Thrombin–Cofactor Interactions
Structural Insights Into Regulatory Mechanisms

Ty E. Adams, James A. Huntington

Abstract—Precise modulation of thrombin activity throughout the hemostatic response is essential for efficient cessation of bleeding while preventing inappropriate clot growth or dissemination which causes thrombosis. Regulating thrombin activity is made difficult by its ability to diffuse from the surface on which it was generated and its ability to cleave at least 12 substrates. To overcome this challenge, thrombin recognition of substrates is largely controlled by cofactors that act by localizing thrombin to various surfaces, blocking substrate binding to critical exosites, engendering new exosites for substrate recognition and by allosterically modulating the properties of the active site of thrombin. Thrombin cofactors can be classified as either pro- or anticoagulants, depending on how substrate preference is altered. The procoagulant cofactors include glycoprotein Ibα, fibrin, and Na+, and the anticoagulants are heparin and thrombomodulin. Over the last few years, crystal structures have been reported for all of the thrombin-cofactor complexes. The purpose of this article is to summarize the features of these structures and to discuss the mechanisms and physiological relevance of cofactor binding in thrombin regulation. (Arterioscler Thromb Vasc Biol. 2006;26:1738-1745.)

Key Words: thrombin ■ hemostasis ■ regulation ■ cofactor ■ specificity

Damage to the blood vessel wall initiates the hemostatic response by exposing subendothelial proteins such as tissue factor and collagen.1,2 Crucial to the formation of the blood clot is the generation of the serine protease thrombin.3 Inability to generate sufficient amounts of thrombin in a timely manner results in hemorrhage, as observed in the bleeding disorders hemophilia A and B,4 whereas overproduction or failure to efficiently inhibit thrombin activity results in thrombosis. Thrombin has a broad range of diverse substrates that includes both pro- and anticoagulant functions (Figure 1). The activation of platelets through cleavage of protease-activated receptors (PARs) 1 and 4 and the cleavage of fibrinogen to fibrin are the primary procoagulant functions of thrombin,2,5 which serve to form the platelet plug and generate the fibrin meshwork that stabilizes the plug at the site of injury. The formation of a stable clot also depends on the ability of thrombin to stimulate its own generation through feedback activation of the protein cofactors V and VIIIa, thereby blunting thrombin generation. Thrombin also participates directly in its final inhibition and clearance from the circulation by specifically recognizing the serpins antithrombin (AT) and heparin cofactor II (HCII).

Thrombin is generated from its zymogen prothrombin through proteolytic cleavage at 2 sites (arginines 271 and 320) by factor Xa. The resulting 37-kDa serine protease thrombin is no longer associated to its Gla and 2 Kringle domains and is thus free to diffuse away from the surface on which it was generated, and its special structural features are used to recognize a broad spectrum of substrates.12-15 The first structure of thrombin, solved in 1989, revealed an overall protein fold very similar to other members of the chymotrypsin family of proteases, including the characteristic catalytic triad of histidine 57, aspartate 102, and serine 195 (chymotrypsin template numbering is used throughout) in an active site cleft.16 The structure of thrombin differs from that of its simpler cousins by the presence of extension loops that line the walls of the active site cleft, the 60- and γ-loops, and 2 regions of positive electrostatic potential known as anion-binding exosites I and II (Figure 2). Biochemical and mutagenesis studies have established that thrombin recognition of substrates invariably involves engagement of the active site cleft and either one17-19 or both20-22 of the exosites (see...
In addition to direct interaction with substrates, thrombin specificity is also regulated through interaction with several cofactors (Table). Cofactor binding regulates thrombin activity toward various substrates by mechanisms such as localization, competition for exosite binding, and allostery. Although much is still unknown about how thrombin specificity is determined, within the last few years all of the thrombin-cofactor crystal structures have been determined (Figure 3). This review summarizes the structural features of the thrombin-cofactor complexes and the significance of cofactors in directing the activity of thrombin.

