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# Targeting Integrin and Integrin Signaling in Treating Thrombosis

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## Abstract

The critical roles of integrins in thrombosis have enabled the successful development and clinical use of the first generation of integrin antagonists as represented by abciximab (Reopro), eptifibatide (Integrilin), and tirofiban (Aggrastat). These integrin $\alpha_{IIb}\beta_3$ antagonists are potent anti-thrombotics, but also have significant side effects. In particular, their induction of ligand-induced integrin conformational changes is associated with thrombocytopenia. Increased bleeding risk prevents integrin antagonists from being used at higher doses and in patients at risk for bleeding. To address the ligand-induced conformational changes caused by current integrin antagonists, compounds that minimally induce conformational changes in integrin $\alpha_{IIb}\beta_3$ have been developed. Recent studies on the mechanisms of integrin signaling suggest that selectively targeting integrin outside-in signaling mechanisms allows for potent inhibition of thrombosis while maintaining hemostasis in animal models.

### Keywords

platelets; integrins; thrombosis; outside-in signaling; Platelet inhibitors

## Introduction

Integrins, a family of cell adhesion receptors, play important roles in cell adhesion, spreading, retraction, migration, anchorage-dependent survival and proliferation. Integrins exist as an α:β heterodimeric complex of transmembrane proteins. In blood platelets, the most abundant integrin is integrin $\alpha_{IIb}\beta_3$. Integrin $\alpha_{IIb}\beta_3$ binds to fibrinogen through the HHLGGAKQAGV sequence in the C-terminus of the fibrinogen γ chain and RGD sequences in the α chain. RGD-like sequences are also present in several other integrin-binding adhesive proteins including vitronectin, fibronectin and von Willebrand factor. In addition, platelets express integrins $\alpha_V\beta_3$, $\alpha_2\beta_1$, $\alpha_6\beta_1$, and $\alpha_5\beta_1$, among which $\alpha_5\beta_1$ and $\alpha_V\beta_3$ also recognize the RGD sequence. Integrin $\alpha_2\beta_1$ and $\alpha_6\beta_1$ bind to collagen and laminin[1]. By binding to adhesive proteins, the integrins mediate platelet adhesion to injured vascular wall and platelet aggregation, which is important for the maintenance of hemostasis, preventing excessive bleeding. The importance of integrin $\alpha_{IIb}\beta_3$ in hemostasis is exemplified in patients suffering from Glanzmann's thrombasthenia, in which genetic

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deficiencies in integrin $\alpha_{IIb}\,\beta_3$ causes bleeding diathesis[2]. Integrin $\alpha_{IIb}\,\beta_3$ is critical for arterial thrombosis[3], which is evident by the protective effects seen in experimental models of thrombosis using either pharmacologic inhibition or genetic deletion/mutation of integrin $\alpha_{IIb}\,\beta_3$[4, 5]; and by the clinical efficacy of $\alpha_{IIb}\,\beta_3$ antagonists[6–8]. However, despite successful clinical use of integrin antagonists as potent anti-thrombotics, their use is primarily limited to patients undergoing percutaneous coronary intervention, mainly due to significant bleeding risk. In fact, increased bleeding risks are a major problem shared by all currently available anti-thrombotic drugs. In this review, we briefly discuss the major problems associated with the currently used integrin antagonists, and new advances in developing the next generation of integrin antagonists.

## Current $\alpha_{IIb}\,\beta_3$ Integrin Antagonists

The three current FDA-approved platelet integrin antagonists are designed to block the ligand binding function of integrin $\alpha_{IIb}\,\beta_3$. Among these drugs, abciximab (Reopro) is a ~48 kilodalton mouse/human chimeric antibody fragment that binds to an epitope near the ligand binding site of $\beta_3$[4, 9–12]; eptifibatide (Integrilin), is a 832 dalton synthetic disulfide-linked cyclic heptapeptide ligand-mimetic, containing an integrin binding sequence, KGD, based on a snake venom peptide, barbourin[9, 12–14]; tirofiban (Aggrastat) is a 495 dalton synthetic compound, engineered to mimic RGD sequence[9, 12, 14–16]. Both eptifibatide and tirofiban are integrin ligand mimetics, which interact with the ligand-binding site of integrin $\alpha_{IIb}\,\beta_3$[12]. Tirofiban appears to be specific for $\alpha_{IIb}\,\beta_3$. Eptifibatide inhibits $\alpha_{IIb}\,\beta_3$ and $\alpha_V\,\beta_3$, and abciximab inhibits $\alpha_{IIb}\,\beta_3$, $\alpha_V\,\beta_3$ and $\alpha_M\,\beta_2$[12, 17, 18]. All three integrin antagonists are administered intravenously. Orally active integrin antagonists were also developed. However, clinical trials of oral integrin antagonists suggested increased mortality instead of beneficial effects[19, 20].

The current integrin antagonists have each demonstrated clear therapeutic benefits in high-risk patients undergoing percutaneous coronary intervention (PCI), as indicated by significant reductions in death and reoccurrence of myocardial infarction[6, 7, 9, 14]. There have also been clinical trials studying the effect of integrin antagonist treatment on patients suffering from acute ischemic stroke. Although, these trials so far have been mainly designed for the purpose of determining safety, and thus the therapeutic efficacy in stroke patients is yet to be conclusively established. In these trials, $\alpha_{IIb}\,\beta_3$ antagonist treatment alone showed no beneficial impact on mortality or debilitating stroke-related outcomes[21, 22], but increased the incidence of symptomatic or fatal intracranial hemorrhage[21, 23], with the exception of a trial of tirofiban[24]. In the tirofiban trial, no significant difference in hemorrhage was found between placebo and tirofiban groups, although the placebo group had significantly more patients also treated with aspirin, which may influence the outcome. Some clinical trials tested a combination of fibrinolytic therapy, using recombinant tissue plasminogen activator (r-tPA), and integrin antagonists, and suggested that integrin $\alpha_{IIb}\,\beta_3$ antagonists may have a beneficial effect by reducing adverse outcome due to stroke[24–26]; although, there is increased risk of hemorrhage, especially with abciximab[25]. In other clinical trials, fibrinolytic therapy, a reduced dose of r-tPA (<0.6 mcg/kg), together with eptifibatide-treatment shows similar bleeding profiles as the normal dose of r-tPA (0.9 mcg/kg) alone[26–28]. Treatment of patients with reduced r-tPA doses in combination with an

integrin antagonist implicate the investigators' consideration of potential hemorrhagic risk of the combination therapy.

