9]. Recently, the successful crystallization of a zebrafish P2X4 receptor (zfP2X4), in the absence [10] and presence [11] of ATP (PDB entry codes: 4DW0 and 4DW1) provides an insight into the mechanism of channel activation. This review focuses entirely on the ATP binding and conformational changes accompanying agonist binding.

ATP Binding Sites

Different from other nucleotide binding proteins, representative peptide segments that generally occured in nucleotide-binding sites [12] are not found in P2XR.

Table 1. Functional study of residues involved in ATP binding

Domain	Receptor	Mutations	Corresponding residues in zfP2X4R	Approaches	References
Body	rP2X2	I67, K69, K71	T68, K70, K72	MTS cross-linking	[16]
Body	**hP2X1**	K68, F185, F291, R292, K309	K70, F188, F297, R298, K316	Partial agonists BzATP and Ap_5A binding	[14]
Body	rP2X4	D280, F230, K190, H286, R278	D283, F233, K193, N289, R281	Homology modeling and site-directed mutagenesis	[20]
Body	rP2X4	K313, Y315	K316, Y318	Site-directed mutagenesis	[34]
Body	**hP2X1**	N290, F291, R292, K309	N296, F297, R298, K316	Radiolabeled 2-azido ATP binding, MTS cross-linking	[15]
Body	**hP2X3**	K65, R281	K72, R298	Site-directed mutagenesis	[18]
Domain	Receptor	Mutations	Corresponding residues in zfP2X4R	Approaches	References
Body	rP2X4	K67, K313, R295	K70, K316, R298	Site-directed mutagenesis and ivermectin modulator	[35]
Body	**hP2X2**	K69, K71, F183, T184, N288, F289, R290, K307	K70, K72, F188, T189, N296, F297, R298, K316	MTS cross-linking	[17]
Body	rP2X4	K67, K69, F185, T186, N293, F294, R295, K313	K70, K72, F188, T189, N296, F297, R298, K316	MTS cross-linking	[17]
Body	**hP2X1**	K70, K68, F92	K72, K70, I94	Homology modeling; Partial agonists BzATP, Ap_5A binding; MTS cross-linking	[36]
Domain	Receptor	Mutations	Corresponding residues in zfP2X4R	Approaches	References
Body	**hP2X3**	K63, K65, G66, T172, F174, K176, N177, N279, F280, R281, K284, R295, K299	K70, K72, G73, T189, L191, K193, N194, N296, F297, R298, K301, R312, K316	Two-electrode voltage clamp; Ca^{2+} imaging	[19]
Body	rP2X2	L186	L191	NCS-ATP affinity labeling	[25]
Head		N140	D145		
Left flipper	rP2X3	S275	A292	Site-directed mutagenesis	[37]
Dorsal fin	rP2X4	L214	L217	MTS cross-linking	[23]

Functional studies identified the ectodomain eight highly conserved residues (Lys70, Lys72, Phe188, Thr189 from one subunit, and Asn296, Phe297, Arg298, Lys316 from another, zebrafish P2X4 numbering) are directly involved in ATP binding. Abundant evidence especially the methanethiosulfonates (MTS) compounds crosslinking studies (see Table 1) have previously confirmed substitutions at these eight positions destroy ATP sensitivity [13-20].

Seen from the X-ray crystallographic structures, these crucial amino acids turned out to be projected to the binding pocket [11]. The vast extracellular domain extends ~70 Å and the ATP binding pocket locates ~45 Å from the cell surface [11]. The architecture of each P2XRs subunits looks like the shape of a dolphin [10, 11]. The extracellular body, head, left flipper, and dorsal fin domains surround the ATP binding cavity (Figure 1). ATP that is positioned at the subunit interfaces adopts an U-shaped conformation [11].