**Glycoprotein Ibα**

A fraction of thrombin generated during the early stages of coagulation binds to platelet glycoprotein (GP) Ibα (GpIbα), 1 of 4 proteins of the GpIB-IX-V platelet receptor complex. GpIbα is a heavily O-glycosylated transmembrane protein that has been hypothesized to be the high-affinity binding site for thrombin on the platelet surface. GpIbα acts as a cofactor by enhancing thrombin cleavage of the platelet receptor PAR-1, resulting in platelet activation. Through binding to the third apple domain of factor XI, GpIbα also stimulates the activation of factor XI by thrombin. Finally, GpIbα can additionally promote platelet activation through enhancing thrombin cleavage of GpV. Several studies using mutations and synthetic peptides identified the thrombin binding site on GpIbα as the negatively charged region 271 to 284, including 3 sulfated tyrosine residues, 276, 278, and 279. The binding site for GpIbα on thrombin has been localized to exosite II, with mutations in this exosite displaying a marked decrease in affinity for GpIbα, whereas similar mutations in exosite I of thrombin showed little or no effect. Exosite II residue arginine 233 is critical for the interaction, with the R233A and the R233A/K236A/Q239A mutations reducing the affinity for GpIbα by 29- and 31-fold, respectively. A complementary study found that thrombin residues in exosite I are involved directly in binding to substrates factor XI and PAR-1 further supporting exosite II as the only cofactor binding site for GpIbα.

In 2003, 2 crystal structures of the N-terminal domain of GpIbα in complex with thrombin were solved. Both structures revealed an interaction between the negatively charged tail of GpIbα and exosite II of thrombin (Figure I in the online data supplement, available at http://atvb.ahajournals.org), with arginine 233 making contacts with several backbone oxygen atoms of the acidic region of GpIbα. Both structures also showed interactions involving GpIbα residues in the acidic region, 275 to 279, including 2 of the 3 sulfated tyrosines at positions 276 and 279. However, the 2 structures showed some important differences as to the precise binding interactions involved. The structure by Dumas et al contained several salt bridges in this region: the sulfate of tyrosine 276 interacts with thrombin residues arginine 126 and lysine 236, with lysine 235 in close proximity; sulfated tyrosine 279 forms a salt bridge with thrombin residue lysine 240, with arginine 93 nearby; and aspartate 277 of GpIbα interacts with arginine 101 of thrombin. Interestingly, in the structure by Dumas et al, sulfation of tyrosine 278 would have allowed an additional salt bridge with lysine 236 (tyrosine 278 was not sulfated in the construct used). The significance of these interactions is illustrated by the charge reversal mutation K236E on thrombin, which reduces affinity for GpIbα by 25-fold. By comparison, the structure by Celikel et al contains only 2 salt bridges: sulfated tyrosine 276 with arginine 126 of thrombin; and aspartate 277 of...
GpIbα with arginine 101. Additionally, lysine 236 hydrogen bonds to tyrosine 278 of GpIbα, yet does not interact with sulfated tyrosine 276. The dependence of affinity on ionic strength suggests the involvement of approximately 4 ionic interactions,\textsuperscript{35} consistent with the structure by Dumas et al more closely representing the interaction that takes place in solution. Although both structures also found contacts between the acidic tail of GpIbα and exosite I of thrombin, for the reasons described above, such an interaction is unlikely to be relevant to the cofactor activity of GpIbα.

**Fibrin**

One of the most important roles of thrombin in blood coagulation is the cleavage of the plasma protein fibrinogen (for review see Mosesson et al\textsuperscript{40}). Fibrinogen is a 340-kDa protein composed of 2 identical subunits of 3 polypeptide chains (Aα, Bβ, and Gγ) linked head-to-head, with the N-terminus of each subunit creating the central E domain (supplemental Figure II). On cleavage of fibrinopeptide A (FpA) by thrombin, the fibrin monomers assemble into protofibrils where subsequent cleavage of fibrinopeptide B (FpB) triggers lateral association into fibrils that provide the scaffold for the growing thrombus.\textsuperscript{17,41–44} These newly generated fibrin polymers can then act as a cofactor for the thrombin activation of the transglutaminase factor XIII, which cross-links the fibrin to strengthen the clot (supplemental Figure II).\textsuperscript{9,45–47} Factor XIII deficiency has been shown to greatly decrease clot strength and leads to severe bleeding diathesis.\textsuperscript{48,49} Fibrinogen/fibrin contains 2 thrombin binding sites.\textsuperscript{50} With a $K_d$ of 2 to 5 $\mu$mol/L (Table), the low-affinity binding site has been shown by mutagenesis, peptide, and modeling studies to encompass residues at the