The benefit of current integrin antagonists over other anti-platelet agents for general antithrombotic therapy is their rapid onset of action, potency, and low inter-patient variability[7, 9, 14]. By contrast, there is significant interpatient variability in response to aspirin (irreversible COX-1 inhibitor) or clopidogrel (P2Y12 inhibitor), mainly due to drug resistance[7, 12, 16]. However, the potent effects of current integrin antagonists are associated with increased bleeding risk[29–31], which can be potentially life-threatening. Bleeding risk limits the use and dose of integrin antagonists, and thus also limits their effectiveness[11, 31]. Abciximab, tirofiban and eptifibatide all cause thrombocytopenia, which may be associated with conformational changes of integrins following the binding of these drugs[32].

## New Inhibitors That Minimally Induce Conformational Changes

### Integrin structure and conformations

Both α and β chains of the $\alpha_{IIb}\beta_3$ complex contain a long extracellular region, a single-pass transmembrane region, and a short cytoplasmic tail. The amino terminal region of the α and β chains interact to form what is known as the 'head', which contains the ligand binding pocket where a conserved structural motif, known as the metal ion-dependent adhesion site (MIDAS) is critical[33]. In $\alpha_{IIb}\beta_3$ the MIDAS is on the $\beta_3$ -subunit and thought to stabilize ligand-binding by coordinating a metal ion with the aspartic acid on RGD-containing ligands[34]. Some other integrin α-subunits contain an additional domain called the interactive domain (I-domain), which also contain a MIDAS[12]. Below the head region are two long 'legs': in $\alpha_{IIb}$, two calf domains and a thigh domain constitute a leg; whereas in $\beta_3$, four integrin epidermal growth factor-like domains, two hybrid domains, and a plexin semaphorin integrin domain form the other leg.

Integrin molecules undergo conformational changes upon receptor activation and ligand-binding[34–36]. Integrin $\alpha_{IIb}\beta_3$ is kept in a resting (low-affinity) state in normal circulation, preventing undesirable thrombus formation. This state is maintained by interactions between the α and β chains within the transmembrane and membrane proximal cytoplasmic domains which constrain the ectodomain[37]. The resting state has been suggested to correspond to the 'bent' conformation as revealed in crystal structure and electron microscopy (EM)[33, 38, 3940]. Integrin activation induces the separation of α and β transmembrane and cytoplasmic domains and "un-bending" of the ectodomain, resulting in an 'extended' active conformation[4142]. The extended conformation with a 'closed' configuration, wherein the $\beta_3$ head and hybrid domain form an acute angle, represents an active intermediate affinity state, which is recognized by the RGD sequence or HHLGGAKQAGV sequence in fibrinogen. Binding of ligand recognition sequences induces further conformational changes, resulting in an 'open' head piece conformation, which is the high affinity state[35, 38, 39, 41–44] (Fig. 1A–C). Between these major conformational states, six different intermediate states have also been suggested, based on crystal structures of the ectodomain of $\alpha_{IIb}\beta_3$[43]. EM studies using intact $\alpha_{IIb}\beta_3$ in a nanodisc suggest different pictures of 'bent' and 'extended' conformations[4045]. Different from models obtained from crystal structures of integrin ectodomains, electron microscopy analyses of intact $\alpha_{IIb}\beta_3$ show that the resting integrin

headpiece points away from the membrane and that the intermediate extended integrin conformation contains crossed legs[40, 45]. The differences in models of resting and activated integrin structure are possibly due to the contribution of the transmembrane/cytoplasmic domains to integrin conformation[39]. The ligand-induced conformational changes are physiologically important because they: (1) expose new epitopes and binding sites on integrins (ligand-induced binding sites, LIBS)[36, 46]; (2) enable the initial interaction of 'resting' integrins with the exposed RGD-like sequence in certain ligands (such as immobilized fibrinogen) to transform integrins into a high affinity form (ligand-induced integrin activation), bypassing the need for inside-out signaling[47]; and (3) are important for integrin clustering and "outside-in" signaling[44, 48].

### Integrin antagonists that minimally affect integrin conformation

Tirofiban and eptifibatide are RGD mimetics, and thus cause "ligand-induced conformational changes"[49, 50], resulting in exposure of LIBS and ligand-induced integrin activation[51], although these monomeric RGD-like peptides or compounds in general do not appear to directly induce integrin outside-in signaling[47, 49, 52]. The conformational changes induced by ligand mimetic antagonists are thought to be important for the adverse effect of thrombocytopenia[32]. Abciximab also induces LIBS and thrombocytopenia[5354]. The ability of these antagonists to induce an active conformation of integrins carries the risk of possible pro-thrombotic effects after antagonist dissociation[5020]. There were some reports of such antagonist-induced prothrombotic effects[50, 52, 55]. Recently, new small molecule integrin antagonists have been developed that exhibit increased specificity and potency without exposing $\beta_3$ LIBS epitopes[56, 57]. RUC-1 and its more potent derivative RUC-2, inhibit the ligand binding function of integrins, platelet aggregation and in vivo thrombus formation, and importantly they do not induce integrin activation[56, 57]. RUC-1 interacts with $\alpha_{IIb}$ whereas RUC-2 appears to interact with $\beta_3$ $Mg^{2+}$ coordinating sites. Interestingly, unlike RUC-1 and current integrin antagonists, RUC-2 competes with $Mg^{2+}$ for binding to the $\beta_3$ -subunit, and its inhibitory effects are attenuated by adding exogenous $Mg^{2+}$ [57].