1)Negatively charged α, β, and γ phosphates groups form salt bridges with the side-chain positively charged nitrogen of K70 (strictly conserved, lower body of subunit A, contacts with the α, β, and γ-phosphate, K67 in rP2X4R, Figure 1), K72 (strictly conserved, lower body of subunit A, contacts with the γ-phosphate, K69 in rP2X4R), N296 (strictly conserved, upper body of subunit B, contacts with β-phosphate, N293 in rP2X4R), R298 (strictly conserved, upper body of subunit B, contacts with the γ-phosphate, R295 in rP2X4R), and K316 (strictly conserved, upper body of subunit B, contacts with the β- and γ-phosphate, K313 in rP2X4R), consistent with previous studies that anticipated a direct involvement of these residues in agonist binding [21]. These ionic interactions with β- and γ-phosphate groups may provide an explanation why P2X receptors preferentially accommodate ATP over ADP and AMP [22];

2)The adenine base lies deeper in the ATP-binding cavity and forms multiple hydrogen bonds with the K70, T189 (strictly conserved, lower body of subunit A, T186 in rP2X4R), L191 (lower body of subunit A, L188 in rP2X4R)

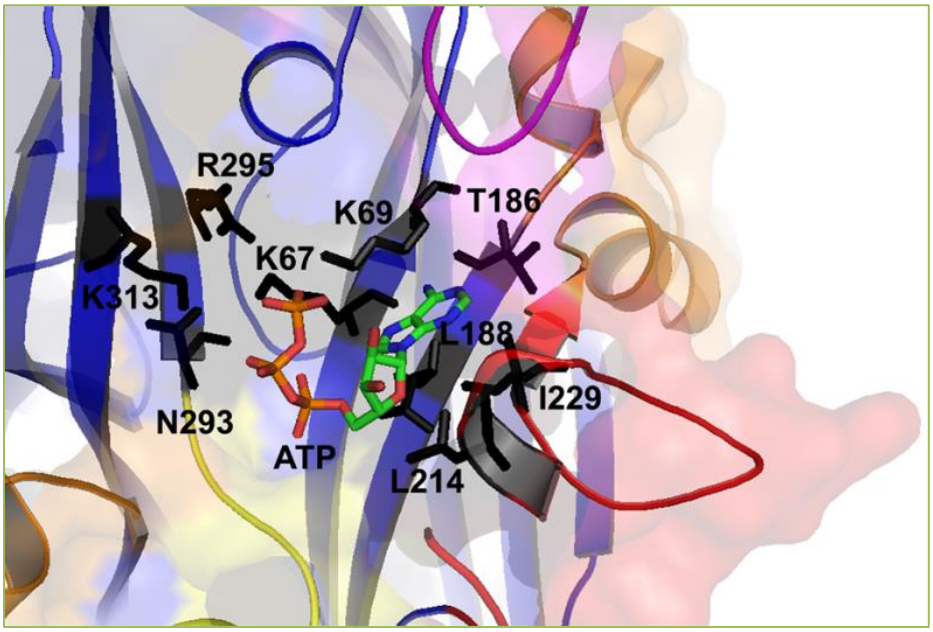


Figure 1. The ATP binding pocket. A detailed view of residues involved in direct interactions with ATP is shown from homology model of the rP2X4R, based on PDB 4DW1. The ATP molecule (shown as sticks) is surrounded by the extracellular head (pink), left flipper (yellow) of one subunit, dorsal fin domain (red) of another subunit and the body. Right flipper (orange).

and I232 (dorsal fin of subunit A, I229 in rP2X4R) [11]. The direct hydrogen bonding interactions between backbone and side-chain oxygen of T189 and N1 and N6 nitrogen atoms of adenine account for the base specificity, explaining why P2X receptors selectivity of P2X receptors for ATP, rather than CTP, GTP, and UTP [22];

3)The two oxygen atoms (O2 and O3) of the ribose ring make hydrophobic contact with L217 (dorsal fin of subunit A, L214 in rP2X4R), which is also proved by our recent functional studies [23, 24]. Mutant receptors L217C caused a larger right shift in the ATP concentration-response curve. What's more, a negatively charged MTS modification completely inhibited the L217C current induced by ATP, indicating L217 is oriented in the ATP-accessible binding cavity proper [24]. The dramatic change in ATP sensitivity was also observed in substitutions of L214 of rP2X4R. Disruption the non-polar hydrophobic interaction by introducing serine mutation markedly attenuated ATP potency [23]. The ATP binding cavity can be also occupied by ATP analogues such as TNP-ATP (whose ribose moiety was added a trinitrophenyl group). Mutant receptors L214A has been shown to reduce the inhibitory effect and contribute to the reversible block by TNP-ATP [23].

