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Review Article

A comprehensive review of the ten main platelet receptors involved in platelet activity and cardiovascular disease

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Abstract: Cardiovascular disease (CVD) is a major cause of death worldwide. Although there are many variables that contribute to the development of this disease, it is predominantly the activity of platelets that provides the mechanisms by which this disease prevails. While there are numerous platelet receptors expressed on the surface of platelets, it is largely the consensus that there are 10 main platelet receptors that contribute to a majority of platelet function. Understanding these key platelet receptors is vitally important for patients suffering from myocardial infarction, CVD, and many other diseases that arise due to overactivation or mutations of these receptors. The goal of this manuscript is to review the main platelet receptors that contribute most to platelet activity.

Keywords: Cardiovascular disease, platelet receptors, GPIb-IX-V, $P2X_1$, GPVI, α2β1, TXA_2, GPIIb/IIIa, PAR1, PAR4, $P2Y_1$, $P2Y_{12}$

Introduction

Platelets play an integral role in the development of cardiovascular disease (CVD), which is a leading cause of death for a large portion of the world. The cause of CVD, which often manifests into myocardial infarction or stroke, can be attributed to a variety of variables such as diet, genetics, tobacco use, and cholesterol levels. Platelets are also responsible for the development of acute coronary syndrome (ACS) due to ST elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), and unstable angina. For healthy individuals, circulating platelets are largely in the inactive form having little interaction with vessel walls. Both cardiovascular disease and acute coronary syndrome are merely two examples of pathologic events that can occur due to the untimely or unwanted activation of platelets.

Platelets are small, disk-shaped, anucleate cell fragments with a primary role of hemostasis and clotting of blood. Platelets also play a role in innate immunity. Developed from megakaryocytes in the bone marrow, platelets have a life span of roughly 10 to 12 days [1]. There has been great attention on the development of antiplatelet medications due to the high number of deaths attributed to CVD or ACS every year. In 2012, sales of antiplatelet pharmaceuticals totaled 12.5 billion dollars worldwide. In the same year for the United States, sales of antiplatelet medications accounted for nearly 17 percent of worldwide sales or 5.1 billion dollars [2]. With an increasing number of people suffering from platelet-related medical conditions, the development of new drugs that can inhibit platelets via the numerous platelet receptors is an active area of research and development.

Platelets play a role in the development of acute coronary syndrome by stimulating an inflammatory response within the atherosclerotic plaque [3, 4]. Activated platelets have the ability to

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release inflammatory mediators, such as chemokines and cytokines, and induce the release of inflammatory modulators from leukocytes and endothelial cells [5]. For example, platelets interact with macrophages through P-selectins on the platelet surface, which then enhances the activation of transcriptional nuclear factor-kB (NF-kB), further inducing the release of chemokines and cytokines. At the same time, platelets respond to these inflammatory mediators and are then induced to continue releasing inflammatory mediators, creating a vicious cycle of inflammation as well as the formation of an atherosclerotic plaque. Within an atherosclerotic plaque, the interaction between platelets induces the release of adhesion molecules and chemokines from leukocytes and endothelial cells, leading to the production of reactive oxygen species. Additionally, platelets contribute to further activation of leukocytes, which cumulatively leads to destabilization of the plaque and triggers the onset of acute coronary syndrome. Among the cytokines that platelets use in this inflammatory process are CD40 ligand (CD40L), P-selectins, and integrins [5]. A recent study suggested that circulating neutrophils have the ability to stimulate the bone marrow to release more immature platelets from megakaryocytes, which boosts the development of CVD [6]. These immature platelets can enhance the inflammatory process, thus increasing the risk of arterial thrombosis, ischemia, and myocardial infarction. Furthermore, abnormalities in platelet function (e.g., increased sensitivity to agonists) have been observed in patients with hypertension, which is a known risk factor for ACS [7].

There are several different types of transmembrane receptors located on the surface of platelets. These receptors include integrins (αIIbβ3, α2β1, α5β1, α6β1, αVβ3), glycoprotein toll-like receptors (TLRs), leucine-rich repeat receptors, seven-pass-transmembrane domain receptors (also known as G protein-coupled receptors or GPCRs), immunoglobulin superfamily proteins (glycoprotein VI, FcγRIIA), tyrosine kinase receptors, C-selectin receptors, and various other types [8]. Despite the numerous receptors located on the surface of platelets, much attention has been paid to several specific receptors due to their potential to attenuate platelet activity. The receptors responsible for the initial recruitment of platelets (GPIb-IX-V), adhesion and aggregation (GPVI, α2β1, GPIIabβ3), and amplification ($P2X_1$, $P2Y_1$, $P2Y_{12}$, PAR-1, PAR-4, TPα) have all been extensively studied [9].

The purpose of this article is to compile an up-to-date review of the main platelet receptors that are largely responsible for platelet activity. Although there are a variety of other receptors located on the surface of platelets, the 10 aforementioned receptors are considered to be the most important related to CVD, ACS, and other platelet-related disorders due to the unwanted or overactivation of platelets in circulation (**Figure 1**). This review will discuss the hemostatic role of platelets in healthy individuals, how platelets are involved in ACS and CVD, the crystal structure of the aforementioned main platelet receptors bound to their respective ligands, and the function of these receptors in platelet activation.

Platelets, hemostasis, and cardiovascular disease

A fundamental role of platelets is hemostasis of blood. Hemostasis is a multi-component process that is mainly initiated by three factors: vascular contraction, platelet adhesion, and activation of coagulation factors [10]. It is largely the responsibility of the endothelium to prevent unwanted platelet activation by secreting nitric acid along with prostaglandin I_2 [11]. When an injury occurs in a blood vessel, vasoconstriction quickly follows. Normal vasoconstriction is an important process to minimize blood loss while also slowing blood flow around the injury. This process allows for platelets to adhere to the subendothelium.

Platelet adhesion is the next major component of blood hemostasis. The initial adhesion is dependent on von Willebrand factor (vWF) binding to glycoproteins, which are located on the surface of platelets. The purpose of vWF is to slow platelets and allow them the time needed to bind fibrinogen. The binding of fibrinogen enables platelets to further adhere to the subendothelium via glycoprotein Ia/IIa (GPIa/IIa). A conformational change following the adhesion to platelets marks the beginning of platelet activation [12]. With this conformational change, collagen receptors become activated, and thromboxane A_2 is synthesized by platelets and

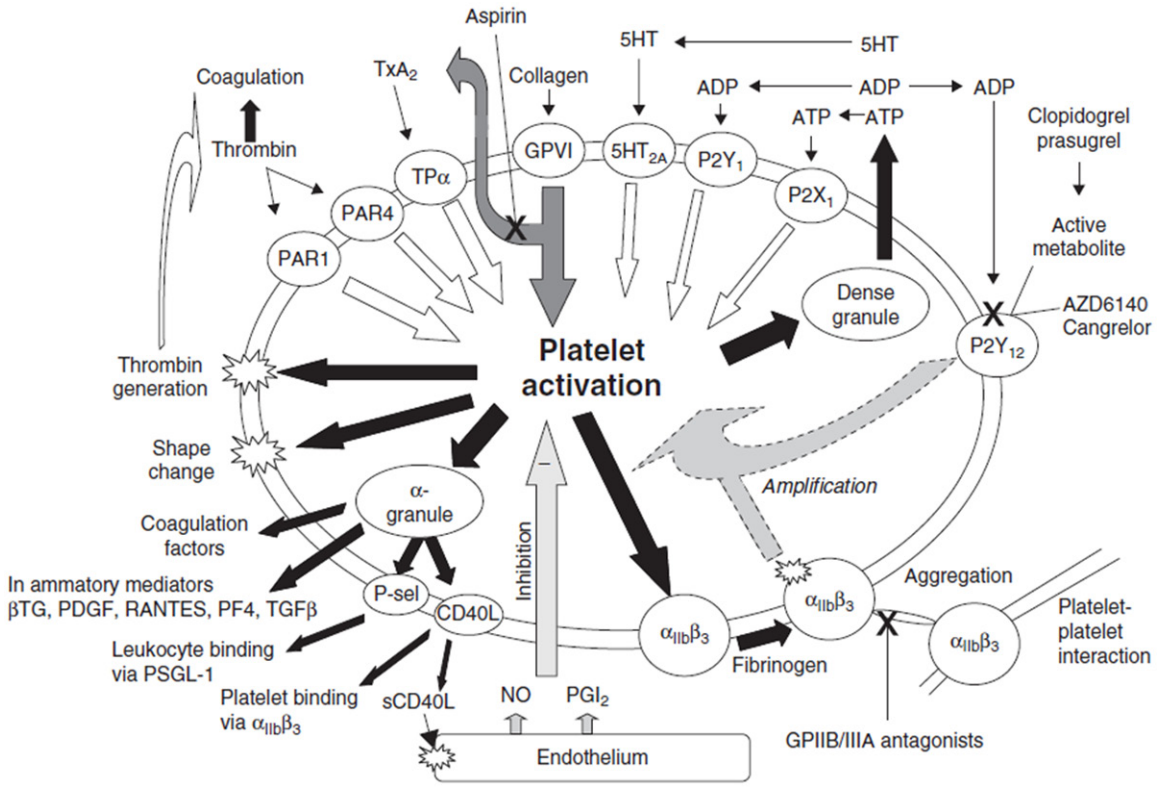

Figure 1. The main platelet receptors and ligands which subsequently lead to the activation of αIIbβ3 [83].

released. The release of TXA_2 serves to aid in platelet aggregation.