## Targeting Integrin Signaling

### Inside-out signaling

Platelets circulating in blood vessels are normally in a resting state and become activated and adherent only when exposed to the site of vascular injury or platelet agonists. Platelet agonists elicit platelet activation signals via various receptor-mediated intracellular signaling pathways[58]. These intracellular signals converge to transform $\alpha_{IIb}$ $\beta_3$ from a 'resting' state to an 'activated' state[1, 58]. This process is called inside-out signaling (Fig. 2). A key requirement for integrin inside-out signaling is the induction of the binding of talin to the membrane proximal half of the $\beta_3$ cytoplasmic domain which includes an important NPXY motif. Talin-binding induces unclasping of the transmembrane and cytoplasmic domains of $\alpha_{IIb}$ and $\beta_3$, and thus integrin activation[59–62]. This talin-dependent integrin activation is facilitated by kindlin, which interacts with the C-terminal region of the $\beta_3$ cytoplasmic domain[62, 63]. It is conceivable that disruption of talin/kindlin binding to integrin $\alpha_{IIb}$ $\beta_3$ or disruption of the signal responsible for the induction of talin/kindlin binding would also

inhibit integrin activation and thus thrombus formation, as evidenced by talin1 gene deletion or mutations[63, 64].

## Inhibitors of inside-out signaling

Current platelet inhibitors including ADP receptor antagonists (e.g. clopidogrel), cyclooxygenase inhibitors (e.g. aspirin), and thrombin receptor inhibitors (e.g. vorapaxar), primarily exert their effects by inhibiting early receptor signaling pathways that initiate inside-out signaling and integrin activation[16]. Pharmacological inhibition of inside-out signaling was demonstrated with cell-permeable peptides containing talin binding sequences[65, 66]. A cell permeable peptide corresponding to $\alpha_{IIb}$ residues 1000–1008 important in talin binding and $\beta_3$ interaction, were also used to inhibit integrin activation[67]. Because inhibition of inside-out signaling results in the loss of the activation of the ligand binding function of integrins, it is expected that the inhibitors of inside-out signaling should show characteristics similar to that of integrin antagonists, which inhibit both thrombosis and hemostasis. Indeed, talin1 deletion or mutational disruption of talin-binding site ($\beta_3$ L746A) protected mice from thrombosis, but they still displayed impaired hemostasis as shown by prolonged tail bleeding times in these mice[6364]. However, one report suggests that partial inhibition of talin binding to the integrin $\beta_3$ NPXY motif caused defective thrombus formation, with only minor bleeding side effect[68]. It remains to be investigated whether partial inhibition of $\alpha_{IIb}$ $\beta_3$ activation may also result in less potent anti-thrombotic effects or whether finding the right balance between potent anti-thrombotic effects and hemorrhagic side effects may allow anti-thrombotic therapy with proper control of bleeding risk.

## Outside-in signaling

Ligand binding to integrins not only mediates platelet adhesion and primary aggregation but also induces signal transduction into cells that triggers the activation of vast intracellular signaling networks and cytoskeleton reorganization[58, 69]. This process is known as outside-in signaling. Outside-in signaling leads to a series of cellular responses including platelet spreading, stable adhesion, granule secretion and clot retraction, which greatly amplify platelet aggregation and thrombus size[69, 70] (Fig. 2).

Several protein tyrosine kinases have been shown to be important in outside-in signaling including focal adhesion kinase, ILK, and Syk, Src family kinases (SFK)[58, 69]. In particular, integrin $\beta_3$ -bound c-Src[71], is now recognized as a key early signaling molecule. Following integrin ligation, c-Src has been shown to phosphorylate two NXXY motifs in the $\beta_3$ tail[58, 69]; and induce activation of the phosphoinositide 3-kinase (PI3K) pathway[72], inhibition of RhoA[73], and activation of the Syk-ITAM pathway[74]. Src-dependent transient inhibition of RhoA and activation of PI3K is necessary for platelet spreading on integrin ligands[72]. The PI3K pathway and the Syk-ITAM pathway stimulate granule secretion[72, 74]. Tyrosine phosphorylation in $\beta_3$ may also help recruit phosphotyrosine-binding proteins, such as SHC and myosin heavy chain[58, 69]. Phosphorylation at $Y^{747}$ also regulates talin binding and thus the direction and dynamics of integrin signaling[64], and phosphorylation at $Y^{759}$ protects $\beta_3$ from calpain cleavage[75]. These events are important for controlling the switch between platelet spreading and retraction[73]. Interestingly, the role of c-Src requires its interaction with the $\beta_3$ cytoplasmic domain. Deletion of the c-Src binding RGT sequence in

the C-terminus of $\beta_3$ abolished the ability of c-Src to mediate cell spreading even when constitutively active c-Src was expressed[76]. Thus, it appears that targeting c-Src binding to integrins may selectively inhibit integrin outside-in signaling. This notion is supported by a study using $\beta_3$ -RGT-deleted integrin-expressing mice, which are defective in platelet responses associated with outside-in signaling, and protected against arterial thrombosis, but display only a mild defect in inside-out signaling[76].