However, recent two experiments by irreversibly forming covalent bonds between single cysteine substitutions and sulfhydryl-reactive compound (NCS-ATP) of the P2X2R [25] and by predicting conformational motions through computational approach [26] suggest the existence of more than one ATP binding modes. What is more, different ATP binding orientations might represent distinct functional states. Current evidence speculate that if the adenine ring is proximal to the cell surface with the ATP entering into its pocket, the receptor is active; otherwise, a closed, potentially desensitized state is dominant if the adenine ring is distal to the cell surface. It raises a question. Does the ATP adopt the only conformation during the channel activation? That is to say, besides the mode showed in crystal structure, whether the second ATP-bound conformation exists or not. These probably need additional studies.

Movements of the binding jaw from close to open

Comparing the closed [10] and open [11] structures indicates that ATP binding drives several substantial rearrangements. At the binding site, ATP binding first disrupts its original steady electrostatic interactions, promotes pocket closure, which results in the head domain descends and dorsal fin domain rises [11]. That is to say, agonist binding contracts the original open jaws, which has been previously suggested by the approach of engineering metal bridges between H120 (located at the head domain) and H213 (located at the dorsal domain) (rat P2X2 numbering) [27, 28]. At the same time, the left flipper is pushed out from the pocket and far away from the dorsal fin domain of adjacent subunit [24]. Together, with the dorsal fin domain up and the left flipper outward of intrasubunit, a lateral pull is exerted on the lower body,