Platelet aggregation can be initiated by many different molecules such as TXA_2, ADP, collagen, serotonin, thrombin, and platelet aggregation factor. Platelet aggregation is controlled predominantly by fibrinogen, which binds platelets to one another via glycoprotein IIb/IIIa. Platelet adhesion to the vessel wall is a crucial step for platelet aggregation to occur. GPIIb/IIIa binds to the immobilized fibrinogen in an irreversible manner, providing the time platelets need to stabilize and adhere to the vessel wall. Thereafter, platelets recruit and bind other platelets to form a hemostatic plug [13]. Platelet aggregation is affected by shearing forces seen from the increased velocity of blood flowing in coronary vessels. Platelets are capable of binding to each other via von Willebrand receptors at shear rates of 1,000 to 10,000 s^{-1}. At shear rates greater than 10,000 s^{-1}, platelet aggregation can occur without activation, in the presence of soluble von Willebrand receptors. Nonetheless, platelets will be unstable without activation via the GPIIb/IIIa receptor [13].

Cardiovascular disease begins as atherosclerosis, which is a process of accumulation of platelets, cellular debris, cholesterol, fat, and other substances within arterial walls. This accumulation and inflammation lead to plaque formation, and increasing plaque within the arterial wall and lumen can subsequently cause the narrowing of blood vessels. Excess plaque accumulation can also result in the dislodgment of plaque from vessel walls, thus causing a complete blockage and ultimately, myocardial infarction, stroke, or even death [13]. Platelets are also though to play a role in the pathogenesis of aortic stenosis, which has similarities to atherosclerosis [14, 15].

In the following sections of this paper, we will discuss and review each of the 10 key platelet receptors responsible for the majority of platelet activity.

GPIb-IX-V

The GPIb-IX-V receptor complex, numbering nearly 25,000 copies per platelet, is responsible for the initiation of platelet recruitment [16].

GpIb-IX is a hetero-tetrameric structure composed of GPIbα, GPIbβ, and GPIX in a 1:2:1 ratio. The GPIbα unit is linked strongly via disulfide bonds to a pair of GPIbβ units, and the GPIX unit is affixed via transmembrane interaction. Studies have shown an interaction with a fourth subunit, GPV, in a 1:1 ratio; however, this association is relatively weak and can be easily interrupted [17]. The above transmembrane proteins belong to the leucine-rich family of proteins and are encoded by genes corresponding to chromosomes 17p12, 22q11.2, 3q29, and 3q21, respectively. The accumulation of functional GPIb-IX-V is highly dependent on chaperone protein gp96 found in the endoplasmic reticulum [9].

GPIbα, encoded by the GPIBA gene, is the largest subunit of the GPIb-IX complex and is responsible for most of GpIb-IX ligand binding, including thrombin, αMβ2, and plasma von Willebrand factor (VWF) [18, 19]. The extracellular portion of GPIbα is made up of a ligand-binding domain at the N-terminal, a macro glycopeptide region, and a region close to the membrane surface with a mechanosensitive domain (MSD) and cysteine residues that bind to GPIbβ via disulfide bonds [20]. The crystal structure of GPIbα has shown that the vWF-A1 domain comes in contact with His12, Ser11, Glu14, and Asn16 located in the N-terminal portion; His37, Lys152, Glu 128, Asp175, Thr176, Phe199 located in the leucine-rich repeats (LRRs); and Glu225, Asn226, Tyr228, and Ser241 located in the C-terminal portion [21]. The ligand-binding domain can be likened to a cupped hand, with the palm representing the beta-sheet of the LRR, the fingertips representing the N-terminal disulfide loop, and the thumb representing the C-terminal disulfide loop. The C-terminal loop remains vital for vWF binding and is found to contain at least five gain-of-function variants of GP1BA mutations.

After the occurrence of vascular injury, subendothelial collagen fibrils and other factors including vWF, laminin, and proteoglycans are released into circulation. Subendothelial vWF is bound to collagen VI, whereas collagen III and I aid in the further recruitment of additional vWF. The increase in vWF allows for the creation of a vWF matrix that has the ability to bind GPIbα. This binding allows for the rolling of platelets along the subendothelium. As platelets fixed to the subendothelium become exposed to shear stress from blood flow, the mechanosensitive domain (MSD) unfolds, exposing a trigger sequence that acts as a physical transducer for receptor activation. This trigger model ensures a ligand that binds with high affinity, but low unbinding force cannot withhold binding during shear stress and would not unfold the MSD [22]. The A1 domain on vWF is known to be the site of binding to the GPIbα complex [23]. Certain peptide inhibitors of the Gp1bα-vWF A1 may be used to study this interaction in the background of pathology.

GPIb-IX activation is implicated in other platelet physiology including inside-out signaling, platelet microparticle formation, platelet degranulation, as well as desialylation via NEU1 [22]. Despite our growing understanding of intracellular mediators of signaling, there is still a significant knowledge gap in the complete mechanism of GPIb-IX activation as it is unclear how GPIbβ plays a role in modulation.

TXA_2

The thromboxane receptor, abbreviated TXA_2R or TP, plays an important role in the initiation and amplification of platelets. The main ligand of this receptor, thromboxane, belongs to the family of eicosanoids, which includes many other molecules such as leukotrienes, prostaglandin, and epoxygenases. All of these molecules are derived from arachidonic acid (AA) via the cyclooxygenase pathway [24].

The thromboxane receptor belongs to the G-protein-coupled receptor (GPCR) superfamily and is expressed in a variety of cells such as platelets, monocytes, macrophages, and smooth muscle [24]. The gene responsible for encoding this receptor is located on chromosome 19.p13.3 [9]. The TP receptor is a membrane-bound heptamer with a mass of 57 kilodaltons (kDa). TP receptors of humans exist in two isoforms: TPα and TPβ, with the former being expressed on the surface of platelets. The difference between isoforms TPα and TPβ lies in the C-terminal cytoplasmic region. The expression of TPβ is limited to endothelial cells. TPα is a crucial player in platelet activation and amplification. Successful binding of the ligand thromboxane to the TPα isoform triggers signal transduction via several G-proteins such as G_q and $G_{12/13}$ and subsequent activation of down-

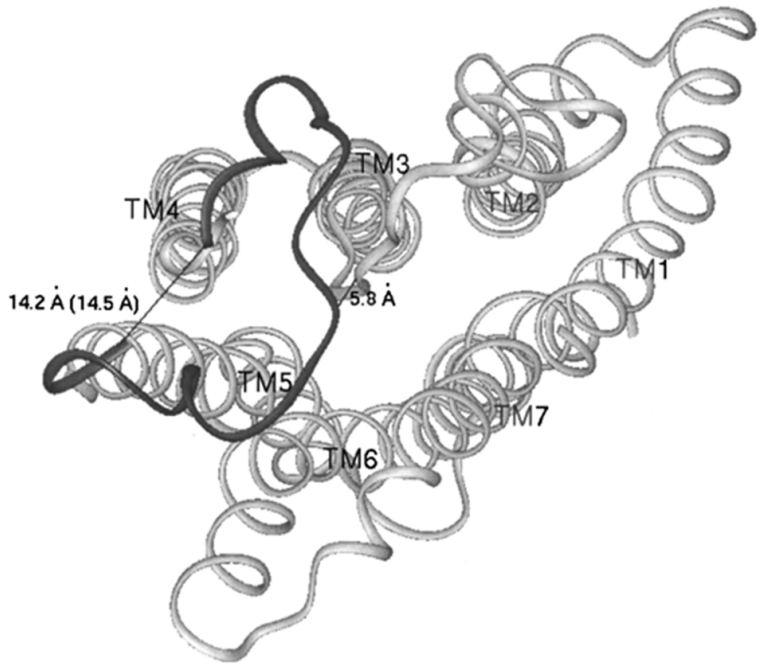

Figure 2. Thromboxane receptor model generated based on the crystallographic structural data associated with bacteriorhodopsin. The C-EL2 is displayed as the dark thin line connecting TM4 and TM5 [84].

stream signaling pathways that include phospholipase C and RhoA, promoting platelet activation. Mutation within the thromboxane receptor has been shown to result in increased bleeding time [24].

Due to difficulties associated with crystallizing mammalian GPCRs largely because of their membrane-bound proteins, analysis of the human TP receptor is based on molecular modeling and crystallographic data associated with bacteriorhodopsin or bovine rhodopsin. The TP receptor is composed of seven transmembrane (TM) domains, three extracellular loops (ELs), and three intracellular loops (ILs). Early studies to uncover the residues responsible for ligand binding using bovine rhodopsin suggested a potential ligand-binding site on the seventh TM domain as point mutations within this segment lead to decreased ligand-binding activity. Further research has implicated that all regions except TM2 play some role in TPR-ligand binding [25].

Another study utilizing photo-affinity labeling and site-specific antibodies suggested that the C-terminal segment of extracellular loop 2 (C-EL2) within the thromboxane receptor, most notably the region spanning Cys183-Asp193, is a highly important region for ligand binding. One nuclear magnetic resonance study looking closer into this region of the TP receptor revealed Val179, Thr186, Leu185, and Leu187 were potential residues involved in ligand binding. Furthermore, mutagenesis studies have indicated that Asp193 is necessary for agonist and antagonist binding while Phe184, Ser191, and Thr186 are only necessary for antagonist binding [25]. Vaccines containing C-EL2 peptides in mice stimulated the production of anti-CEL2 TPR antibodies which selectively inhibited TPR-mediated platelet aggregation without additional bleeding risk. Additionally, the antibodies inhibited the formation of platelet-leukocyte aggregates, which have been shown to contribute to the development of atherosclerosis and athero-thrombotic disease. No effects on platelet aggregation secondary to ADP or other proaggregatory factors were noted, suggesting a different pathway of activity [26]. While the TXA_2-TPR signaling pathway has been known to play a significant role in platelet function *in vivo*, this study expands our understanding of the role of C-EL2 in TPR-dependent platelet activation and sheds light on the potential therapeutic use of a thromboxane-A_2 receptor-based vaccine to decrease risk of thrombogenesis.