The most proximal signaling mediator of outside-in signaling identified so far is Gα13. Gα13 directly interacts with an ExE motif in the cytoplasmic domain of integrin β-subunits, and this binding is required for c-Src activation and Src-dependent outside-in signaling[77]. Gα13 binding to $\beta_3$ occurs only during early phase outside-in signaling. The Gα13 binds to an ExE motif located near talin binding sites of $\beta_3$. However, the ExE motif is not required for talin binding, and Gα13-binding is not involved in integrin activation[66]. Thus, suppression of Gα13 expression or disruption of the Gα13-binding site in $\beta_3$ selectively inhibits the early phase of outside-in signaling responsible for stabilization and amplification of a thrombus, but does not affect inside-out signaling nor the ligand binding function of integrins.

## Selective inhibitors of outside-in signaling

Recent conceptual advances in integrin outside-in signaling reveal the potential in developing selective inhibitors of integrin outside-in signaling as new anti-thrombotic drugs. A major advantage for targeting outside-in signaling is that inhibition of outside-in signaling should not affect primary platelet adhesion and aggregation, which is critical for hemostasis, but should limit the size of a thrombus to prevent vessel occlusion (Fig. 3). Our laboratory has recently demonstrated the potential of such an approach with a myristoylated ExE motif peptide that selectively inhibits Gα13-integrin interaction[66]. This inhibitor selectively inhibits outside-in signaling, platelet spreading and the second wave of platelet aggregation without affecting primary platelet aggregation. Importantly, this inhibitor potently inhibits occlusive thrombosis in mouse models in vivo without affecting bleeding time, unlike eptifibatide, which dramatically prolongs bleeding time[66]. Thus, selective inhibitors of outside-in signaling as a new antithrombotic strategy have the potential to selectively inhibit arterial thrombosis without causing excessive hemorrhage. Although, it is still important to consider and investigate potential off-target effects caused by selective targeting of Gα13.

Since SFK is a required signal downstream of Gα13 in outside-in signaling, inhibitors of SFK could potentially be effective inhibitors. However, SFK play multiple roles in platelets and other cells. For example, SFK is important in the ITAM pathway and GPIb-IX signaling pathways[58], and thus is important in inside-out signaling, which limits the value of SFK inhibitors as selective outside-in signaling inhibitors. However, blocking the interaction between SFK and integrins may selectively inhibit outside-in signaling. A myristoylated peptide inhibitor derived from the c-Src-binding sequence of $\beta_3$, abolished platelet spreading without affecting ADP-induced fibrinogen binding[78]. However, disruption of the $\beta_3$ c-Src-binding site in mice provided protection from thrombosis, but also mildly affected hemostasis[76].

## Conclusions

All current integrin antagonists function by blocking the binding of integrin ligands[9, 12, 34]. These inhibitors induce conformational changes in integrins, which are associated with thrombocytopenia and possibly other adverse effects. New inhibitors with minimal conformational effects may potentially help resolve this issue. A major problem associated with the current integrin antagonists is that at doses where they exhibit high potency they also increase the risk of hemorrhage. Emerging evidence suggests that selective inhibition of outside-in signaling has the potential to have potent antithrombotic effects without causing bleeding.

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## References

1. Hynes RO. Integrins: Bidirectional, allosteric signaling machines. Cell. 2002; 110:673–687. [PubMed: 12297042]
2. Nurden AT, Fiore M, Nurden P, Pillois X. Glanzmann thrombasthenia: A review of ITGA2B and ITGB3 defects with emphasis on variants, phenotypic variability, and mouse models. Blood. 2011; 118:5996–6005. [PubMed: 21917754]
3. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. New England Journal of Medicine. 1992; 326:242–250. [PubMed: 1727977]
4. Coller B, Scudder L. Inhibition of dog platelet function by in vivo infusion of f(ab')2 fragments of a monoclonal antibody to the platelet glycoprotein IIb/IIIa receptor. Blood. 1985; 66:1456–1459. [PubMed: 2998514]
5. Hodivala-Dilke KM, McHugh KP, Tsakiris DA, Rayburn H, Crowley D, Ullman C, xE, Mollie, Ross FP, Coller BS, Teitelbaum S, Hynes RO. β3-integrin–deficient mice are a model for glanzmann thrombasthenia showing placental defects and reduced survival. The Journal of clinical investigation. 1999; 103:229–238. [PubMed: 9916135]
6. Lincoff, AM.; Topol, EJ. Platelet glycoprotein IIb/IIIa inhibitors in cardiovascular disease. Springer; 2003. Overview of the glycoprotein iib/iiia interventional trials; p. 167-199.
7. Patrono C, Baigent C, Hirsh J, Roth G. Antiplatelet drugs: American college of chest physicians evidence-based clinical practice guidelines (8th edition). Chest. 2008; 133:199s–233s. [PubMed: 18574266]
8. Bosch X, Marrugat J, Sanchis J. Platelet glycoprotein IIb/IIIa blockers during percutaneous coronary intervention and as the initial medical treatment of non-st segment elevation acute coronary syndromes. The Cochrane database of systematic reviews. 2013; 11 Cd002130.
9. Bledzka K, Smyth SS, Plow EF. Integrin $\alpha_{IIb}\beta_3$: From discovery to efficacious therapeutic target. Circ Res. 2013; 112:1189–1200. [PubMed: 23580774]
10. Coller BS. A new murine monoclonal antibody reports an activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa complex. The Journal of clinical investigation. 1985; 76:101–108. [PubMed: 2991335]
11. Coller BS, Folts JD, Smith SR, Scudder LE, Jordan R. Abolition of in vivo platelet thrombus formation in primates with monoclonal antibodies to the platelet GPIIb/IIIa receptor. Correlation with bleeding time, platelet aggregation, and blockade of gpIIb/IIIa receptors. Circulation. 1989; 80:1766–1774. [PubMed: 2598436]