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>$K_d$ (nmol/L)</th>
<th>Substrate and Fold Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GpIbα</td>
<td>50–200</td>
<td>PAR-1 (5–7); Gv (6–10)\textsuperscript{10}; Factor XI (5000)\textsuperscript{18}</td>
</tr>
<tr>
<td>Fibrin</td>
<td>300–7000</td>
<td>Factor XIII (80)\textsuperscript{47}</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>3–10</td>
<td>PC (1000)\textsuperscript{45}; TAFI (1250)\textsuperscript{51}</td>
</tr>
<tr>
<td>Glycosaminoglycan</td>
<td>6000–10 000</td>
<td>AT(1000)\textsuperscript{52}; HCII (1000)\textsuperscript{57}</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>$25 \times 10^5$–$150 \times 10^6$</td>
<td>Fibrinogen (7–20)\textsuperscript{102,103}</td>
</tr>
</tbody>
</table>

References not indicated are included in the text of the article. The magnitude of activation effect is shown in parentheses.

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**Figure 3.** Thrombin-cofactor exosite interactions. The surface representation of thrombin is shown in the standard orientation (left) for exosite I interactions (Protein DataBank entries: fibrin-1QVH; TM-1DX5) and rotated 90° (right) to show exosite II interactions (PDB entries: GPIbα-1P8V, heparin-1XMN). The thrombin residues involved at the cofactor interface (<4 Å distant) are colored as green.
N-terminal flexible tails of both the Aα and Bβ chains of fibrinogen.51–53 Analysis of the chicken fibrinogen structure revealed a negatively charged region composed of residues 35 to 40 of the Aα chain and 68 to 71 of the Bβ chain.54 Additionally, 2 mutagenesis studies identified residues within exosite I, the Na\(^+\)-binding site, and active site of thrombin as potentially important for interaction with fibrinogen.55,56 The crystal structure of active site-inhibited human α-thrombin in complex with the central E domain fragment of human fibrin57 (Figure 3) confirmed the biochemical studies showing thrombin interacting with fibrin via exosite I residues phenylalanine 34, serine 36a, leucine 76, arginine 77a, isoleucine 82, and lysine 110. Although the interface juxtaposes complementary charged regions on thrombin and fibrin, hydrophobic interactions provide the bulk of the binding energy. The high affinity binding site (\(K_d\approx0.1\,\mu\text{mol/L}\)) on fibrin involves the engagement of thrombin exosite II with the fibrin γ' chain (present in approximately 10% of fibrin monomers)58,59 along with the simultaneous binding of exosite I as described above.60 The C-terminal portion of the γ' chain contains negatively charged residues, including sulfated tyrosine, and is assumed to bind equivalently to other exosite II ligands.53,59,61 Whether the binding of thrombin to the γ' chain plays a role in the activation of factor XIII, which has also been shown to bind to the γ' chain of fibrin,62 is unclear.

**Thrombomodulin**

Thrombomodulin (TM) is expressed on the surface of endothelial and cells and binds thrombin with high affinity (Table). Binding to TM alters thrombin specificity from procoagulant substrates toward activation of the anticoagulant protease protein C (Figure 1).63,64 When bound to TM, thrombin also activates the carboxypeptidase B-like enzyme thrombin activatable fibrinolysis inhibitor (TAFI).65 TM is organized into several distinct regions: an N-terminal lectin domain, 6 contiguous epidermal growth factor (EGF)-like domains, an O-glycosylated serine-threonine rich region, a transmembrane region, and a short cytoplasmic tail. The binding site for thrombin has been localized by alanine-scanning mutagenesis and peptide-binding studies to the final 2 EGF domains, EGF56,66–68. Thrombin interacts with TM primarily through exosite I, yet exosite II can also be involved in the presence of a chondroitin sulfate (CS) moiety found within the serine/threonine-rich region on approximately 20% to 35% of TM molecules.69 Although binding of the EGF56 fragment efficiently competes for thrombin binding to full-length TM, it fails to provide any cofactor activity. Addition of the preceding EGF4 domain is necessary for cofactor function67,70,71 and the 3 orders of magnitude increase in rate of activation of protein C.11 EGF4 of TM has been shown to interact directly with protein C,72 providing a supplementary exosite to bring about effective substrate recognition. Further addition of the EGF3 domain is required to stimulate activation of TAFI.73 TM induces a change in thrombin specificity significantly by blocking the binding of procoagulant substrates which depend on an interaction with exosite I. Protein C inhibitor (PCI) has recently been shown to efficiently inhibit thrombin in the presence of TM, presumably through direct interactions between PCI and TM.74