Furthermore, studies using pharmacologic and genetic approaches show that in hemostatic plugs, TXA_2 signaling is predominantly in the outer region of the plug and less prevalent within the core (**Figure 2**). This explains the relatively low risk of *in vivo* bleeding using TXA_2 inhibitors [27].

$P2X_1$

The next receptor of importance for platelet activity is the ATP-gated $P2X_1$ receptor. This receptor belongs to the P2X family of surface receptors, which are ligand-gated, non-selec-

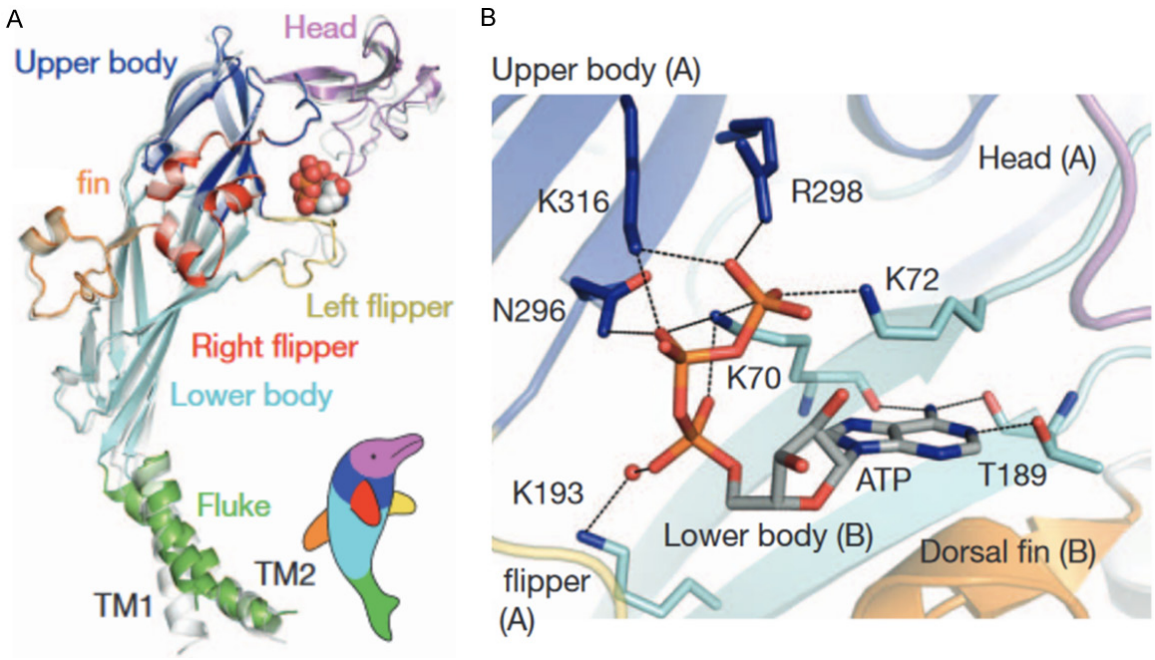

Figure 3. Crystal structure of $P2X_4$ receptor in complex with ATP. A. Representation of $P2X_4$ with colors corresponding to different regions of the dolphin. B. ATP binding pocket [32].

tive cation channels. $P2X_1$ is one of three P2 receptors expressed by platelets, with $P2Y_{12}$ and $P2Y_1$ being the others [28]. This receptor becomes activated when a vascular injury occurs, facilitating a release of ATP. The activation of the $P2X_1$ receptors leads to an influx of Ca^{2+} thereby leading to the conformation change within platelets that is necessary for proper platelet activation. The $P2X_1$ receptors have several subtypes ($P2X_1$-$P2X_7$), numbered according to the chronological order of discovery. The exact mechanism by which this receptor works remains elusive, but $P2X_1$ knockout mice have displayed increased bleeding times and decreased thrombus formation, further indicating this receptor is of high importance [28, 29].

The $P2X_1$ gene is encoded by chromosome 17p13.2 and contains 12 exons responsible for creating a 399-amino acid chain that comprises this receptor [30]. It has been discovered that P2X receptors orient into trimeric structures comprised of the subtypes $P2X_1$-$P2X_7$ forming either homomeric or heteromeric complexes [30, 31]. Using the crystallographic data, it was determined that these subunits share a similar structure with two transmembrane domains (TM1 and TM2) separated by an extracellular area containing 10 cysteine residues. It is within this extracellular domain that ATP is able to bind. The transmembrane domain composes the non-selective cation channel [32]. It was also determined that TM2 but not TM1 is required for proper subunit assembly [31].

ATP is capable of binding to multiple locations within this trimeric receptor. Using the binding site between (A) and (B) (**Figure 3**), it was shown that ATP adopts a U-shape structure upon binding with the negative phosphate substituents, creating a salt bridge and additional hydrophobic contacts [32]. It was determined that Lys70 plays a critical role due to the ammonium substituent being at the center of this ATP "U" conformation and interaction with oxygen moieties on the α, β, and γ phosphates. Asn296 and Lys316 function to serve as further contacts for the β-phosphate while Lys72, Arg298, and Lys316 interact with the γ-phosphate. The adenine base of ATP resides deep within the binding site of this receptor and forms hydrogen bonds with Thr189, Lys70, and Thr189 [32]. These recent discoveries are a step toward a detailed structural and functional understanding of the $P2X_1$ receptor. There are currently no experimentally determined structures of the

receptor, although reasonable assumptions have been made from other P2X structures. Further research in this area can facilitate the development of antithrombotic agents targeting the $P2X_1$ receptor.

$P2Y_1$

The $P2Y_1$ receptor, belonging to the GPCR family of receptors, is commonly expressed in platelets, blood vessels, smooth muscle, neural tissues, testis, ovary, and prostate [9]. As mentioned in the previous sections, $P2Y_1$ is one of the three P2 receptors expressed on the surface of platelets. Similar to $P2Y_{12}$, $P2Y_1$ is activated by the successful binding of the G_q-coupled ADP ligand [33]. Mutagenic studies have indicated that the $P2Y_1$ receptor is necessary for ADP-induced platelet aggregation. Patients with deficient or mutated $P2Y_1$ receptors will have a complete absence of platelet conformational change and aggregation in response to ADP. While it is true that ATP binds to $P2Y_1$ with a lower affinity, ATP acts as a $P2Y_1$ antagonist when expressed at low concentrations. The $P2Y_1$ receptor constitutes approximately 20 to 30 percent of the total ADP binding sites on the surface of platelets [9]. Activation of the $P2Y_1$ receptor results in the mobilization of internal calcium, resulting in platelet conformational change and a weak aggregatory response to ADP [34]. The $P2Y_1$ receptor is encoded by chromosome 3q25 and contains 373 amino acids. Coded by an intronless gene spanning approximately four kilobases (kb) and composed of a single exon containing 3,122 base pairs, this receptor has been shown to play a role in the aggregation response of collagen-induced conformational change when TXA2 formation is inhibited [34, 35]. A recent study showed that the $P2Y_1$ receptor activates G_q protein signaling in mouse and human platelets in the absence of agonist ligand, indicating the constitutive activation of $P2Y_1$ receptors [36]. These findings suggest that modification of constitutive $P2Y_1$ receptor signaling might be involved in susceptibility to thrombosis, and targeting this receptor activation may be a promising strategy for future antiplatelet therapy.

The structure of the $P2Y_1$ receptor contains seven TM domains and is largely similar to other GPCRs previously discussed. The commonly conserved amino acid motif usually observed in class A GPCRs ($DER^{3.50}Y$) is replaced by the residues $HR^{3.50}Y$ in the $P2Y_1$ receptor. This deviation from many common GPCR structures makes the $P2Y_1$ crystal structure the first GPCR with a basic histidine at position 3.49 (according to Ballesteros-Weinstein numbering). The basic histidine residue repels $R149^{3.50}$ leading to the conformation of the side chain being even more extended than other class A GPCRs [35].

Following x-ray crystallography of the $P2Y_1$ receptor complexed with MRS2500 (a nucleotide antagonist), it was determined that the ligand fits into a binding pocket composed of residues from the N-terminus, ECL2, and α helices VI and VII (**Figure 4**) [35]. The adenine ring of the ADP analog is inserted into the narrow opening of $R286^{6.62}$ with L44 falling on either side. N^6H and N^7 also of the adenine ring form hydrogen bonds with the $N283^{6.58}$ side chain. The N-methanocarba ring has hydrophilic interactions with the phenyl Y203 in ECL2. Studies have indicated that the phosphate substituent of the nucleotide antagonist is important for the receptor to bind to the ligand with a high affinity. The 3' phosphate forms hydrogen bonds with $Y110^{2.63}$ and $Y303^{7.32}$ while also forming two salt bridge structures with K46 located in the N-terminus and R195 located in ECL2. The 5' phosphate forms a salt bridge with $R310^{7.39}$ and hydrogen bonds with T205 located in ECL2 and the aromatic $Y306^{7.35}$. Mutagenic studies have indicated that mutations in residues L44A, $Y110^{2.63}F$, Y203A, T205A, and $N283^{6.58}$ reduce the binding affinity for the nucleotide antagonist [37]. These results highlight the critical role of these residues in ligand binding of the $P2Y_1$ receptor, furthering our understanding of the structural biological features of this key platelet receptor.