12. Cox D, Brennan M, Moran N. Integrins as therapeutic targets: Lessons and opportunities. Nature reviews. Drug discovery. 2010; 9:804–820.
13. Scarborough RM. Development of eptifibatide. American Heart Journal. 1999; 138:1093–1104. [PubMed: 10577440]
14. Schneider DJ. Anti-platelet therapy: Glycoprotein IIb/IIIa antagonists. Br J Clin Pharmacol. 2011; 72:672–682. [PubMed: 21906121]
15. Lynch JJ Jr, Cook JJ, Sitko GR, Holahan MA, Ramjit DR, Mellott MJ, Stranieri MT, Stabilito II, Zhang G, Lynch RJ, et al. Nonpeptide glycoprotein IIb/IIIa inhibitors. 5. Antithrombotic effects of MK-0383. The Journal of pharmacology and experimental therapeutics. 1995; 272:20–32. [PubMed: 7815334]
16. Michelson AD. Antiplatelet therapies for the treatment of cardiovascular disease. Nature reviews. Drug discovery. 2010; 9:154–169.
17. Lele M, Sajid M, Wajih N, Stouffer GA. Eptifibatide and 7E3, but not tirofiban, inhibit $\alpha_v\beta_3$ integrin-mediated binding of smooth muscle cells to thrombospondin and prothrombin. Circulation. 2001; 104:582–587. [PubMed: 11479257]
18. Simon DI, Xu H, Ortlepp S, Rogers C, Rao NK. 7E3 monoclonal antibody directed against the platelet glycoprotein IIb/IIIa cross-reacts with the leukocyte integrin Mac-1 and blocks adhesion to fibrinogen and ICAM-1. Arteriosclerosis, thrombosis, and vascular biology. 1997; 17:528–535.
19. Cannon CP, McCabe CH, Wilcox RG, Langer A, Caspi A, Berink P, Lopez-Sendon J, Toman J, Charlesworth A, Anders RJ, Alexander JC, Skene A, Braunwald E. Oral glycoprotein IIb/IIIa inhibition with orbofiban in patients with unstable coronary syndromes (OPUS-TIMI16) trial. Circulation. 2000; 102:149–156. [PubMed: 10889124]
20. Chew DP, Bhatt DL, Sapp S, Topol EJ. Increased mortality with oral platelet glycoprotein IIb/IIIa antagonists: A meta-analysis of phase III multicenter randomized trials. Circulation. 2001; 103:201–206. [PubMed: 11208677]
21. Ciccone A, Motto C, Abraha I, Cozzolino F, Santilli I. Glycoprotein IIb/IIIa inhibitors for acute ischaemic stroke. The Cochrane database of systematic reviews. 2014; 3 Cd005208.
22. Mandava P, Thiagarajan P, Kent T. Glycoprotein IIb/IIIa antagonists in acute ischaemic stroke. Drugs. 2008; 68:1019–1028. [PubMed: 18484795]
23. Adams HP, Effron MB, Torner J, Dávalos A, Frayne J, Teal P, Leclerc J, Oemar B, Padgett L, Barnathan ES, Hacke W. Investigators ftA-I. Emergency administration of abciximab for treatment of patients with acute ischemic stroke: Results of an international phase III trial: Abciximab in emergency treatment of stroke trial (AbESTT-II). Stroke; a journal of cerebral circulation. 2008; 39:87–99.
24. Siebler M, Hennerici MG, Schneider D, von Reutern GM, Seitz RJ, Rother J, Witte OW, Hamann G, Junghans U, Villringer A, Fiebach JB. Safety of tirofiban in acute ischemic stroke: The satis trial. Stroke; a journal of cerebral circulation. 2011; 42:2388–2392.
25. Eckert B, Koch C, Thomalla G, Kucinski T, Grzyska U, Roether J, Alfke K, Jansen O, Zeumer H. Aggressive therapy with intravenous abciximab and intra-arterial rtpa and additional pta/stenting improves clinical outcome in acute vertebrobasilar occlusion: Combined local fibrinolysis and intravenous abciximab in acute vertebrobasilar stroke treatment (FAST): Results of a multicenter study. Stroke; a journal of cerebral circulation. 2005; 36:1160–1165.
26. Pancioli AM, Adeoye O, Schmit PA, Khoury J, Levine SR, Tomsick TA, Sucharew H, Brooks CE, Crocco TJ, Gutmann L, Hemmen TM, Kasner SE, Kleindorfer D, Knight WA, Martini S, McKinney JS, Meurer WJ, Meyer BC, Schneider A, Scott PA, Starkman S, Warach S, Broderick JP. Combined approach to lysis utilizing eptifibatide and recombinant tissue plasminogen activator in acute ischemic stroke-enhanced regimen stroke trial. Stroke; a journal of cerebral circulation. 2013; 44:2381–2387.
27. Mangiafico S, Cellerini M, Nencini P, Gensini G, Inzitari D. Intravenous glycoprotein IIb/IIIa inhibitor (tirofiban) followed by intra-arterial urokinase and mechanical thrombolysis in stroke. AJNR. American journal of neuroradiology. 2005; 26:2595–2601. [PubMed: 16286408]
28. Seitz RJ, Hamzavi M, Junghans U, Ringleb PA, Schranz C, Siebler M. Thrombolysis with recombinant tissue plasminogen activator and tirofiban in stroke: Preliminary observations. Stroke; a journal of cerebral circulation. 2003; 34:1932–1935.