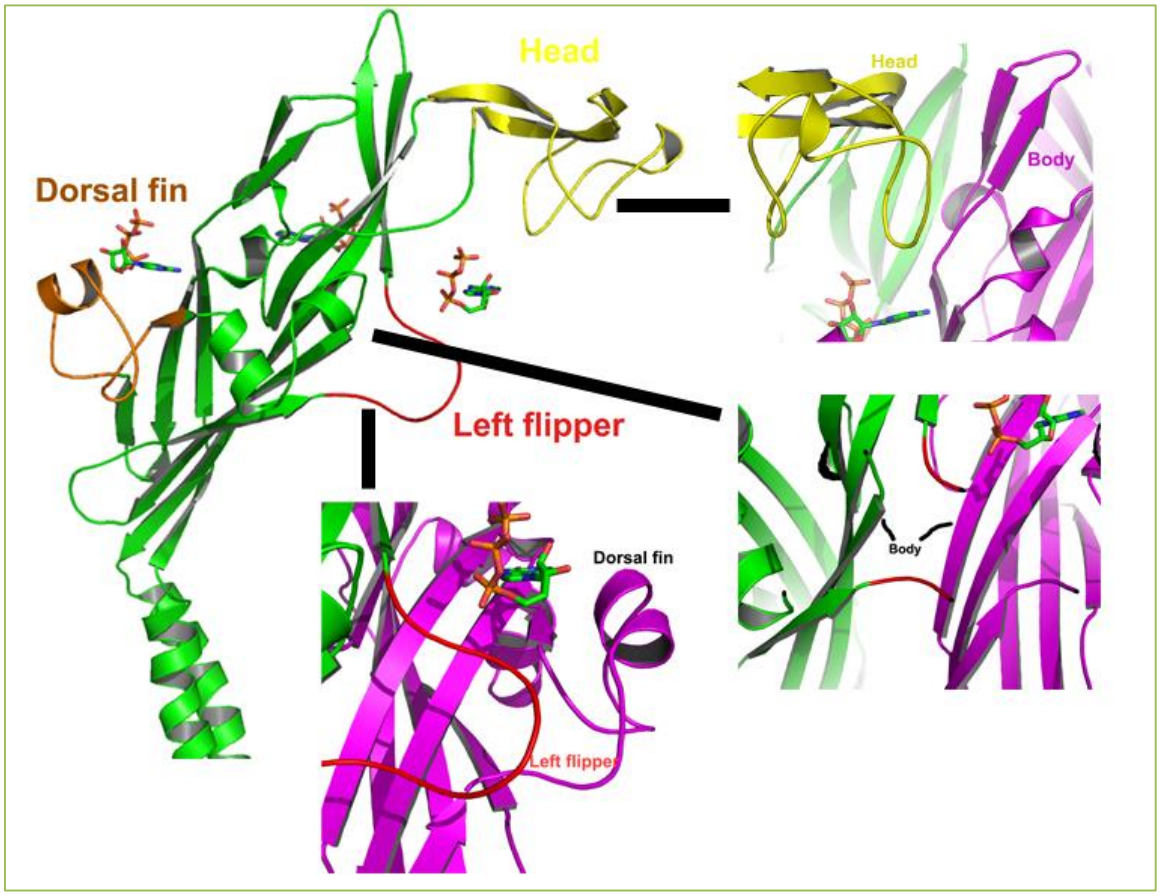


Figure 2. Subunit-subunit interaction. Left, one subunit is shown. Three Panels depict sites of significant interaction with the neighboring subunit (colored pink): head–body, left flipper–dorsal fin, and body–body (labeled respectively).

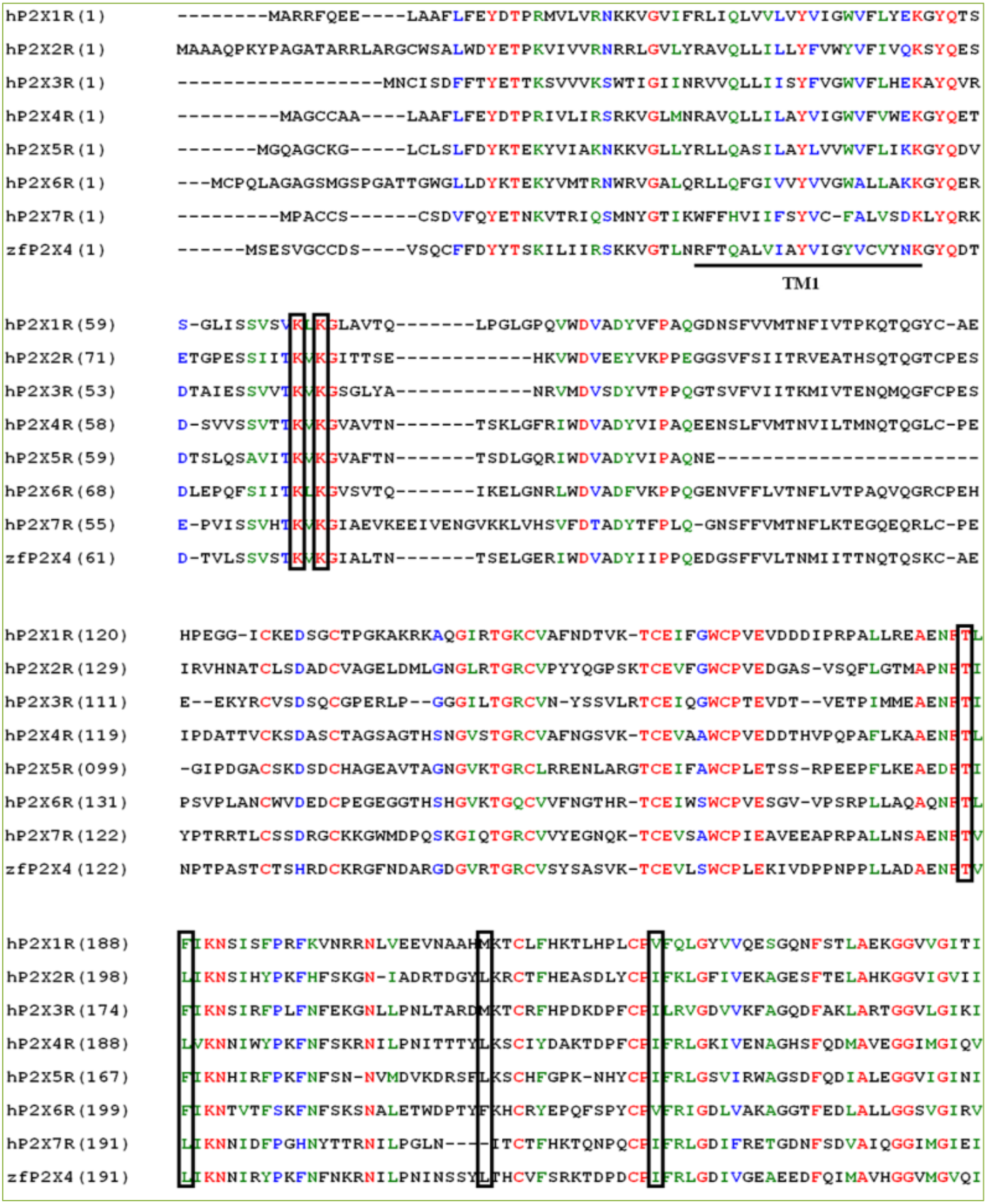
markedly enlarging the extracellular vestibule. Finally, this expanding conformation change of the lower body spreads and sets the out ends of the three TM2 helices apart [29]. The resulting relative rotations of TM domains decrease the spaces between TM1 and TM2 domains, thus enlarge the narrowest central pathway of ion conduction and open the pore, which have been further strengthened by our [30] and Swartz' group [31] recent studies upon the zfP2X4 structure.