$P2Y_{12}$

The $P2Y_{12}$ receptor plays a central role in platelet recruitment, procoagulant activity, clot formation, platelet hemostasis, and aggregation potential. $P2Y_{12}$ belongs to the GPCR family of receptors. As stated above, this receptor is another type of P2 receptor found on the surface of platelets [38]. Rather than being activated by ATP molecules like $P2X_1$, $P2Y_{12}$ is activated by ADP. While ADP binds to $P2Y_{12}$ with the

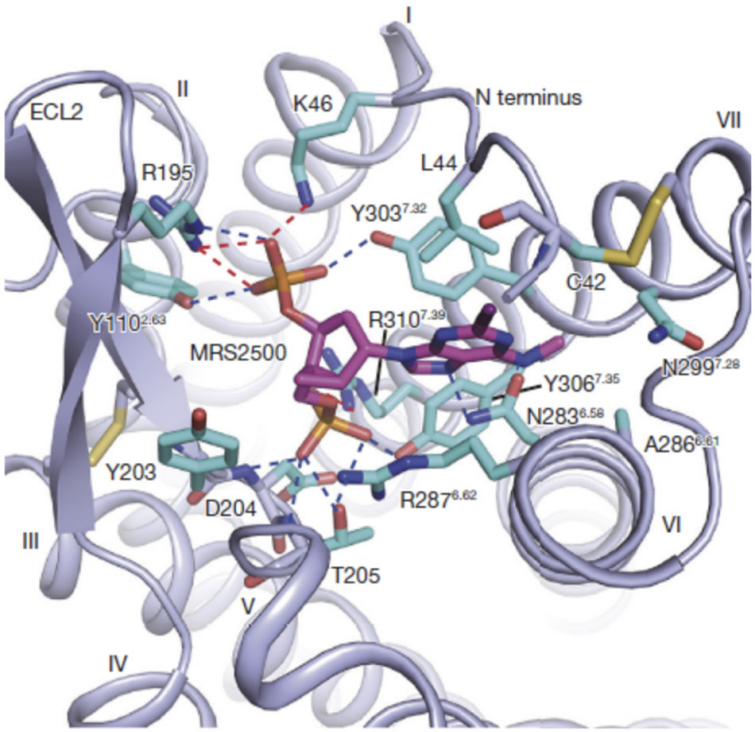

Figure 4. Residues involved in $P2Y_1$ binding of the nucleotide antagonist MRS2500. The magenta carbons of MRS2500, and $P2Y_1$ residues (cyan) involved in ligand binding are shown in stick representation. Salt bridges are indicated by red dashed lines; hydrogen bonds displayed as dashed blue lines. Oxygen atoms, red; nitrogen atoms, dark blue; sulfur atoms, yellow; phosphorus atoms, orange; iodide atoms, purple [37].

highest affinity and is a full agonist of this receptor, ATP binds to the receptor at a much lower rate. Once ADP binds with the $P2Y_{12}$ receptor, it initiates the release of Ca^{2+} consequently leading to conformational changes within the platelet. This allows for platelet activation and initiation of platelet recruitment and aggregation [38]. Patients with mutated or deficient $P2Y_{12}$ show mild to moderate bleeding [9].

In humans, the $P2Y_{12}$ receptor gene maps to chromosome 3q21-q25 and is commonly found in platelets, glial cells, smooth muscle cells, and endothelial cells [9, 38]. This gene, spanning 47 kb and containing three exons and two introns, consists of 342 amino acids [9, 28]. The $P2Y_{12}$ receptor contains four extracellular cysteine residues: C17, C97, C175, and C270. The Cys residues, C97 and C175, are linked via disulfide bonds and are of high importance for the successful expression of this receptor. In the $P2Y_{12}$ receptor, it was found that the intracellular conformational changes are less prominent than extracellular conformational changes [39]. Studies of this receptor have indicated that extracellular conformational changes resulting from successful ligand binding are similar to the conformational changes seen in PAR1. Subtle changes in helices VI and VII were observed in the $P2Y_{12}$R-2MeSADP complex, suggesting that when ADP binds with this receptor, it represents an "agonist-bound inactive state" [39].

The 2MeSADP-binding pocket is composed of residues from helices III, IV, V, VI, and VII as well as residues from ECL2 and the N-terminus (**Figure 5**) [37]. The important residues that constitute the binding pocket are as follows. The adenine moiety of the ADP analog 2MeSADP forms a π-π interaction with the side chain of Y105. The 2-thioether substituent enters the hydrophobic pocket composed of residues F106, L155, S156, and N159. This functions as a cornerstone to maintain the adenine and ribose ring in the most advantageous orientation [39]. Because of this, the 2MeSADP analog of endogenous ADP binds at a slightly higher affinity to $P2Y_{12}$. The binding of $P2Y_{12}$ and the diphosphate of 2MeSADP involve a myriad of positively charged, hydrophilic residues. Determined by sequence analysis and mutagenic studies, the aromatic residue Y259 and cationic residues R256 and K280 are all responsible for the coordination of the ligand's phosphate moiety [31]. These structural findings provide valuable insight into the mechanisms of agonist and antagonist interactions with the $P2Y_{12}$ receptor.

Protease-activated receptors

Protease-activated receptors (PARs) belong to the family of GPCRs, and four types have been identified: PAR1, PAR2, PAR3, and PAR4. PARs are similar in architecture to a majority of other GPCRs with all containing seven transmembrane helices attached by three intracellular and three extracellular loops. PAR1 is one of

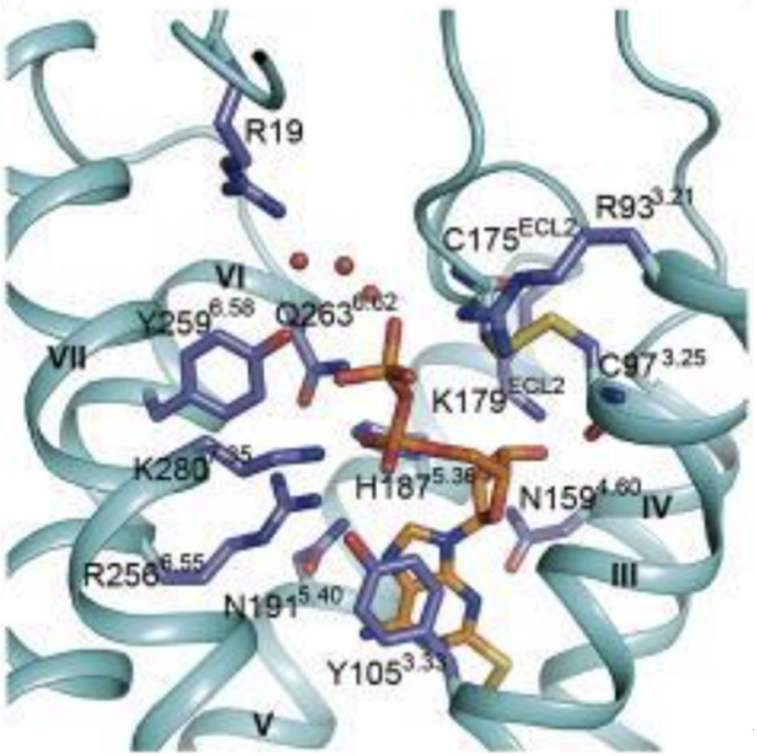

Figure 5. Ligand binding pocket for 2MeSADP. A cartoon representation of the binding pocket on $P2Y_{12}$ that is in complex with ADP [39, 85].

two protease-activated receptors located on the surface of platelets, the other being PAR4. PARs play an additional role for platelets by releasing ADP, thromboxane, serotonin, epinephrine, activation/mobilization of CD-40 and P-selectin, and lastly in the induction of platelet procoagulant activity [40]. PARs are activated via proteolytic cleavage of the N-terminal allowing for the display of a "tethered ligand". This tethered ligand binds intramolecularly to the seventh TM α-helix bundle thereby affecting G-protein activation. The proteolytic cleavage of PAR receptors by thrombin is irreversible. PAR1 is the main receptor responsible for binding thrombin and can become activated by minute amounts of the molecule [40, 41].

PAR1

PAR1 is expressed on red blood cells, epithelium, neurons, astrocytes, and immune cells. It takes part in tumorigenesis and is also expressed in some tumors and malignancies on cancer-related fibroblasts, epithelial cells, blood vessels, myocytes, mast cells, and macrophages. Overexpression of PAR1 has been observed in several cancer types including breast, melanoma, renal, gastric, colon, lung, pancreatic, esophageal, prostate, liver, ovarian, endometrial, head, and neck cancers. PAR1 consists of 415 amino acids and five functional domains: extracellular N-terminal, extracellular loop, seven transmembrane domains, intracellular loop, and intracellular C-terminal [42, 43]. The structural basis of proteolytic PAR1 activation and initiation of signal transduction is not completely understood, and only partial PAR structures in the presence of antagonists are currently available. Further research is warranted to resolve additional questions regarding PAR1 signaling biology, including the mechanism by which PAR dimerization modulates different signaling outputs and the cellular signals that prompt PAR colocalization.

