Unravelling the mechanism and significance of thrombin binding to platelet glycoprotein Ib

Zaverio M. Ruggeri1; Alessandro Zarpellon1; James R. Roberts1; Richard A. Mc Clintock1; Hua Jing1; G. Loredana Mendolicchio2

1Roon Research Center for Arteriosclerosis and Thrombosis, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California, USA; 2IRCCS Istituto Clinico Humanitas, Milan, Italy

Summary
The main question concerning the mechanism of α-thrombin binding to platelet membrane glycoprotein (GPIb) is whether it involves both thrombin exosite I and exosite II. The solution of two independent crystal structures suggests alternative explanations that may actually reflect different modes of binding with distinct pathophysiological significance. With respect to function, it is still unclear whether thrombin binding to GPIb promotes procoagulant and prothrombotic pathways of response to vascular injury or limits such responses by sequestering, at least temporarily, the active enzyme. We review here published information on these topics and touch upon ongoing studies aimed at finding definitive answers to outstanding questions relevant for a better understanding of thrombosis and haemostasis.

Keywords
Thrombin, GPIb, von Willebrand factor, thrombosis, animal models

Remembering Oscar Ratnoff and Ted Zimmerman: The path to thrombin and glycoprotein Ib

Oscar Ratnoff was a giant in the field of blood coagulation studies and his scientific legacy still influences our understanding of haemostasis and thrombosis. As all great scientists, he mentored younger colleagues and some became members of his own league. One was Theodore (Ted) Zimmerman, who worked till his last days to explain the origin of congenital coagulation disorders focusing on haemophilia (1) and von Willebrand disease (2), and greatly contributed to improving diagnosis and treatment of affected patients (3). Oscar studied how the clotting cascade leads to thrombin generation (4), and Ted how von Willebrand factor (VWF) binds to its platelet receptor, glycoprotein (GP) Ib (5). In many ways, they were the inspiration for studying mechanisms and biological significance of the interaction between thrombin and GPIb.

Platelets contribute to the generation and biological activities of thrombin, the final product of the coagulation cascade. In turn thrombin, through protease-activated receptors, is a key player in platelet activation and function (6). In this context, the role of GPIb remains unclear as it provides by far the most abundant thrombin-binding site on the platelet membrane but with poorly understood functional consequences. To address this problem, efforts have been aimed at clarifying the molecular bases of thrombin association with GPIb, with the assumption that structural information might provide the background for functional experiments capable of shedding light on pathophysiological mechanisms. The results obtained to date are briefly reviewed here, following a general introduction on platelet function in health and disease.

Background on platelet biology and glycoprotein Ib structure and function

Mammalian platelets are anucleated fragments of cytoplasm released into blood by bone marrow resident megakaryocytes (7, 8). As such, they can not control gene expression but have a translational machinery for protein synthesis (9). Platelets perform key roles in haemostasis, the defense mechanism that prevents blood loss from traumatic tissue injuries (10, 11), and their function is initiated by adhesion to the vessel wall where alterations of the endothelial cell lining are detected (12). Platelets also control vascular permeability (13), exhibit antimicrobial activities (14, 15), and support cytotoxic T-lymphocytes in clearing viral infection (16), thus contribute to host defense with a role linking haemostasis and inflammation (17, 18). In this regard, mammalian platelets function like the nucleated thrombocytes of all other vertebrates (19–21). Platelets and the haemostatic system, however, can not discriminate between traumatic wounds and other pathologic vas-
cular conditions. As a consequence, chronic vascular diseases can acutely induce massive platelet responses, usually initiated by the occurrence of destabilising events such as rupture of an atherosclerotic plaque, which lead to the formation of intravascular occluding aggregates and fibrin clots (thrombi) (22). Under such circumstances, the same processes that underlie the beneficial haemostatic function become a life-threatening disease mechanism, that by curtailing the supply of blood to vital organs, may precipitate ischaemic syndromes of the heart and brain resulting in death. In a similar fashion, inflammatory signals originating from pathological processes within organs may induce local platelet reactivity. Activated platelets, in turn, attract cytotoxic T-lymphocytes capable of causing tissue damage, as documented in a mouse model of viral hepatitis in which platelet depletion prevents the destruction of hepatocytes expressing a specific hepatitis B virus antigen (23, 24). Experimental animal studies, therefore, suggest that knowledge of the mechanisms regulating platelet interactions with other vascular cells may have far reaching consequences in advancing the comprehension and treatment of serious diseases.