In 2000, the crystal structure of the complex between human thrombin and the minimal cofactor fragment of TM, EGF456, revealed the features of the TM–thrombin interaction and corroborated earlier biochemical information (Figure 3).75 A reasonably small contact interface of \(\approx900\,\text{Å}^2\) is seen between exosite I of thrombin and EGF domains 5 and 6 of TM. Although the surfaces at the interface display charge complementarity, which may aid in steering the 2 fragments together,76 only a single salt bridge exists between lysine 110 of thrombin and aspartate 461 of TM. The majority of binding energy is supplied by hydrophobic contacts, exemplified by isoleucine 414 and 424 on TM; isoleucine 414 inserts into a hydrophobic cavity on thrombin surrounded by phenylalanine 34, tyrosine 76, and isoleucine 82, and isoleucine 424 interacts with leucine 65 of thrombin. Confirming the importance of these hydrophobic contacts, mutation of isoleucine 414 and 424 to alanine reduced the affinity of TM for thrombin by 25- to 30-fold.68 The TM-thrombin complex crystal structure revealed no obvious conformational changes within the active site of thrombin, but the presence of a bound active-site inhibitor clouds this issue. However, allostery may play a role in switching the substrate preference of thrombin, as several biochemical studies, including fluorescence and amide exchange studies, suggest a tightening of the active site in response to TM binding.77–79 The cofactor effect of TM may also be partially attributable to an improved accessibility of the activation peptide of TM-bound protein C.80,81

**Heparin and Other Glycosaminoglycans**

Final inhibition of thrombin is primarily the responsibility of members of the serpin family of serine protease inhibitors.82 One common feature of these serpins that inhibit thrombin is the ability to bind to and be activated by heparin-like glycosaminoglycans (GAGs). GAGs, such as heparan sulfate, chondroitin sulfate, and derman sulfate, are long-chain sulfated polysaccharides attached to proteoglycans that coat the surface of cells lining the vascular and extravascular spaces. Heparin, the widely used anticoagulant drug, is a relatively small and uniform GAG secreted by mast cells. GAGs bind to thrombin with micromolar affinity and have been shown to significantly accelerate the inhibition of thrombin by serpins through a bridging mechanism.83,84 The 2 principal circulating thrombin inhibitors are the serpins antithrombin (AT) and heparin cofactor II (HCII). The rate of thrombin inhibition by AT is enhanced by \(\approx1000\)-fold in the presence of heparin and heparan sulfates,85,86 and inhibition by HCII is accelerated to a similar degree by either heparin or derman sulfate.87,88 Mutagenesis studies have identified the heparin binding site of thrombin as exosite II and includes (in order of importance) arginine 93, lysine 236, lysine 240, arginine 101, and arginine 233.89–91 Biochemical work has also shown a minimal heparin binding site size of 6 monosaccharide residues, with binding strongly dependent on ionic strength, suggesting a nonspecific electrostatic association of 5 to 6 ionic interactions.92 Whereas smaller heparins can activate AT toward factors IXa and Xa, activation of thrombin inhibition is only realized for a heparin molecule of at least 18 monosaccharide units in length.93 This is attributable to the requirement that a single heparin molecule bridge AT
and thrombin simultaneously. A similar bridging mechanism is in operation for the GAG acceleration of HCII inhibition of thrombin\textsuperscript{94}; however, allosteric activation of HCII also plays an important role.\textsuperscript{95,96}