Relative movements between subunits especially at the subunit interface during closed to open process have been suggested by many investigations. The main contacts between subunits in the extracellular domains exist at the head-to-body, body-to-body and left-flipper-to-dorsal-fin (Figure 2) [10]. The latter two interactions have been proved to be involved in channel activation. Cysteines introduced into the body domain [29, 32] or the outer ends of TMs [29] restrict the relative movement of adjacent subunits and prevents the ATP-induced channel opening. Along similar lines, engineered disulfide crosslinking and zinc bridges between V288 (located at the left flipper) and T211 (located at the dorsal fin domain) in rat P2X4R significantly influenced the activation of the receptor [33]. Deleting the nine residues of the left flipper in zfP2X4R disrupted ATP efficiency [24]. All of these results are consistent with the movement predicted from the crystal structures.

Conclusions

In this review, the existing functional studies regarding ATP binding with the available crystal structure data have been emphasized. In human, the amino acid involved in ATP binding are strictly conserved compared with zebrafish P2X4R (Figure 3). The ATP signaling through P2XR plays an important part in the nervous system such as functioning of the five senses [6]. The 3D structure provides a global picture on the agonist binding and channel activation, which can favor the R&D of novel drugs. The aid of structure-based small molecule design tools will provide a better opportunity to develop new small molecules targeting into P2XR. For long term progress, it is best to obtain other P2X structures and find subtype-specific small molecules sites.

Acknowledgments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hP2X7R(1)
zfP2X4(1)
TM1
hP2X1R(59)
hP2X2R(71)
hP2X3R(53)
hP2X4R(58)
hP2X5R(59)
hP2X6R(68)
hP2X7R(55)
zfP2X4(61)
hP2X1R(120)
hP2X2R(129)
hP2X3R(111)
hP2X4R(119)
hP2X5R(099)
hP2X6R(131)
hP2X7R(122)
zfP2X4(122)
hP2X1R(188)
hP2X2R(198)
hP2X3R(174)
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zfP2X4(191)