The GPIb-IX-V receptor is an abundant platelet membrane component with multiple functions supported by a unique structure. It consists of a hetero-oligomeric protein complex assembled in megakaryocytes from four distinct gene products. These subunits are type-I leucine-rich transmembrane proteins: GPIbα (610 residues), GPIbβ (181 residues), GPIX (160 residues) and GPV (544 residues; all these values refer to the human proteins). There are ∼20,000 copies of GPIbα per platelet (25) but the stoichiometry of the complex is still debated (26). The N-terminal 290 residues of GPIbα, which include the binding sites for several ligands, form eight leucine-rich repeats (residues 19–204) flanked on either end by disulfide-bridge-containing loops: Cys4–Cys17 in the N-terminal flank; Cys209–Cys248 and Cys211–264 in the C-terminal flank (residues 205–268). The latter region also contains a functionally important anionic segment (residues 269–287) that includes three sulfated tyrosine residues at positions 276, 278 and 279 (27). The loop linking the amino terminal and mucine-like carbohydrate-rich macroglycopeptide domains may be inherently flexible, as suggested by the observation that it is disordered (i.e. with no clearly defined structure) in the crystal structure of the complex with VWF-A1 (28, 29), but ordered (i.e. with a defined structure) when bound to α-thrombin (30, 31).

The GPIb-IX-V complex interacts with VWF to form the primary link for platelet adhesion under elevated shear stress (22). In addition to VWF, GPIb-IX-V binds other ligands. The interaction with α-thrombin has been extensively studied. However, in spite of the known crystal structure (30, 31) and an interest spanning over more than thirty years (32), the functional significance of α-thrombin binding to GPIbα remains controversial. Interpretations vary with respect to the affinity of the interaction (33, 34), the pro-thrombotic or anti-thrombotic significance (35, 36), and the very mechanism of binding (37). Even less definitive information, in both structural and functional terms, has been obtained on the GPIb interactions with P-selectin (38), Mac-1 (integrin αMβ2) (39), coagulation factors XI (40, 41) and XII (42), and high-molecular-weight kininogen (43, 44). It is reasonable to assume that binding to P-selectin and Mac-1 support platelet-leukocyte contacts (45, 46), while binding to contact phase factors may play a role in generating procoagulant activity that appears to be independent of GPIbα interactions with VWF (47) and thrombin (unpublished personal observation). These, however, remain hypotheses to be proven as the corresponding biological functions involve multiple and potentially alternative pathways. Recently, evidence has been presented that platelets interact with protein C (PC) and activated protein C (APC), suggesting a role for GPIb in the regulation of this anticoagulant pathway (48). A clarification of these points will help comprehend how platelets in general, and GPIb in particular, may contribute to inflammatory and infectious processes. In conclusion, available evidence indicates that the GPIb-IX-V complex is a key regulator of diverse functions during haemostatic and inflammatory responses, orchestrating the activities of different ligands and, possibly, modulating the access of α-thrombin to different substrates.

### Mechanism of α-thrombin binding to GPIbα

It is striking that a bimolecular interaction studied by numerous investigators for more than 30 years (32, 36), and for which there are two known crystal structures (30, 31), remains poorly understood with respect to mechanisms involved and functional significance. It is generally accepted that GPIb is the major α-thrombin binding site on the platelet surface, with evidence for both high and intermediate affinity sites. In this regard, there are unsolved questions concerning the discrepancy between the number (∼15,000–20,000) of GPIb molecules expressed on human platelets (49) and that (∼6,000) of high and moderate affinity thrombin binding sites reported in the literature; also uncertain is the existence of low affinity and high capacity sites that may not be related to GPIb (50, 51). These issues still await a definitive answer, possibly difficult to attain because modifications of α-thrombin residues caused by the labelling necessary for direct binding studies may alter affinity and stoichiometry of the interaction with GPIbα.

### Exosite I, exosite II or both?

Different investigators using a variety of experimental approaches have concluded that both exosite I and exosite II of α-thrombin bind to GPIbα. For example, glycolalycin (the soluble, platelet-derived fragment comprising most of the GPIbα extracytoplasmic domain) competes with recognised exosite I ligands, such as fibrinogen, for α-thrombin binding (52). Hirudin, a selective and high-affinity exosite I ligand (53), interferes with α-thrombin binding to GPIbα (54) specifically competing at the 269–287 sequence (55). Nevertheless, at least four exosite I residues have been mutated without appreciable effects on GPIbα binding (37). On the other hand, molecules that associate with thrombin exosite II clearly in-
hhibit the interaction with GPIbα (56–59), and in this case muta-
genesis directed at exosite II residues has provided concordant re-
results (37, 60).