The recent crystal structure of human thrombin bound to an 8-monosaccharide heparin fragment revealed the molecular basis of the interaction (Figure 3).\textsuperscript{97} The structure shows how thrombin engages heparin through the interposing of positively charged residues (histidine 91, arginine 93, arginine 101, arginine 126, arginine 165, lysine 236, and lysine 240) with the negatively charged sulfate groups of heparin in a predominantly ionic interaction. A length of 6 monosaccharide units is necessary to achieve full occupancy of the heparin binding site,\textsuperscript{92} and only 6 of the 8 monosaccharide units can be seen in the crystal structure. In 2004, 2 crystal structures of the thrombin–AT–heparin ternary complex were solved, using catalytically inert thrombin.\textsuperscript{98,99} Both structures were crystallized using a heparin mimetic containing 3 fully sulfated glucose units as the thrombin binding site. The structure by Li et al\textsuperscript{98} showed binding of the heparin mimetic to thrombin consistent with that seen using the natural fragment and with the mutagenesis data described above. Specifically arginine 93 is of crucial importance making 3 ionic interactions to sulfate oxygens as well as several other hydrogen bonds to the GAG. Lysine 236 participates in 2 interactions with sulfate moieties, and arginine 101 and 233 each make 1 interaction. The partially modeled lysine 240 could potentially provide an additional 3 interactions. Incomplete density for the heparin mimetic in the structure by Dementiev et al\textsuperscript{99} makes direct comparison difficult, but no sulfate density was observed on the single monosaccharide unit modeled in the heparin binding region. Also, the structure by Dementiev et al suggests histidine 230 contributes significantly to the interaction with heparin, whereas, in the thrombin–heparin structure by Carter et al\textsuperscript{97} described above, only a single water-mediated interaction between histidine 230 and heparin is observed. The chondroitin sulfate moiety (CS) found on the serine/threonine-rich domain of TM is likely to bind thrombin in a similar manner to that observed in the crystal structure of thrombin bound to heparin.\textsuperscript{100}

### Sodium Ion

Another potential cofactor for the modulation of thrombin activity is the monovalent cation sodium (Na\textsuperscript{+}). The binding site for Na\textsuperscript{+} was first identified in 1995 by Di Cera et al by substituting the more electron-rich rubidium into crystals grown in the presence of Na\textsuperscript{+}, and it was concluded that most of the preceding crystal structures of thrombin had placed a water molecule into the position now known to be occupied by Na\textsuperscript{+}.\textsuperscript{101} Na\textsuperscript{+} was found coordinated with octahedral geometry by the backbone carbonyl oxygens of arginine 221a and lysine 224 and 4 conserved water molecules (supplemental Figure III). When bound to Na\textsuperscript{+}, thrombin exists in a prothrombotic state capable of efficient cleavage of procoagulant substrates like fibrinogen and PAR-1.\textsuperscript{21,22,102,103} In the absence of Na\textsuperscript{+}, thrombin displays a generally reduced catalytic efficiency for these and other substrates yet maintains the ability to effectively activate protein C in the presence of TM.\textsuperscript{103} It has been assumed that the 2 states, Na\textsuperscript{+}-bound and Na\textsuperscript{+}-free, represent 2 distinct thrombin conformations, termed the “fast” and “slow” forms, respectively. Several biochemical and crystallographic studies have attempted to clarify the precise nature of the conformational change responsible for the activation of thrombin on Na\textsuperscript{+} binding.\textsuperscript{104–113} As many structures of Na\textsuperscript{+}-bound thrombin have already been deposited in the protein data bank, crystallization efforts have recently focused on the Na\textsuperscript{+}-free slow form. In 2002, a report by Pineda et al described a structure of thrombin crystallized in the absence of Na\textsuperscript{+} that displayed minor changes in the side chain positions of residues in the active site.\textsuperscript{106} Disappointingly, the conformation of the Na\textsuperscript{+}-binding site and of other regions previously shown by mutagenesis studies to undergo conformational rearrangement on Na\textsuperscript{+} binding remained unchanged relative to the fast form (eg, tryptophan 215, which is known to undergo a significant change in environment\textsuperscript{105}). Subsequent crystallization of wild-type and variants of thrombin in the presence of different monovalent cations such as choline, potassium, and lithium have resulted in an collection of thrombin structures displaying an array of conformational alterations with respect to the Na\textsuperscript{+}-bound form.\textsuperscript{107–110,112,114,115} Just a few of these conformational changes include disruption of the Na\textsuperscript{+}-binding loop, constriction and blockage of the active site cleft, reorientation of the catalytic serine 195, and loss of the oxyanion hole caused by flipping of glycine 193. Whether some or all of these changes are critical for the transition between the slow and fast forms is still unclear; however, release of Na\textsuperscript{+} appears to lead to an increase in conformational flexibility within thrombin. This suggests that in the absence of Na\textsuperscript{+} coordination, thrombin exists as an ensemble of conformational states whose collective activities have traditionally been taken to be that of a single slow form.\textsuperscript{115} Whatever the Na\textsuperscript{+}-induced conformational transition may be, the physiological relevance of Na\textsuperscript{+} as an allosteric effector molecule depends on differential binding in the plasma. Accordingly, it has been reported that the affinity of thrombin for Na\textsuperscript{+} is highly temperature dependent, with a $K_d$ of $\approx 25$ mmol/L at room temperature and near the physiological Na\textsuperscript{+} concentration of $\approx 140$ mmol/L at 37°C.\textsuperscript{116} However, with the plasma concentration of Na\textsuperscript{+} highly regulated, it is difficult to imagine how its interaction with thrombin could serve a true cofactor function in the regulation of hemostasis.