Analysis of a crystallized PAR1 receptor in complex with vorapaxar, a highly selective PAR1 antagonist, has revealed additional information about this important platelet receptor. Crystallographic data have indicated that vorapaxar binds relatively close to the extracellular surface in comparison to other GPCR ligands [44]. The binding site for the vorapaxar-PAR1 complex is composed of residues from TM domains 3-7 in conjunction with residues from ECL2 and ECL3 (**Figure 6**). The residues involved in the binding site come together to form a tunnel spanning the receptor. There is an opening for this tunnel located between TM4 and TM5. The other opening, between TM6 and TM7, binds to the ethyl carbamate tail of vorapaxar [44]. The structural reasoning behind the selective binding of vorapaxar to PAR1 versus PAR2 or PAR4 remains unclear. Almost all residues that contact vorapaxar in PAR1 are conserved in the other PAR subtypes. The Cys175 of TM3 and Cys254 of ECL2 form a disulfide bond. The ECL2 loops orient outward forming two antiparallel β-strands which are held in place by hydrogen bonds between His255 of ECL2 and Tyr353 of TM7, as well as Asp256 of ECL2 and Tyr95 in the N-terminus [44]. Access to the

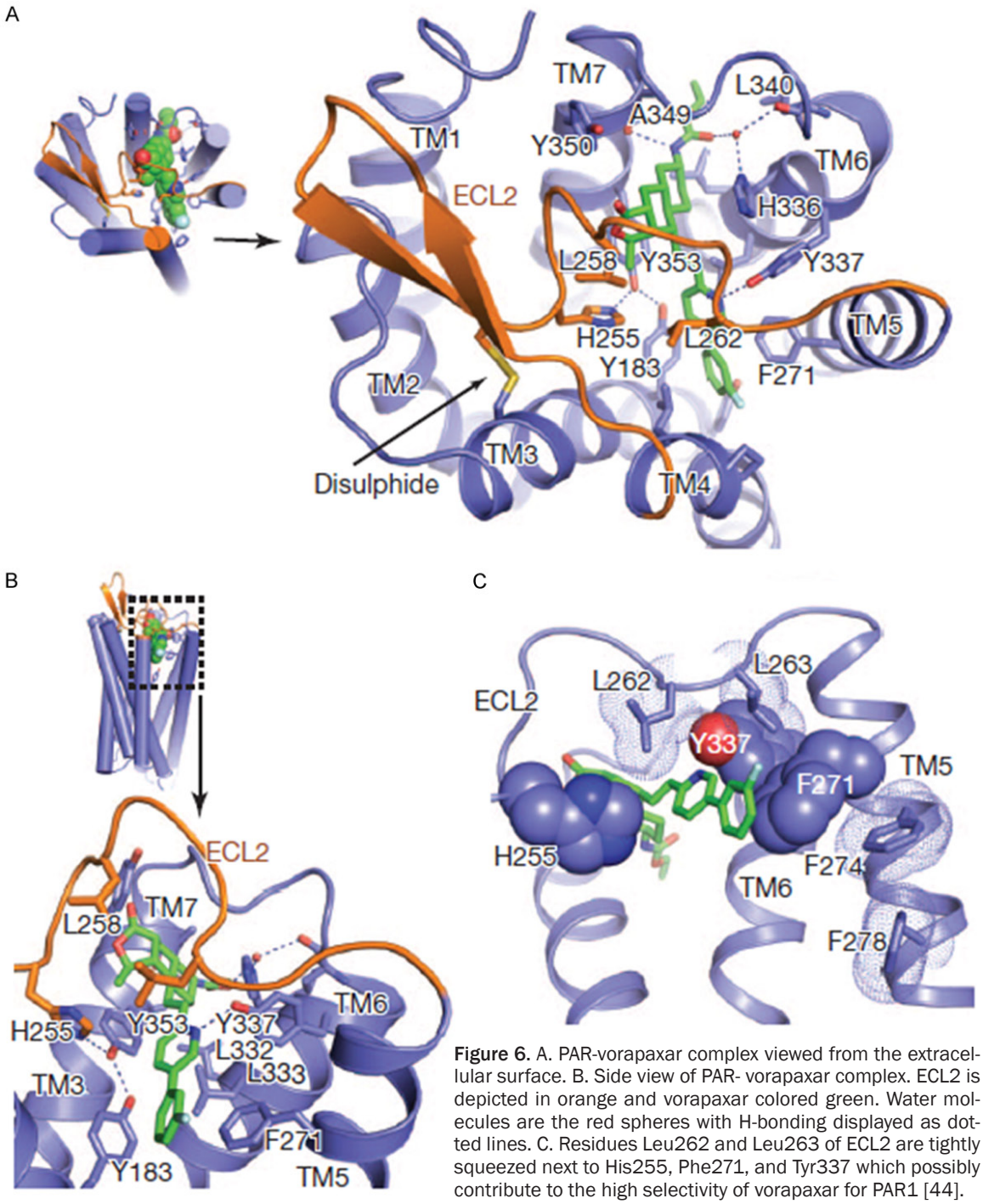

Figure 6. A. PAR-vorapaxar complex viewed from the extracellular surface. B. Side view of PAR- vorapaxar complex. ECL2 is depicted in orange and vorapaxar colored green. Water molecules are the red spheres with H-bonding displayed as dotted lines. C. Residues Leu262 and Leu263 of ECL2 are tightly squeezed next to His255, Phe271, and Tyr337 which possibly contribute to the high selectivity of vorapaxar for PAR1 [44].

binding pocket of PAR1 by vorapaxar is seemingly impeded by the centrally located ECL2. The residues of PAR1 which are most important for binding vorapaxar include Y183, Y353, H336, Y337, F271, and L340 [44].

PAR2

Although PAR2 is not directly involved in platelet aggregation, it is active in many different facets, including modulating inflammatory processes such as obesity and metabolism. It also functions as a sensor for proteolytic enzymes in the setting of infection. PAR2 is also a GPCR but is not a proteolytic substrate for thrombin, unlike PAR1, PAR3, and PAR4. It is instead activated by multiple components of the coagulation cascade including trypsin, tryptases, and other trypsin-like proteases. Trypsin was identified as the main proteolytic activator at the

N-terminus of PAR2 with the exposed tethered ligand, SLIGRL in rodents or SLIGKV in humans. Over the years, the list of proteases capable of PAR2 activation and/or disarming the receptor has grown and now includes tryptase, tissue factor/factor VIIa complex, matriptase, thrombin, cathepsins, kallikreins, and human leukocyte elastase. Each protease offers unique PAR2 cleavage in a way that may promote allosteric modulation and biased agonism [45]. Studies by Cheng et al. showed that PAR2 and PAR1 share 36 percent sequence identity and are particularly similar in structure [46]. Their differences, however, are observed in ECL2, ECL3, and the extracellular portions of TM5 and TM6. In PAR2, the top four helical turns of TM5 move by 5.9Å toward the TM domain core. Further, ECL2 is tethered to the top of TM3 through hydrogen bonding between $His227^{ECL2}$ and $Tyr156^{3.33}$; this interaction is notably absent in PAR1. TM6 moves by 4.7Å toward the core of the receptor on the extracellular side, whereas TM7 moves outward by 1.8Å. $Tyr326^{7.35}$ is subsequently pulled away from $His227^{ECL2}$, and this breaks the hydrogen bond observed in PAR1. These movements in TM5, TM6, and TM7 result in the complete elimination of the vorapaxar binding pocket in PAR2 [46]. These studies elucidate the multiple allosteric sites present on PAR2, expanding our knowledge base for future drug design.

PAR3

PAR3 is not as substantially researched as other PARs. It is also known to be a GPCR and is activated by thrombin. The results of an *in vitro* study by Ahmed and Macara suggested that PAR3 could be an exocyst receptor essential for mammary cell survival. The findings were not concrete, but their research did show that silencing of PAR3 in mammary cells was not compatible with cell survival [47]. When PAR3 is silenced, it triggers phosphoinositide trisphosphate depletion and decreased Akt phosphorylation, which leads to apoptosis of the cell. Electron microscopy analysis has demonstrated that PAR3 along with atypical protein kinase C and PAR6 form the tripartite partition defect polarity complex, which is a major regulator of cellular polarity. This PAR complex also plays a role in neural development where it is thought to be essential for the differentiation of neurites into axons and dendrites [48]. While studies have attempted to characterize the non-polymerized building blocks of the PAR complex, very little is known about the structure of PAR3 itself, warranting further investigation.

PAR4

Thrombin's effects on human platelets are mediated primarily by two PARs, PAR1 and PAR4. For a long time, PAR1 was known to be a high-affinity thrombin receptor, and PAR4 was considered to be a backup to PAR1. Research later showed the importance and uniqueness of PAR4 in platelet activation and antiplatelet therapy [49]. Unlike PAR1, PAR4 structurally lacks a hirudin-like thrombin binding site and instead contains an anionic sequence downstream of the thrombin cleavage site. Because of this difference, PAR1 was shown to have a rapid initial signal, while PAR4 has a slower and more prolonged response to thrombin [49]. Encoded by genes found on chromosome 19p12, PAR4 is another primary signaling receptor for platelets. The activation of PAR4 produces a signal with a long-lasting effect that aids in stable blood clot formation. Carrying a similar mechanism to PAR1, PAR4 becomes activated by the cleavage of the receptor's N-terminus by thrombin to form a tethered ligand. Unlike PAR1, PAR4 does not bind to thrombin with a high affinity likely due to the receptor missing a hirudin-like binding sequence [50]. As a consequence, PAR4 only becomes activated in the presence of high concentrations of thrombin. Successful activation of PAR4 induces a sustained steady increase in $[Ca^{2+}]$, which constitutes the majority of intracellular calcium influx [51]. Experimental studies using bioluminescence energy transfer have shown that PAR1 and PAR4 work in tandem [50]. It has been proposed that following the cleavage of PAR1 by thrombin, residual amounts of thrombin are still bound to PAR1 via the hirudin-like sequence and cleave the adjacent PAR4. These experiments also indicated that thrombin induces the formation of PAR1-PAR4 heterodimers [50]. Given the complex arrangement of receptors and signaling molecules on the platelet surface, future research will need to examine the interaction between these PARs and other GPCRs to mediate signaling *in vivo*.