Two independently obtained crystal structures of the complex 
between GPIbα amino terminal domain (GPIbα-N), comprising 
290 (30) or 288 (31) residues of the mature protein, and α-throm-
bin reveal the existence of two distinct contact sites that, in the 
latter, involve exosites I or II with adjacent residues (Fig. 1A, B).
Superposition of the structures shows considerable differences 
between the two (Fig. 1C). Thrombin interacting through exosite I is 
rotated by nearly 180°, with axis of rotation centred on GPIbα
Leu196, such that contacts involve different residues in the two 
crystals. Moreover, a shift of ∼90° in the C-terminal loop of 
GPIbα-N (beginning at residue 246) changes the relative position 
of thrombin interacting through exosite II, which in one of the two 
crystals establishes additional contacts with GPIbα residues in 
the leucine-rich repeat region (for details, see below). Of note, in either 
case the interaction with GPIbα causes no significant conforma-
tional changes in α-thrombin (30, 31). It is important to consider 
that both crystal asymmetric units contain a 1:1 complex with only 
one or the other contact interface, since it is impossible for a 
GPIbα-N/α-thrombin pair to interact through both sites concur-
rently. However, pairs of symmetry-related complexes can be vis-
ualised showing that in the crystal lattice each α-thrombin and 
GPIbα molecule interacts with two distinct binding partners (Fig.
1). This structural evidence is no proof that both interaction sites 
are functionally relevant, as one could result from crystal packing 
with no contribution to complex formation. Thrombin mutagen-
enesis studies reported above (37), and the fact that residues involved 
in exosite I-mediated interactions are different in two distinct 
crystals while those involved in exosite II-mediated interactions 
are common, suggest that the latter are more likely to be function-
ally relevant. Such arguments, however, are not definitive; thus, 
while it seems clear that the α-thrombin exosite II/GPIbα-N inter-
face plays a key role in the interaction, as confirmed also recently 
(61), a function of the exosite I interface in the setting of thrombin 
binding to platelet GPIb remains a possibility.

Comparison of two α-thrombin/GPIbα-N crystal structures

The α-thrombin exosite I/GPIbα-N interface differs substantially 
in the Celikel et al. and Dumas et al structures (1OOK and 1P8V,
respectively; Fig. 2). In 1OOK, GPIbα-N residues interacting 
with α-thrombin at this site are located in leucine-rich repeats VI,
VII and VIII; in the α-helix comprising residues 218–223; and in 
the anionic loop between Asp269 and Asp277. These residues contact

Figure 1: Comparison of two crystal structures of α-thrombin in com-
plex with GPIbα-N. The main-chain (α-carbon) tracing is shown with tape 
(for α-helices and β-strands) or tube (for loops) representation, and relevant 
side chains are drawn in ball-and-stick; α-thrombin is shown with space fil-
ing in A and C (left), Th-I and Th-II designate α-thrombin molecules inter-
acting through exosite I or II, respectively. Distinctive colours are used to iden-
tify different molecules and are consistent in all panels (1OOK: GPIbα-N in 
cyan, Th-I in light green, Th-II in green; 1P8V: magenta, yellow and orange, 
respectively). The two crystal structures are represented with the same orien-
tation of GPIbα-N in A or α-thrombin in B. A molecule related by crystal sym-
metry is included in both panels to illustrate that one GPIbα-N can interact 
with two α-thrombin molecules (A) and, in turn, one α-thrombin with two 
GPIbα-N molecules (B). In B it is apparent that the α-thrombin active site is 
blocked by PPACK in 1OOK and DFP in 1P8V; both inhibitors are represented 
in ball and stick with yellow colour. C) Superposition of the two structures 
based on GPIbα on the left or α-thrombin on the right. The two GPIbα mole-
cules on the left superpose well, except for residues beyond Pro269 where 
the anionic tails go in separate directions ∼90° apart (see also Fig. 5A). As a 
consequence, the corresponding Th-II molecules (coloured in green and red) 
are in different positions. The two superposed GPIbα molecules shown on 
the left correspond to those below thrombin on the right, where it is evident 
that the anionic tail interacts with exosite I in only one of the two structures.
This is the consequence of a 180° rotation around GPIbα-N Leu196 relative to 
Th-I. The two GPIbα molecules seen on top of thrombin are at an ∼90° angle 
as a consequence of the anionic tail change of direction after Pro265.
numerous α-thrombin residues including Lys36-Glu39; Trp60d and Asp60e; Leu65-Met84 (all part of exosite I); Lys110; and Lys145-Ser153 (Fig. 2A). Note that in all figures shown here, as in Celikel et al. and most publications (62), α-thrombin residues are numbered according to chymotrypsinogen homology, thus residues in inserted sequences have the same number followed by a lower case letter in alphabetical order; in contrast, Dumas et al. number sequentially from residue 1 of the B chain. Correspondence between the two numbering schemes is not straightforward, and awareness of the discrepancy is important when comparing the two crystal structures. In 1P8V, relevant contacts involve Lys36(21), Lys109(106) and Arg77a(73) in α-thrombin (in parentheses the numbers from Dumas).