29. Platelet glycoprotein IIb/IIIa receptor blockade and low-dose heparin during percutaneous coronary revascularization. The New England journal of medicine. 1997; 336:1689–1696. [PubMed: 9182212]
30. Inhibition of platelet glycoprotein IIb/IIIa with eptifibatide in patients with acute coronary syndromes. The pursuit trial investigators. Platelet glycoprotein IIb/IIIa in unstable angina: Receptor suppression using integrilin therapy. The New England journal of medicine. 1998; 339:436–443. [PubMed: 9705684]
31. Kereiakes DJ, Kleiman NS, Ambrose J, Cohen M, Rodriguez S, Palabrica T, Herrmann HC, Sutton JM, Weaver WD, McKee DB, Fitzpatrick V, Sax FL. Randomized, double-blind, placebo-controlled dose-ranging study of tirofiban (MK-383) platelet gpIIb/IIIa blockade in high risk patients undergoing coronary angioplasty. Journal of the American College of Cardiology. 1996; 27:536–542. [PubMed: 8606262]
32. Bougie DW, Rasmussen M, Zhu J, Aster RH. Antibodies causing thrombocytopenia in patients treated with RGD-mimetic platelet inhibitors recognize ligand-specific conformers of $\alpha_{IIb}\beta_3$ integrin. Blood. 2012; 119:6317–6325. [PubMed: 22490676]
33. Xiong JP, Stehle T, Diefenbach B, Zhang R, Dunker R, Scott DL, Joachimiak A, Goodman SL, Arnaout MA. Crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$. Science (New York, N Y.). 2001; 294:339–345.
34. Xiao T, Takagi J, Coller BS, Wang JH, Springer TA. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. Nature. 2004; 432:59–67. [PubMed: 15378069]
35. Takagi J, Petre BM, Walz T, Springer TA. Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. Cell. 2002; 110:599–511. [PubMed: 12230977]
36. Du X, Gu M, Weisel JW, Nagaswami C, Bennett JS, Bowditch R, Ginsberg MH. Long range propagation of conformational changes in integrin $\alpha_{IIb}\beta_3$. The Journal of biological chemistry. 1993; 268:23087–23092. [PubMed: 7693683]
37. Vinogradova O, Velyvis A, Velyviene A, Hu B, Haas T, Plow E, Qin J. A structural mechanism of integrin $\alpha_{IIb}\beta_3$ "inside-out" activation as regulated by its cytoplasmic face. Cell. 2002; 110:587–597. [PubMed: 12230976]
38. Zhu J, Zhu J, Negri A, Provasi D, Filizola M, Coller BS, Springer TA. Closed headpiece of integrin $\alpha_{IIb}\beta_3$ and its complex with an $\alpha_{IIb}\beta_3$-specific antagonist that does not induce opening. Blood. 2010; 116:5050–5059. [PubMed: 20679525]
39. Kim C, Schmidt T, Cho E-G, Ye F, Ulmer TS, Ginsberg MH. Basic amino-acid side chains regulate transmembrane integrin signalling. Nature. 2012; 481:209–213.
40. Choi WS, Rice WJ, Stokes DL, Coller BS. Three-dimensional reconstruction of intact human integrin $\alpha_{IIb}\beta_3$: New implications for activation-dependent ligand binding. Blood. 2013; 122:4165–4171. [PubMed: 24136164]
41. Kim M, Carman CV, Springer TA. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. Science (New York, N.Y.). 2003; 301:1720–1725.
42. Lau TL, Kim C, Ginsberg MH, Ulmer TS. The structure of the integrin $\alpha_{IIb}\beta_3$ transmembrane complex explains integrin transmembrane signalling. Embo j. 2009; 28:1351–1361. [PubMed: 19279667]
43. Zhu J, Zhu J, Springer TA. Complete integrin headpiece opening in eight steps. The Journal of cell biology. 2013; 201:1053–1068. [PubMed: 23798730]
44. Li R, Mitra N, Gratkowski H, Vilaire G, Litvinov R, Nagasami C, Weisel JW, Lear JD, DeGrado WF, Bennett JS. Activation of integrin $\alpha_{IIb}\beta_3$ by modulation of transmembrane helix associations. Science (New York, N Y.). 2003; 300:795–798.
45. Eng ET, Smagghe BJ, Walz T, Springer TA. Intact $\alpha_{IIb}\beta_3$ integrin is extended after activation as measured by solution x-ray scattering and electron microscopy. The Journal of biological chemistry. 2011; 286:35218–35226. [PubMed: 21832081]
46. Parise LV, Helgerson SL, Steiner B, Nannizzi L, Phillips DR. Synthetic peptides derived from fibrinogen and fibronectin change the conformation of purified platelet glycoprotein IIb/IIIa. Journal of Biological Chemistry. 1987; 262:12597–12602. [PubMed: 2957377]