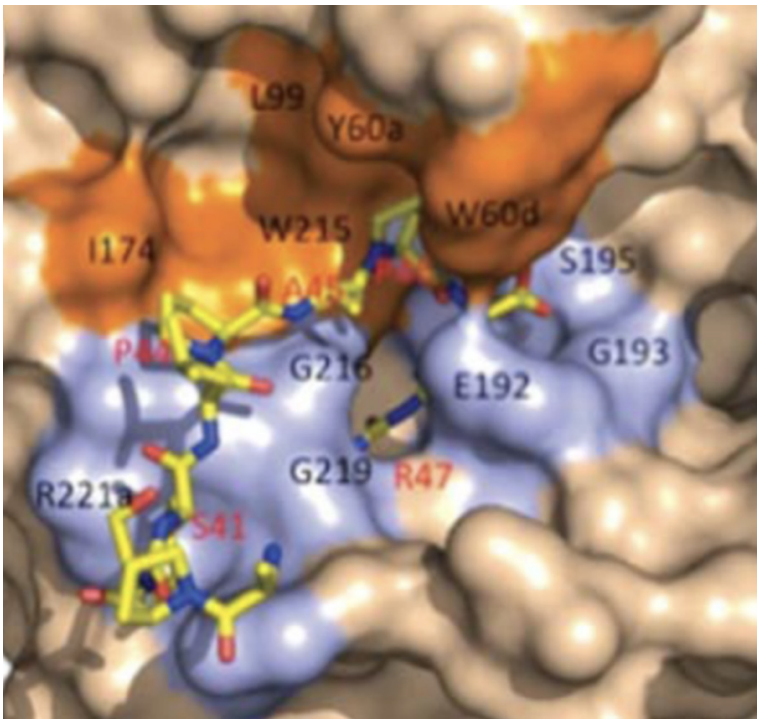

Figure 7. Crystal structure of human PAR4-thrombin complex. Thrombin is displayed as a stick model with the hydrophobic residues of PAR4 in orange and polar regions in periwinkle [52].

The crystal structure of the thrombin-PAR4 complex has yielded valuable information regarding the action of PARs. Analysis of the crystal structure demonstrates that the guanidinium substituent belonging to R47 of PAR4 interacts with the side chain of D189 and the backbone oxygen of thrombin residue G219 (**Figure 7**). The C-terminus of R47 binds to the backbone nitrogen atoms of G193 and S195 located in the oxyanion hole, with the backbone nitrogen of R47 interacting with S214. P46 of PAR4 exhibits numerous van der Waals interactions with catalytic H57, Y60a, and W60d of the 60-loop along with L99 and W215 in the aryl binding site of thrombin. A45 of PAR4 binds to the backbone oxygen and nitrogen of G216 in thrombin [52].

GPVI

The GPVI platelet receptor, which binds the ligand collagen, is necessary for stable platelet adhesion to occur. It is expressed exclusively on megakaryocytes and platelet membranes. GPVI is composed of 319 amino acids with a molecular weight of 62 kDa [53]. This receptor is also responsible for the secretion of granules which serve to activate nearby platelets, thereby initiating thrombus formation. GPVI is a 60-65 kDA transmembrane glycoprotein belonging to the immunoglobulin superfamily of proteins. This receptor is one of two collagen receptors found on the surface of platelets [9]. When platelets are in their inactive state, this receptor is expressed in its monomeric form. Upon platelet activation, there is increased expression of the dimeric form of GPVI on the platelet surface. It is this dimeric GPVI that has the highest affinity toward collagen [54]. There was some uncertainty regarding whether GPVI's configuration in the membrane of resting and activated platelets is a monomer or a dimer. A recent study by Clark et al. suggests that GPVI is expressed as a mixture of monomers and dimers in cells, and there is a possibility that ligands only bind to the dimeric form [53]. The experimental techniques used in this study provide valuable evidence, but additional complementary approaches such as structural studies (e.g., X-ray crystallography, cryo-electron microscopy) could further enhance our understanding of the dimeric and monomeric forms of GPVI. Secondly, the study primarily focuses on *in vitro* experiments, and the relevance of these findings in the context of platelet function *in vivo* should be explored in future studies, including animal models and clinical investigations.

The gene encoding the GPVI receptor is found on chromosome 19 in the leukocyte receptor cluster. The active binding site was uncovered using collagen-like peptides. The binding site is composed of two immunoglobulin domains distanced approximately 90° apart. The N-terminal (D1) and C-terminal bear resemblance to p58KIR. Due to difficulties associated with the crystallization of this receptor bound to its natural ligand, a collagen-like peptide along with a computational approach were utilized to determine the active site. Using this model, it was uncovered that there are two molecules of GPVI

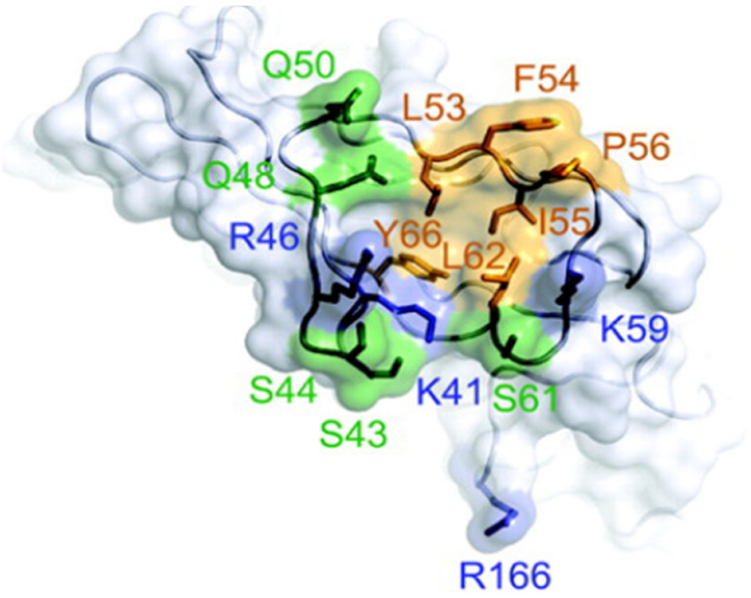

Figure 8. Stereo view of the residues of D1 of the GPVI receptor in contact with collagen-like peptides as indicated by computer modeling. Colors on image depict chemical properties: green are polar, yellow are hydrophobic, and blue are positively charged [55].

per subunit. The ground of the binding pocket is formed by L53, F54, P56, L62, and Y66 [55]. The polar residues S43, S44, Q48, Q50, and S61 and basic residues K41, R46, K59, and R166 form the periphery (**Figure 8**). It has been discovered that the active site for this receptor resides primarily within a cluster of basic residues K41, K59, R60, and R166 [55]. New studies on the signaling cascade of GPVI indicate that cross-linking induces tyrosine phosphorylation, which subsequently leads to the recruitment and binding of tyrosine kinases and further downstream signaling [53].

α2β1

The receptor α2β1 (also known as VLA-2, CD49b, or GPIa-IIa) is the other collagen receptor located on the surface of platelets. This receptor is second only to αIIbβ3 in level of expression, with an estimated 2,000 to 4,000 copies per platelet [56]. CD49b is an integrin receptor that forms a heterodimer with the α2 subunit (150 kDa), which is non-covalently linked to the β1 subunit (130 kDa). The 200-residue inserted sequence of the I domain facilitates this receptor's recognition of collagen IV, collagen VI, and transmembrane collagen XIII [9, 57]. Successful binding of collagen to this receptor allows for platelets to adhere to the vessel wall under flow conditions. Using platelets from animal models deficient in the α2 subunit resulted in the inability of this receptor to bind collagen under static or free-flowing conditions. Human patients with deficient α2 subunit showed increased bleeding times, further indicating the importance of this subunit [57].

The growing number of studies on this receptor have demonstrated the importance of the I domain within the α2 subunit of the α2β1 receptor. The I domain is an Mg^{2+} or Mn^{2+} metal-ion-dependent adhesion site (MIDAS) which is critical for the recognition of collagen (**Figure 9**). The MIDAS binding site is integrated by side chains from three main loops located on the upper surface of the domain along with glutamic acid from the middle strand of collagen [19]. The important residues of this motif are D151, which forms a water-induced bond to Mg^{2+} or Mn^{2+}, and Ser residues S153 and S155, which directly bind via the hydroxyl oxygens on loop 1 (L1). Loop 2 (L2) contains residue T221 which directly binds to the metal ion through its hydroxyl oxygen. Loop 3 (L3) contains residues D254 and E256 that form water-induced bonds to the metal ion. Through mutagenesis studies, it has been elucidated that residues D151, D254, and S153 are of the utmost importance for collagen binding [19]. The three loops of the MIDAS site also provide the residues responsible for the recognition and binding of collagen. It is largely the middle strand of collagen that mediates the binding of collagen to α2β1. The middle strand glutamate binds to the metal ion, while arginine, also of the middle strand, forms a salt bridge with D219 of L2. Hydrogen bonding occurs with the collagen main chain from N154 and Y157 of L1 and H258 of L3. The collagen sequence GFOGER has been shown to be the high-affinity binding motif for the I domain [19].