Figure 2: Stereo images comparing residues interacting at the α-thrombin exosite I/GPⅠbα-N interface in two crystal structures. The structures of Celikel et al. (1OOK, panel A) and Dumas et al. (1P8V, panel B) are presented in the same orientation relative to α-thrombin in the complex. Molecules are coloured and represented as in Figure 1 (Th-I, as identified in Fig. 1, is shown here). Residues with >10% buried surface are shown with thicker bonds. Labels for α-thrombin residues are underlined, and the corresponding sequence numbering is based on analogy with chymotrypsinogen in both panels. Note that the 148-loop in α-thrombin and the anionic region after Lys279 in GPⅠbα-N interact extensively in 1OOK, while the α-thrombin 148-loop is disordered in 1P8V.
et al.) and Lys237, Tyr228, Glu225 and Ser194 in GPIbα (Fig. 2B). The most notable difference at this interface is the absence in 1P8V of any involvement of the anionic GPIbα sequence between Asp269 and Asp287 (compare Fig. 2A and B), which includes three sulfated Tyr residues (Tyr276, Tyr278 and Tyr279) known to play a prominent role in α-thrombin binding (27). Of the three, Tyr279 establishes close bonds with residues in the α-thrombin loop Leu144-Gly150 flanking the exosite I loop, while Tyr276 establishes additional contacts with exosite I residues (Fig. 2A). It is noteworthy that Tyr276 and Tyr279, along with Thr273, are the only GPIbα residues that can interact with both exosite I and II (Fig. 3), albeit in distinct α-thrombin molecules. As a result of the differences outlined here, the α-thrombin exosite I/GPIbα-N interface is more extensive in 1OOK than 1P8V, with multiple salt bridges and hydrophobic interactions that may support a tight bimolecular complex once established.

The exosite II/GPIbα-N interface, unlike that involving exosite I, is similar in the two structures (Fig. 4), even though the corresponding thrombin molecules show a positional shift relative to GPIbα-N caused by a different conformation of the anionic region in the latter (see Fig. 1). The main GPIbα-N residues contacting α-thrombin exosite II are Tyr276 and Asp277. Note that the latter, Asp274 and Leu275, are the only GPIbα residues that can interact exclusively with α-thrombin exosite II (Fig. 3). The α-thrombin residues that interact strongly are Arg101, Arg126 and Lys236 (Fig. 4). Bimolecular contacts at this interface are more extensive in 1P8V than 1OOK. A substantial difference between the two structures is represented by contacts between residues on the convex face of GPIbα, within the Arg126-Gly170 region corresponding to leucine-rich repeats V-VII, and thrombin residues from the α-helix including Arg126(123) and Glu127(124). These contacts extending over ~800 Å² is 40% of the total 2000 Å² covered by the GPIbα-exosite II interface in 1P8V, are absent in 1OOK. As a consequence, the relative surface extension of the contact areas involving exosite II or exosite I is opposite in the two structures, the former being predominant in 1P8V and the latter in 1OOK.