47. Du X, Plow EF, Frelinger AL III, O'Toole TE, Loftus JC, Ginsberg MH. Ligands "activate" integrin $\alpha_{IIb}\beta_3$ (platelet gpIIb/IIIa). Cell. 65:409–416. [PubMed: 2018974]
48. Hato T, Pampori N, Shattil SJ. Complementary roles for receptor clustering and conformational change in the adhesive and signaling functions of integrin $\alpha_{IIb}\beta_3$. The Journal of cell biology. 1998; 141:1685–1695. [PubMed: 9647659]
49. Cox D, Smith R, Quinn M, Theroux P, Crean P, Fitzgerald DJ. Evidence of platelet activation during treatment with a gpIIb/IIIa antagonist in patients presenting with acute coronary syndromes. Journal of the American College of Cardiology. 2000; 36:1514–1519. [PubMed: 11079651]
50. Hantgan RR, Stahle MC. Integrin priming dynamics: Mechanisms of integrin antagonist-promoted $\alpha_{IIb}\beta_3$:PAC-1 molecular recognition. Biochemistry. 2009; 48:8355–8365. [PubMed: 19640007]
51. Jennings LK, Haga JH, Slack SM. Differential expression of a ligand induced binding site (LIBS) by gpIIb/IIIa ligand recognition peptides and parenteral antagonists. Thrombosis and haemostasis. 2000; 84:1095–1102. [PubMed: 11154119]
52. Bassler N, Loeffler C, Mangin P, Yuan Y, Schwarz M, Hagemeyer CE, Eisenhardt SU, Ahrens I, Bode C, Jackson SP, Peter K. A mechanistic model for paradoxical platelet activation by ligand-mimetic $\alpha_{IIb}\beta_3$ (gpIIb/IIIa) antagonists. Arteriosclerosis, thrombosis, and vascular biology. 2007; 27:E9–E15.
53. Berkowitz SD, Harrington RA, Rund MM, Tcheng JE. Acute profound thrombocytopenia after C7E3 fab (abciximab) therapy. Circulation. 1997; 95:809–813. [PubMed: 9054735]
54. Peter K, Straub A, Kohler B, Volkmann M, Schwarz M, Kübler W, Bode C. Platelet activation as a potential mechanism of GPIIb/IIIa inhibitor-induced thrombocytopenia. The American Journal of Cardiology. 1999; 84:519–524. [PubMed: 10482148]
55. Peter K, Schwarz M, Ylänne J, Kohler B, Moser M, Nordt T, Salbach P, Kübler W, Bode C. Induction of fibrinogen binding and platelet aggregation as a potential intrinsic property of various glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) inhibitors. Blood. 1998; 92:3240–3249. [PubMed: 9787160]
56. Blue R, Murcia M, Karan C, Jirouskova M, Coller BS. Application of high-throughput screening to identify a novel $\alpha_{IIb}$-specific small- molecule inhibitor of $\alpha_{IIb}\beta_3$-mediated platelet interaction with fibrinogen. Blood. 2008; 111:1248–1256. [PubMed: 17978171]
57. Zhu J, Choi W-S, McCoy JG, Negri A, Zhu J, Naini S, Li J, Shen M, Huang W, Bougie D, Rasmussen M, Aster R, Thomas CJ, Filizola M, Springer TA, Coller BS. Structure-guided design of a high-affinity platelet integrin $\alpha_{IIb}\beta_3$ receptor antagonist that disrupts $Mg^{2+}$ binding to the MIDAS. Science Translational Medicine. 2012; 4:125ra132.
58. Li Z, Delaney MK, O'Brien KA, Du X. Signaling during platelet adhesion and activation. Arteriosclerosis, thrombosis, and vascular biology. 2010; 30:2341–2349.
59. Ma YQ, Qin J, Wu C, Plow EF. Kindlin-2 (Mig-2): A co-activator of $\beta_3$ integrins. The Journal of cell biology. 2008; 181:439–446. [PubMed: 18458155]
60. Wegener KL, Partridge AW, Han J, Pickford AR, Liddington RC, Ginsberg MH, Campbell ID. Structural basis of integrin activation by talin. Cell. 2007; 128:171–182. [PubMed: 17218263]
61. Tadokoro S, Shattil SJ, Eto K, Tai V, Liddington RC, de Pereda JM, Ginsberg MH, Calderwood DA. Talin binding to integrin beta tails: A final common step in integrin activation. Science (New York, N.Y.). 2003; 302:103–106.
62. Moser M, Nieswandt B, Ussar S, Pozgajova M, Fassler R. Kindlin-3 is essential for integrin activation and platelet aggregation. Nature medicine. 2008; 14:325–330.
63. Nieswandt B, Moser M, Pleines I, Varga-Szabo D, Monkley S, Critchley D, Fassler R. Loss of talin1 in platelets abrogates integrin activation, platelet aggregation, and thrombus formation in vitro and in vivo. J Exp Med. 2007; 204:3113–3118. [PubMed: 18086864]
64. Petrich BG, Fogelstrand P, Partridge AW, Yousefi N, Ablooglu AJ, Shattil SJ, Ginsberg MH. The antithrombotic potential of selective blockade of talin-dependent integrin $\alpha_{IIb}\beta_3$(platelet gpIIb/IIIa) activation. The Journal of clinical investigation. 2007; 117:2250–2259. [PubMed: 17627302]
65. Liu XY, Timmons S, Lin YZ, Hawiger J. Identification of a functionally important sequence in the cytoplasmic tail of integrin $\beta_3$ by using cell-permeable peptide analogs. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:11819–11824. [PubMed: 8876221]