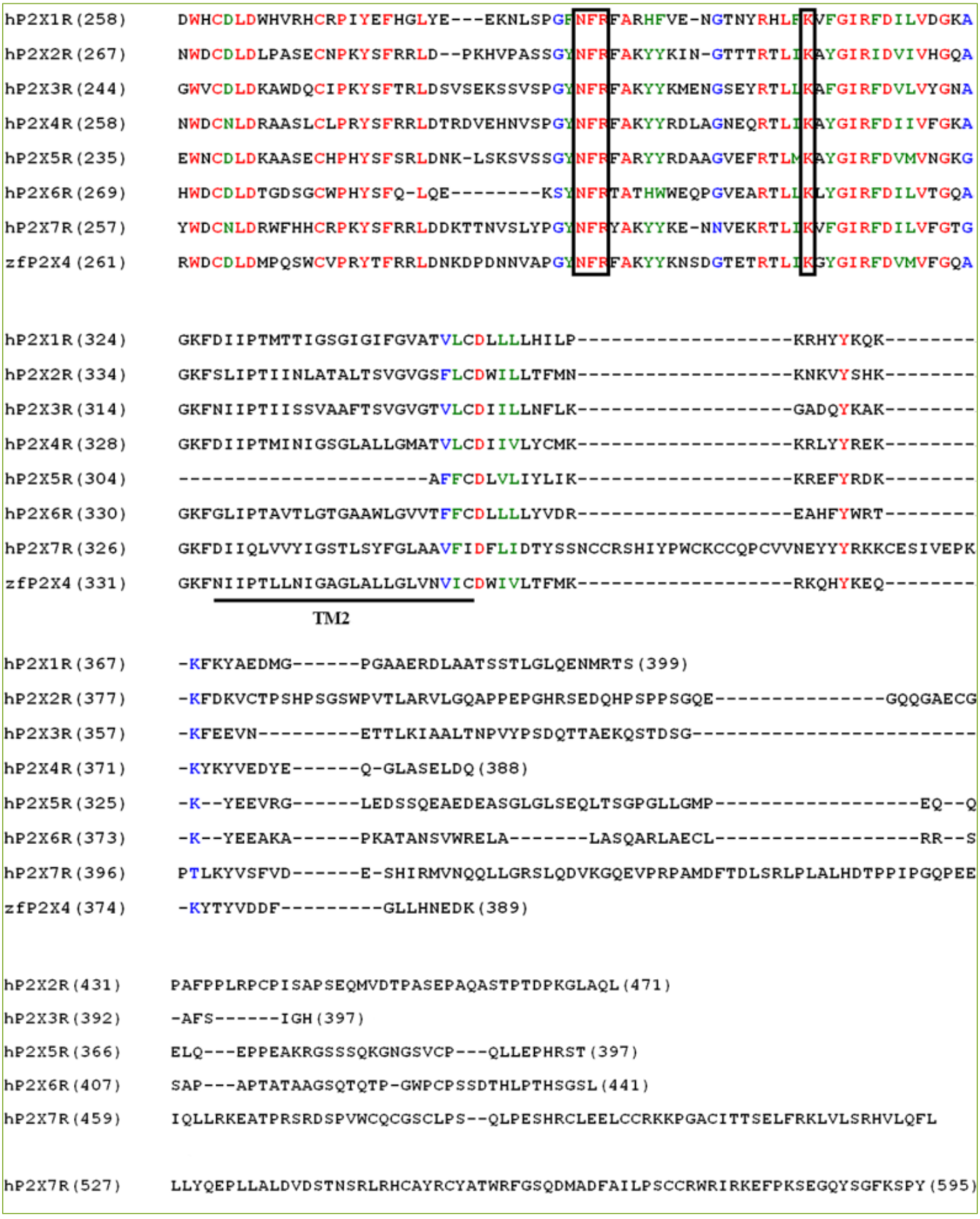
hP2X1R(258) DWHCDLDWHVRHCRPIYEFHGLYE---EKNLSPGFNFRFARHFVE-NGTNYRHLFKVFGIRFDILVDGKA
hP2X2R(267) NWDCDLDLPASECNPKYSFRRLD--PKHVPASSGYNFRFAKYYKIN-GTTTRTLIKAYGIRIDVIVHGQA
hP2X3R(244) GWVCDLDKAWDQCIPKYSFTRLDSVSEKSSVSPGYNFRFAKYYKMENGSEYRTLLKAFGIRFDVLVYGNA
hP2X4R(258) NWDCNLDRAASLCLPRYSFRRLDTRDVEHNVSPGYNFRFAKYYRDLAGNEQRTLIKAYGIRFDIIVFGKA
hP2X5R(235) EWNCDLDKAASECHPHYSFSRLDNK-LSKSVSSGYNFRFARYYRDAAGVEFRTLMKAYGIRFDVMVNGKG
hP2X6R(269) HWDCDLDTGDSGCWPHYSFQ-LQE--------KSYNFRTATHWWEQPGVEARTLLKLYGIRFDILVTGQA
hP2X7R(257) YWDCNLDRWFHHCRPKYSFRRLDDKTTNVSLYPGYNFRYAKYYKE-NNVEKRTLIKVFGIRFDILVFGTG
zfP2X4(261) RWDCDLDMPQSWCVPRYTFRRLDNKDPDNNVAPGYNFRFAKYYKNSDGTETRTLIKGYGIRFDVMVFGQA
hP2X1R(324) GKFDIIPTMTTIGSGIGIFGVATVLCDLLLLHILP----------KRHYYKQK--------
hP2X2R(334) GKFSLIPTIINLATALTSVGVGSFLCDWILLTFMN----------KNKVYSHK--------
hP2X3R(314) GKFNIIPTIISSVAAFTSVGVGTVLCDIILLNFLK----------GADQYKAK--------
hP2X4R(328) GKFDIIPTMINIGSGLALLGMATVLCDIIVLYCMK----------KRLYYREK--------
hP2X5R(304) ----------AFFCDLVLIYLIK----------KREFYRDK--------
hP2X6R(330) GKFGLIPTAVTLGTGAAWLGVVTFFCDLLLLYVDR----------EAHFYWRT--------
hP2X7R(326) GKFDIIQLVVYIGSTLSYFGLAAVFIDFLIDTYSSNCCRSHIYPWCKCCQPCVVNEYYYRKKCESIVEPK
zfP2X4(331) GKFNIIPTLLNIGAGLALLGLVNVICDWIVLTFMK----------RKQHYKEQ--------
TM2
hP2X1R(367) -KFKYAEDMG------PGAAERDLAATSSTLGLQENMRTS(399)
hP2X2R(377) -KFDKVCTPSHPSGSWPVTLARVLGQAPPEPGHRSEDQHPSPPSGQE----------GQQGAECG
hP2X3R(357) -KFEEVN---------ETTLKIAALTNPVYPSDQTTAEKQSTDSG----------