**GPIbα and α-thrombin: Functional consequences of the interaction**

The pathophysiological relevance of α-thrombin binding to platelet GPIbα is still uncertain, and the picture emerging from ongoing studies with targeted animal models is not yet defined. A key question is: Are the consequences of thrombin binding to GPIbα prothrombotic or antithrombotic? Or can either response occur depending on different modes of interaction? In this respect, whether and how exosite I is involved in binding to GPIbα may have important implications, as most substrates of α-thrombin proteo-
lytic activity need to be bound to exosite I to be cleaved efficiently (63) and GPIb could act as a competing inhibitor. The balance of effects resulting from α-thrombin binding to GPIb in vivo could be complex, because thrombin substrates act in both procoagulant and anticoagulant pathways. Moreover, efficient α-thrombin inhibition by regulatory anticoagulant pathways requires exosite II availability, such that binding to GPIb through this interface might prolong the half-life of proteolytic activity with potentially variable consequences on both procoagulant and anticoagulant pathways.

A step forward towards addressing these issues has been taken with a published study reporting on the functional consequences of the mutation Tyr276Phe in human GPIbα expressed within the GPIb-IX-V complex of murine platelets (64). In essence, in vivo findings support the conclusion that this substitution results in a reduced platelet ability to form thrombi. The tail bleeding time of the animals expressing mutant and wild-type human GPIbα was not significantly different. Nonetheless, in different models of vascular injury thrombi formed more slowly, were of smaller size and less stable in mice expressing mutant as compared to normal human GPIbα. It is not significantly different. Nonetheless, in different models of vascular injury thrombi formed more slowly, were of smaller size and less stable in mice expressing mutant as compared to normal human GPIbα.

Effects of thrombin on the VWF-GPIb interaction

One possible consequence of α-thrombin binding to GPIbα is interference with VWF binding that, in view of the role played by the latter in early prothrombotic adhesive events (22), could greatly influence the functional balance resulting from the thrombin-GPIb interaction. In this respect, information based on the crystal structures of GPIbα-N in complex with VWF A1 domain (28, 29) or α-thrombin (30, 31) indicates that the mode of interaction between α-thrombin and GPIbα may potentially lead to opposite effects on VWF. Only thrombin bound through the exosite I inter-
face may influence VWF binding, as molecules bound through the exosite II interface in either of the known complex conformations (see Fig. 1) can not sterically hinder VWF A1. Of the two possible conformations of the α-thrombin exosite I/GPIbα-N association (see Figs. 1 and 2), the one seen in 1P8V is not compatible with concurrent binding of the VWF A1 domain. In contrast, the conformation seen in 1OOK can accommodate both VWF A1 domain and α-thrombin on the same GPIb molecule and actually create the possibility of close interactions between the two ligands with the potential for reciprocally enhancing effects on binding to the receptor.

The results of studies we are currently performing suggest that VWF binding to platelet GPIb certainly is not inhibited and may actually be enhanced in the presence of α-thrombin. Contrasting results have been obtained, however, with a mutant containing the substitutions Trp215Ala and Glu217Ala, designated WE-thrombin (65). This mutant has been reported to activate selectively protein C and display a potent antithrombotic effect in primates. Using a flow assay, immobilised WE-thrombin was found to support platelet tethering and rolling as efficiently as wild-type thrombin and the phenomenon was blocked by an anti-GPIb antibody, a result taken to indicate normal WE-thrombin interaction with GPIb. In contrast, WE-thrombin but not wild type thrombin abrogated platelet tethering and rolling on immobilised VWF as well as platelet deposition onto collagen under shear flow, suggesting that the mutant but not wild-type thrombin interacts with platelets GPIb in a manner that leads to inhibition of GPIb-dependent VWF function (65). These functional results suggest some intriguing conclusions. i) Thrombin binds to GPIb in a manner that may interfere with VWF binding. ii) The thrombin-GPIb interaction must involve the exosite I interface, as this is the only conformation of the complex that may lead to inhibition of VWF binding (see above). iii) The fact that wild-type thrombin does not inhibit VWF binding to GPIb, as shown by our unpublished data and control results in the WE-thrombin study, indicates that the interaction with GPIb mediated by the exosite I thrombin interface may indeed occur through two distinct conformations, one seen by Dumas et al. (1P8V, inhibitory) and the other by Celikel et al. (1OOK, non-inhibitory). Whether the latter actually may enhance VWF binding to GPIb is an additional possibility to evaluate.