GPIIb/IIIa

GPIIb/IIIa (integrin aIIbβ3) is one of the most predominant and important platelet receptors

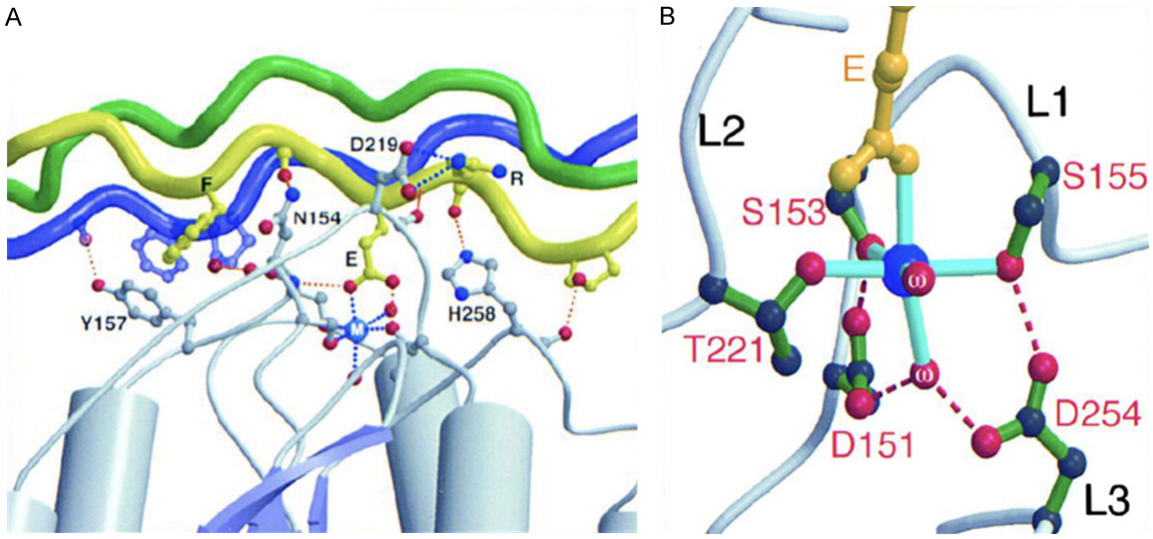

Figure 9. A. Close-up showing the I domain and collagen interface of receptor α2β1. Side chains are displayed as balls and sticks with the metal ion labeled as M. B. Close-up view of MIDAS domain. The metal ion is displayed as a blue ball while H-bonding is displayed as dashed-lines and water molecules depicted with the symbol ω [19].

located on the surface of platelets. Due to this receptor's specificity to platelets and its constitutive expression at approximately 80,000 copies per platelet, this receptor has long been an important target for antithrombotic agents [58]. GPIIb/IIIa is a heterodimer formed by one subunit each of IIb and IIIa, which are non-covalently associated [59]. The 130 kDa α-subunit and the 95 kDa β-subunit are coded by separate genes ITGA2B and ITGB3, which are both located on chromosome 17 and are independently expressed [59, 60]. Defects in the GPIIb/IIIa receptor can lead to Glanzmann's thrombasthenia, a rare autosomal recessive bleeding disorder [60, 61].

The crystal structure of GPIIb/IIIa is based on structures of similar integrin proteins αVβ3 and αIIbβ3. The α-subunit of αIIbβ3 contains four extracellular domains (a β-propeller domain, a thigh domain, and two calf domains), a single-pass transmembrane domain, and a cytoplasmic tail. The β-propeller domain serves as the primary domain for binding ligands [62]. The N-terminus of the α-subunit is made of amino acid repeats that orient into seven blades giving rise to the β-propeller model of αIIbβ3. Cationic binding sites within these blades are formed from aspartic acid, asparagine, and backbone oxygen atoms which function to maintain the rigidity of the β-propeller [63].

The β-subunit of αIIbβ3 comprises seven extracellular domains (a β3A domain, a hybrid domain, four epidermal growth factor (EGF) domains, and a membrane-proximal β-tail domain (βTD domain)), a single-pass transmembrane domain, and a cytoplasmic tail. The extracellular domains contain many highly conserved cysteine residues which form disulfide bonds. These disulfide bonds rearrange upon activation by forming free thiols and undergoing a disulfide exchange reaction [62]. The β-subunit N-terminus forms the β3A domain composed of six β-sheets surrounded by eight α-helices. The β3A domain contains three metal binding sites that allow the binding of different ligands (**Figure 10**). The MIDAS is formed by Asp119, Asp251, Glu220, Ser121, and Ser23. The ADMIDAS (adjacent to MIDAS) metal adhesion site orients its metal ion through Asp126, Asp127, and Ser123. The LIMBS (ligand-induced metal binding site) is formed by Asn215, Asp158, Asp217, Pro219, and Glu220 [64]. Determined from the structure of αVβ3, the main ligand binding site is in between the seven blades of the β-propeller of the αV-subunit and the β3-subunit. It is suspected that the same main ligand binding site exists between the αIIb-subunit and β3-subunit of GPIIb/IIIa. The A-domain metal binding sites have been shown to be necessary for the binding of fibrinogen and vWF. The MIDAS binds to aspartic acid in the bound ligand, and the ADMIDAS regulates ligand binding. Furthermore, the LIMBS facilitates stabilization of the ligand-receptor complex [64]. Interaction between the α and β sub-

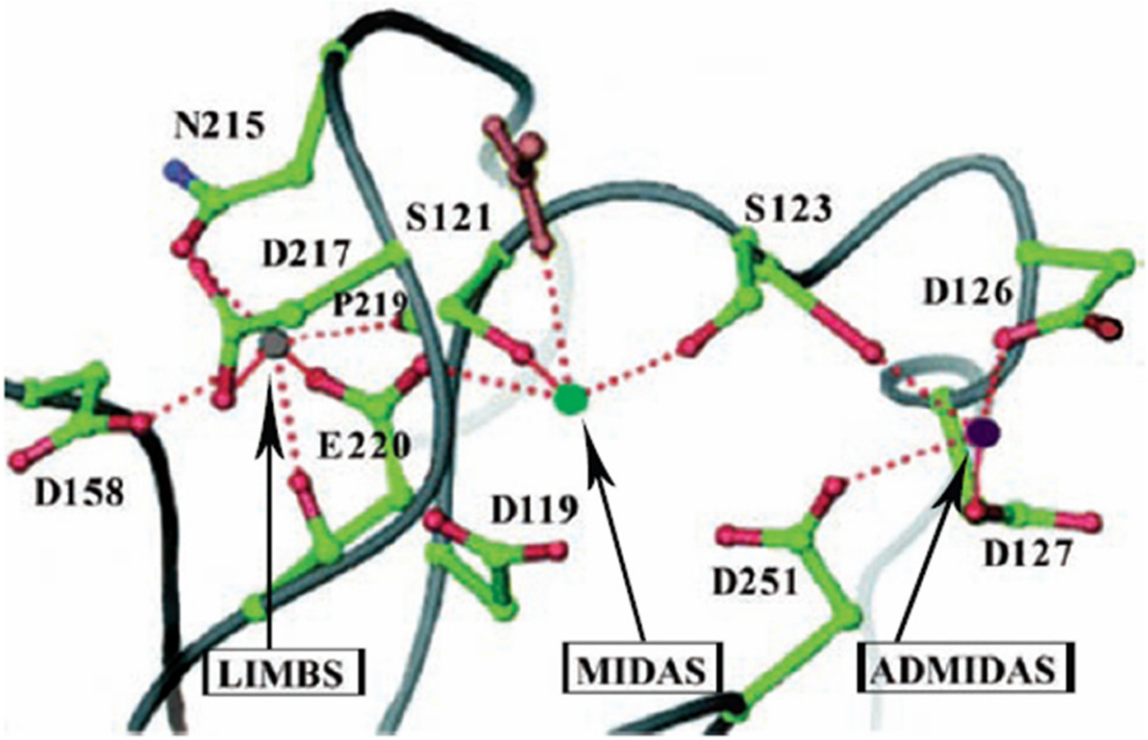

Figure 10. β3 A-domain metal binding sites of GPIIb/IIIa. Carbon atoms shown in green, nitrogen atoms in blue, and oxygen atoms in red. The bound cations of the LIMBS, MIDAS, and ADMIDAS are displayed as gray, green, and blue spheres respectively [64].

units of aIIbβ3 is mediated by the β-propeller domain and β3A domain, respectively [62].

To prevent uncontrolled platelet aggregation and unwanted thrombus formation, the activation of GPIIb/IIIa is tightly regulated through inside-out and outside-in signaling. The receptor has a low affinity for ligands in its resting state due to poor accessibility of the ligand binding site [60, 62]. Platelet activation is caused by different factors such as thromboxane A_2, ADP, subendothelial collagen, and thrombin [58]. All of these triggering agonists result in an increase in intracellular calcium concentration $[Ca^{2+}]_i$ through Ca^{2+} release from intracellular stores as well as from the extracellular environment through the plasma membrane. This subsequently initiates the steps of platelet activation, including conformational change and inside-out activation of the GPIIb/IIIa receptor [63].

During inside-out signaling, the binding of agonists such as ADP or thrombin to GPCRs triggers signaling pathways that involve key proteins such as protein kinase C (PKC), phosphoinositide 3-kinase (PI3K), and diacylglycerol-regulated guanine nucleotide exchange factor I (CalDAG-GEFI). This leads to the recruitment of actin cytoskeletal proteins kindlin and talin which interact with the cytoplasmic tails of GPIIb/IIIa [62]. The cytoplasmic tails are connected by hydrophobic and electrostatic bonds, which must be completely disrupted for the full activation of this receptor. The displacement of these bonds is facilitated by the talin head (talin-H) [65]. The cytoplasmic tails and transmembrane domains of the α2 and β3 subunits separate. Additionally, the extracellular domain of GPIIb/IIIa extends outward, exposing binding sites. This conformational change allows the receptor to bind to fibrinogen and other ligands with high affinity and avidity [66].