### Conclusions

This review focuses on how thrombin interacts with a number of different cofactors throughout a “lifecycle,” which begins with its generation in response to vascular injury and concludes with its irreversible inhibition by serpins after the formation of a stable clot. The cofactors described in this report play a critical role in steering thrombin specificity throughout its lifecycle. The lifecycle of thrombin was nicely outlined in the recent review by Lane et al.\textsuperscript{15} In the early stages of the hemostatic response, thrombin activity is heavily weighted toward procoagulation. Thrombin, via exosite II interactions, preferentially binds GpIb\alpha on the surface of platelets, cleaving PARs to activate platelets and release partially activated factor V from α granules. This causes several rounds of amplification of thrombin generation fol-
lowed by positive feedback stimulation through activation of additional factor V and factor VIII. As more thrombin is produced, it begins to associate with the amassing fibrinogen within the platelet plug. Because of its weak affinity for fibrinogen \( (K_a = 7 \, \text{mol/L}) \), thrombin relies on the high concentration of fibrinogen in plasma (15 \, \text{mol/L}) and the increased local concentration of fibrinogen near the site of injury. Thrombin remains bound to fibrin through exosite interactions after cleavage of fibrinogen. Fibrin then acts as a cofactor for thrombin activation of factor XIII, as well as providing the substrate for factor XIIIa cross-linking. Once the growing thrombus spreads beyond the site of injury, the relative abundance of cofactors shifts from those of procoagulant (GpIbα and fibrin) to those of anticoagulant (TM and GAGs) activities. The tight-binding \( (K_a = 3 \, \text{mol/L}) \) TM expressed on the surface of the endothelium effectively competes with the weak-binding fibrinogen for exosite I of thrombin. Heparan sulfate, which coats the endothelium, can also effectively compete with GpIbα for exosite II and assist in clearance of active thrombin through inhibition by AT and HCII. In this way, cofactors have evolved to use both exosites of thrombin and direct the enzyme toward procoagulant activity, anticoagulant activity, or inhibition. The thrombin-cofactor interactions, now fully revealed by crystallographic structures, are of critical importance for directing the activity of thrombin throughout its brief, but eventful, existence and thus help to maintain the hemostatic balance.

**Sources of Funding**

Funding was provided by the British Heart Foundation, the Sir Isaac Newton Trust, and the Medical Research Council (UK).

**Disclosure(s)**

None.

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Arterioscler Thromb Vasc Biol. 2006;26:1738-1745; originally published online May 25, 2006;
doi: 10.1161/01.ATV.0000228844.65168.d1

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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Supplemental Figures

**Figure I: Thrombin-GP1bα Interactions.** Ribbon diagram comparing two crystal structures of the thrombin-GP1bα complex (PDB entries 1P8V, 1OOK). Superimposing thrombin (grey) reveals the two binding modes of GP1bα (Dumas *et al.* in cyan and Celikel *et al.* in gold). Close-ups of the binding regions are shown in the boxes.
Figure II: Fibrin-Assisted Thrombin Activation of Factor XIII. Two thrombin molecules (grey surfaces) bind to fibrinogen (α-, β-, γ-chains shown in red, blue, and yellow ribbon, respectively. PDB entries 1QVH, 1M1J) followed by cleavage of fibrinopeptide A (FpA) and formation of protofibrils. Cleavage of fibrinopeptide B (FpB) leads to lateral polymerization of fibrin followed by factor XIII (green, PDB entry 1F13) binding to the γ-chain of a fibrin molecule positioning the zymogen for activation by thrombin.
Figure III: Sodium Ion Binding to Thrombin. The octahedral coordination sphere of sodium ion (Na\(^{+}\)) consists of six ligands, including two backbone carbonyl oxygens from the protein (arginine 221A and lysine 224) and four water molecules (PDB entry 1JOU).