It is intriguing to think that thrombin properties, as evidence by the WE mutation, may influence the conformation of the complex with GPIb. In this regard, GPIbα residues in the anionic region between Asp269 and Asp287 may play a key role as they have been shown to assume at least three different conformations (Fig. 5A) allowing: i) No binding of α-thrombin but permissive of VWF A1 domain binding. ii) Binding of one α-thrombin molecule through exosite I with no exosite I interactions (seen in 1P8V); in this case, the latter binds to another GPIbα molecule in a conformation not permissive of concurrent VWF A1 domain binding. iii) Binding of

Figure 5: Possible modes of interaction between α-thrombin and GPIbα. A) The anionic region of GPIbα-N following Pro265 (see Fig. 3) is shown in three different conformations, seen in published crystal structures, that are compatible with binding of no, one, or two thrombin molecule(s). This GPIbα-N region has an ordered conformation in three out of nine crystal structures known so far, including one uncomplexed (67) and two complexed with α-thrombin (30, 31); it is disordered in the remaining six structures, including complexes with VWF A1 domain. Note that in the ordered free GPIbα-N conformation (1GW 8-B, red), this GPIbα-N region is stabilised in a closed conformation not compatible with α-thrombin binding. In the presence of bound α-thrombin, it may be stabilised by one molecule, interacting through exosite II (1P8V, pink) or two molecules, one interacting through exosite I and the other through exosite II (1OOK, cyan; only the former is shown for clarity). The transition from one conformation to the other may have biological implications. For example, the conformation seen in 1P8V may correspond to that of a stable soluble complex; the conformation seen in 1OOK may be that of α-thrombin bound to GPIbα on platelets, and is compatible with VWF A1 domain binding. B) Putative interaction on platelets, showing the binding of one α-thrombin molecule to two GPIbα molecules involving both thrombin exosites. The spatial orientation of thrombin and GPIbα amino terminal domains is in agreement with the crystal structure shown on the left (1OOK), while the remaining portion of the GPIbα molecule is depicted arbitrarily. C) Putative interaction in solution between α-thrombin and GPIbα-N (amino terminal fragment) mediated solely by exosite II. The representation of putative stable and unstable complex conformations is based on the crystal structures shown on the left (superposition of 1OOK and 1P8V showing Th-H; see Fig. 1) but is hypothetical since the correspondence between a specific conformation and stability of the interaction has not been proven experimentally.

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two α-thrombin molecules, one through interactions involving exosite II and the other exosite I (seen in 1OOK); in this case, con-
formation of the complex allows concurrent VWF A1 domain binding. These considerations, and the potentially relevant func-
tional consequences on haemostasis and thrombosis they entail, make it even more compelling to arrive at a definition of the mode of interaction between α-thrombin and platelet GP Ib in the presence or absence of VWF, as well as to elucidate reciprocal effects of one GP Ib ligand on the other.

Ongoing and future studies addressing unresolved questions

We are currently testing the hypothesis that thrombin binding to GP Ib involves both exosite I and II (Fig. 5B, C). Available structural information, as discussed above, indicates that the possibility exists and directs the use of mutagenesis strategies and specific inhibitors to obtain definitive evidence corroborated by functional results. An important consideration in this regard is that GP Ibα is an integral membrane protein and, thus, has constrained mobility on the platelet surface where it can be expressed at high density. A soluble fragment may not properly represent such conditions; consequently, functional studies are ongoing to compare the characteris-
tics of α-thrombin binding to GP Ibα immobilised on a sur-
face or in solution, considering that the latter reflects the procedure followed to obtain the complex required for crystallisation. The hypothesis being tested, corroborated by preliminary results, is that the exosite II contact site may be sufficient to form a soluble complex but not to bind to immobilised GP Ib; the latter may require initiation by exosite II-mediated interactions but also a necessary consolidation supported by exosite I-mediated inter-
actions (Fig. 5B, C). These ongoing studies involve recombinant GP Ibα fragments with wild-type or mutated sequence and gen-
eration of genetically modified mouse platelets in which human GP Ibα, again with wild-type or mutated sequence, replaces the murine counterpart in the GP Ib-IX-V complex (66). These tools will allow interrogating the role of specific residues in mediating α-thrombin binding, for example targeting specifically residues that should be crucial for interactions involving the exosite I or exo-
site II interfaces. Of particular discriminative value in this regard will be the mutation of Tyr to Phe, a substitution that prevents sulfation of a residue key to exosite I-mediated binding, or Asp to Asn, replacing a residue that is distinctly central to exosite II-mediated interactions. The results obtained with these ongoing studies should lead to a definitive clarification of the mechanism supporting thrombin binding to GP Ib and set the stage for plan-
ning and interpreting functional studies aimed at elucidating the potentially diverse consequences of this fascinating interaction.

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