The clustering of integrins via various ligand and activation-dependent mechanisms is the start of outside-in signaling. The density of integrins increases when a soluble ligand is present at the extracellular surface, leading to the clustering and activation of tyrosine kinases such as c-Src, which associates with the β3-integrin tail. Other downstream signaling pro-

teins and cytoskeletal proteins such as Syk, focal adhesion kinase (FAK), and RhoGAP subsequently become activated through tyrosine phosphorylation [66]. The successful completion of bidirectional signaling (inside-out and outside-in) enables platelets to form cross-linkages between themselves and surrounding platelets via fibrinogen, thus allowing long chains of platelets to form [67, 68].

Platelets play a fundamental role in hemostasis by forming a plug at sites of vascular injury, preventing excessive blood loss. The GPIIb/IIIa (integrin aIIbβ3) receptor is one of the most important platelet receptors involved in this process. It plays a crucial role in platelet adhesion by binding to fibrinogen and other RGD-containing ligands such as fibronectin and vWF [69]. When an injury occurs to blood vessels, platelets form aggregates held together by fibrinogen-GPIIb/IIIa interaction, which restricts their movement and ultimately leads to clot formation. Activation of GPIIb/IIIa is the final and most critical step of the platelet activation mechanism, which is tightly regulated through inside-out and outside-in signaling to prevent uncontrolled platelet aggregation and unwanted thrombus formation. Studies have suggested that endogenous and exogenous estrogen may inhibit GPIIb/IIIa receptor activation, which could in part explain the higher rates of cardiovascular disease seen in postmenopausal women and the cardioprotective effect of early estrogen replacement therapy [13].

Serotonin

The 5-HT_{2a} receptor is encoded by the HTR2A gene located on chromosome 13. This gene spans approximately 66 kB and is composed of seven exons responsible for the 471-amino acid sequence that composes this receptor [70]. 5-HT_{2a} is a member of the GPCR family, similar to other receptors described in previous sections. Computer modeling of this receptor was performed by Sencanski et al. based on the β2-adrenergic receptor due to 23 percent similarity. Ligand docking studies were performed with enantiomers (2R, 4S and 2S, 4R) of *trans*-4-phenyl-N, N-dimethyl-2-aminotetralin (PAT) and 4-chlorophenyl-PAT, a novel 5-HT_{2C} agonist/5-$HT_{2A/2B}$ receptor antagonist. These studies revealed that the binding pocket for this residue is composed of the following residues: D3.32, S3.36, W6.48, F6.51, F6.52, and Y7.43 (**Figure 11**) [71]. Ligand models with (2S, 4R)-PAT indicated that this molecule forms branched hydrogen bonds with the carboxylate substituent of D3.32 as well as the *para*-hydroxyl group of Y7.43 [72].

5-HT_{2a} GPCR is activated at the site of injury by serotonin (5-HT). Upon activation, platelets release 5-HT from stores of internal dense granules subsequently activating other platelets. This action also stimulates vascular contraction which serves to promote thrombus formation [73]. Unfortunately, significant measures of this action have proven difficult. Satoh et al. speculate that this difficulty may be due to limitations in optical density-based platelet aggregometry. Przyklenk et al. postulate that this associated difficulty is due to a lack of targeting specificity of the 5-HT_{2a} receptor subtype by the agonists being tested [74]. Tests of the novel 5-HT_{2a} receptor antagonist APD791 (3-Methoxy-N-(3-(1-methyl-1H-pyrazol-5-yl)-4-(2-morpho-linoethoxy)phenyl)benzamide) have shown this compound to be an effective, orally active, antagonist of the 5-HT_{2a} receptor. APD791 shows a binding affinity 2,000 times greater for receptor subtype 5-HT_{2a} than 5-HT_{2b} or 5-HT_{2c}, as well as inactivity when tested against other G-protein-coupled receptors [75]. The tests of APD791 supported the theory that 5-HT_{2a} is an augmentative activator. When pretreated with APD791, platelet-rich plasma from an *in vivo* canine model did not display the 5-HT_{2a}-amplified aggregation, suggesting that the receptor is a viable target for cardiovascular drugs as thrombosis was effectively blocked without increased bleeding time [74]. Similar results were reported in prior animal studies and some human studies using other 5-HT_{2a} receptor antagonists [76-80].

However, Bampalis et al. investigated the effect of serotonin receptor antagonists on human *in vitro* platelet activation in blood exposed to physiological stimuli and reported conflicting results. Although this group supported the findings that 5-HT_{2A} receptor antagonists inhibit platelet aggregation in the presence of exogenous serotonin, their experiments indicated that endogenous 5-HT released as a result of platelet stimulation does not promote the formation of platelet aggregate structures or adhesion stimulated by atherosclerotic plaque

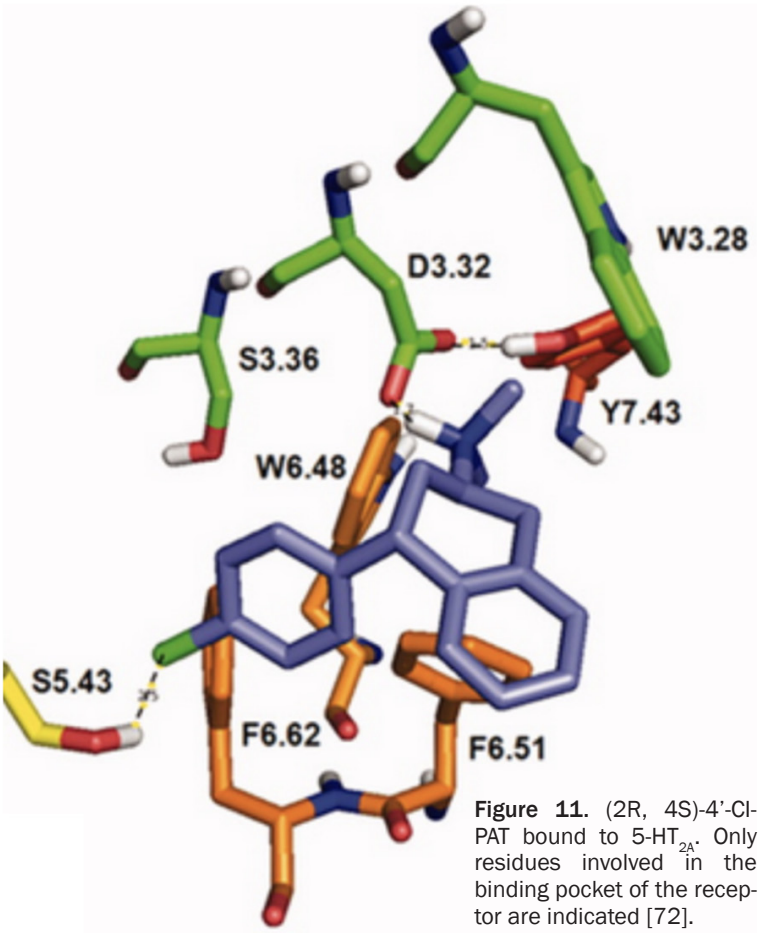

Figure 11. (2R, 4S)-4'-Cl-PAT bound to 5-HT_{2A}. Only residues involved in the binding pocket of the receptor are indicated [72].

under arterial flow conditions [81]. Thus, Bampalis et al. concluded that the 5-HT_{2A} receptor is not a prime target for antiplatelet therapy due to the antagonism of the receptor having no effect on platelet activation as a result of collagen, thrombin, or pathologic stimuli of atherosclerotic plaque. One prior double-blinded randomized crossover study of a 5-HT_{2A} receptor antagonist in patients with Raynaud phenomenon showed a nonsignificant decrease in platelet aggregation *in vitro* in response to exogenous serotonin and no change in platelet aggregation in the presence of ADP, collagen, and other aggregating agents [82]. However, the results may not be generalizable to healthy individuals or those with other platelet-related conditions. This study was also limited by a small sample size (n=23) like prior human studies in this area. Furthermore, it is possible that the structure and function of human 5-HT_{2A} receptors differ from other species studied previously. For example, the 5-HT_{2A} receptor may have a higher affinity to 5-HT in some animal species, which could explain, at least in part, the lower sensitivity of platelets to 5-HT_{2A} receptor antagonists observed in these human studies. Further research is needed to fully determine the role of the 5-HT_{2A} receptor in thrombus formation.

Conclusions

Cardiovascular disease and acute coronary syndrome have been major contributors to morbidity and mortality for a large portion of the world. CVD in the form of myocardial infarction, heart failure, stroke, etc. has been attributed to numerous variables including race and genetics. ACS manifesting itself in the form of STEMI, NSTEMI, or unstable angina is seemingly on an upward trend. Both CVD and ACS are attributed to the untimely, unwanted, or overactivation of platelets. Although platelets largely circulate in the body in their inactive form, the aforementioned pathologic conditions are a direct result of platelet function.

The creation of new and improved pharmaceuticals which target platelet receptors and contain antithrombotic potential is a highly active area of research and development. Although this paper reviewed the 10 major platelet surface receptors all leading to the activation of GPIIb/IIIa, other types of platelet receptors can mediate platelet activity and are also potential drug targets. Due to platelets being responsible for a broad range of biological processes unrelated to hemostasis, locating druggable targets with minimum adverse side effects has proven to be difficult. Due to limitations and the extensive research required to understand the role of newly identified receptors, difficulties associated with differentiating receptor subtypes and x-ray crystallization or in-silico modeling of potential targets renders antithrombotic agents challenging to synthesize. Research on serotonin receptor antagonism, which seemingly

influences platelet aggregation, is ongoing. The inhibition of this receptor might be one of the many future antithrombotic targets despite the conflicting studies published thus far.

Disclosure of conflict of interest

None.

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