hP2X4R(371) -KYKYVEDYE------Q-GLASELDQ(388)
hP2X5R(325) -K--YEEVRG------LEDSSQEAEDEASGLGLSEQLTSGPGLLGMP----------EQ--Q
hP2X6R(373) -K--YEEAKA------PKATANSVWRELA-------LASQARLAECL----------RR--S
hP2X7R(396) PTLKYVSFVD------E-SHIRMVNQQLLGRSLQDVKGQEVPRPAMDFTDLSRLPLALHDTPPIPGQPEE
zfP2X4(374) -KYTYVDDF---------GLLHNEDK(389)
hP2X2R(431) PAFPPLRPCPISAPSEQMVDTPASEPAQASTPTDPKGLAQL(471)
hP2X3R(392) -AFS------IGH(397)
hP2X5R(366) ELQ---EPPEAKRGSSSQKGNGSVCP---QLLEPHRST(397)
hP2X6R(407) SAP---APTATAAGSQTQTP-GWPCPSSDTHLPTHSGSL(441)
hP2X7R(459) IQLLRKEATPRSRDSPVWCQCGSCLPS--QLPESHRCLEELCCRKKPGACITTSELFRKLVLSRHVLQFL
hP2X7R(527) LLYQEPLLALDVDSTNSRLRHCAYRCYATWRFGSQDMADFAILPSCCRWRIRKEFPKSEGQYSGFKSPY(595)

Figure 3. Sequence alignment for seven hP2X receptors and zfP2X4 receptor. Protein sequences are obtained from NCBI (hP2X1, NP_002549; hP2X2, NP_733782; hP2X3, NP_002550; hP2X4, NP_002551; hP2X5, NP_778255; hP2X6, NP_005437; hP2X7, NP_002553; zfP2X4, NP_705939). Solid underlines indicate the two transmembrane domains (TM1 and TM2). The highly conserved amino acids are colored red. The strongly and weakly similar amino acids sequences are colored green and blue, respectively. Open boxes indicate amino acids directly involved in ATP binding. The human P2X5R cDNAs reported are missing exon 10 (which encodes amino acids including the TM2 domain).

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Conflicting interests

The authors have declared that no competing interests exist.

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