66. Shen B, Zhao X, O'Brien KA, Stojanovic-Terpo A, Delaney MK, Kim K, Cho J, Lam SC, Du X. A directional switch of integrin signalling and a new anti-thrombotic strategy. Nature. 2013; 503:131–135. [PubMed: 24162846]
67. Koloka V, Christofidou ED, Vaxevanelis S, Dimitriou AA, Tsikaris V, Tselepis AD, Panou-Pomonis E, Sakarellos-Daitsiotis M, Tsoukatos DC. A palmitoylated peptide, derived from the acidic carboxyl-terminal segment of the integrin $\alpha_{IIb}$ cytoplasmic domain, inhibits platelet activation. Platelets. 2008; 19:502–511. [PubMed: 18979362]
68. Stefanini L, Ye F, Snider AK, Sarabakhsh K, Piatt R, Paul DS, Bergmeier W, Petrich BG. A talin mutant that impairs talin-integrin binding in platelets decelerates $\alpha_{IIb}\beta_3$ activation without pathological bleeding. Blood. 2014; 123:2722–2731. [PubMed: 24585775]
69. Shattil SJ, Newman PJ. Integrins: Dynamic scaffolds for adhesion and signaling in platelets. Blood. 2004; 104:1606–1615. [PubMed: 15205259]
70. Shen B, Delaney MK, Du X. Inside-out, outside-in, and inside-outside-in: G protein signaling in integrin-mediated cell adhesion, spreading, and retraction. Curr Opin Cell Biol. 2012; 24:600–606. [PubMed: 22980731]
71. Arias-Salgado EG, Lizano S, Sarkar S, Brugge JS, Ginsberg MH, Shattil SJ. Src kinase activation by direct interaction with the integrin β cytoplasmic domain. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:13298–13302. [PubMed: 14593208]
72. O'Brien KA, Gartner TK, Hay N, Du X. ADP-stimulated activation of AKT during integrin outside-in signaling promotes platelet spreading by inhibiting glycogen synthase kinase-3β. Arteriosclerosis, thrombosis, and vascular biology. 2012; 32:2232–2240.
73. Flevaris P, Stojanovic A, Gong H, Chishti A, Welch E, Du X. A molecular switch that controls cell spreading and retraction. The Journal of cell biology. 2007; 179:553–565. [PubMed: 17967945]
74. Boylan B, Gao C, Rathore V, Gill JC, Newman DK, Newman PJ. Identification of fcgammariia as the itam-bearing receptor mediating $\alpha_{IIb}\beta_3$ outside-in integrin signaling in human platelets. Blood. 2008; 112:2780–2786. [PubMed: 18641368]
75. Xi X, Flevaris P, Stojanovic A, Chishti A, Phillips DR, Lam SC, Du X. Tyrosine phosphorylation of the integrin $\beta_3$ subunit regulates $\beta_3$ cleavage by calpain. The Journal of biological chemistry. 2006; 281:29426–29430. [PubMed: 16935858]
76. Ablooglu AJ, Kang J, Petrich BG, Ginsberg MH, Shattil SJ. Antithrombotic effects of targeting $\alpha_{IIb}\beta_3$ signaling in platelets. Blood. 2009; 113:3585–3592. [PubMed: 19005179]
77. Gong H, Shen B, Flevaris P, Chow C, Lam SC, Voyno-Yasenetskaya TA, Kozasa T, Du X. G protein subunit Gα13 binds to integrin $\alpha_{IIb}\beta_3$ and mediates integrin "outside-in" signaling. Science (New York, N.Y.). 2010; 327:340–343.
78. Su X, Mi J, Yan J, Flevaris P, Lu Y, Liu H, Ruan Z, Wang X, Kieffer N, Chen S, Du X, Xi X. RGT, a synthetic peptide corresponding to the integrin $\beta_3$ cytoplasmic C-terminal sequence, selectively inhibits outside-in signaling in human platelets by disrupting the interaction of integrin $\alpha_{IIb}\beta_3$ with Src kinase. Blood. 2008; 112:592–602. [PubMed: 18398066]

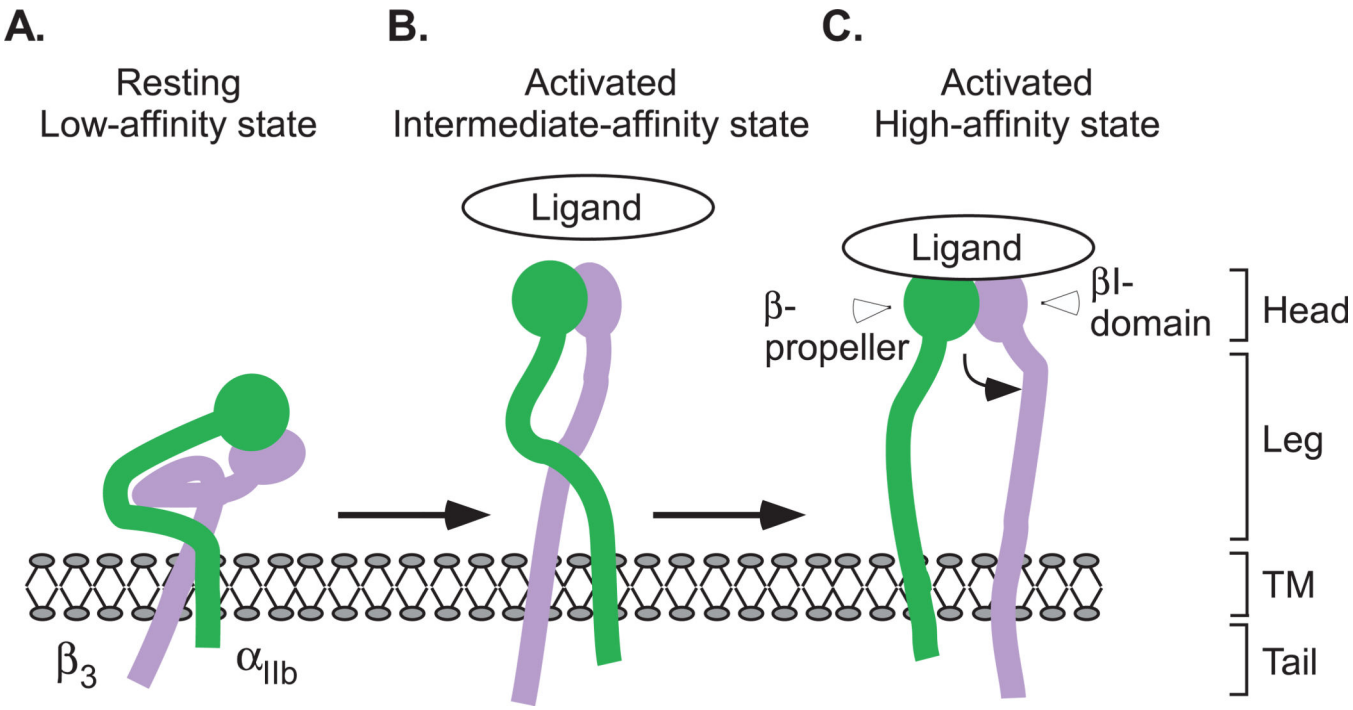


**Figure 1. Conformational states during integrin activation and ligand binding**

(A) The resting (low-affinity) state is maintained by interactions between the α and β chains within the transmembrane and membrane proximal cytoplasmic domains which constrain the ectodomain. (B) The activated (intermediate affinity) state has an extended conformation with a 'closed' configuration. (C) Binding of ligand recognition sequences induces further conformational changes, resulting in an 'open' high affinity state. TM, transmembrane domain. Curved black arrow indicates the swing-out motion of the β3 subunit hybrid domain upon full integrin activation.

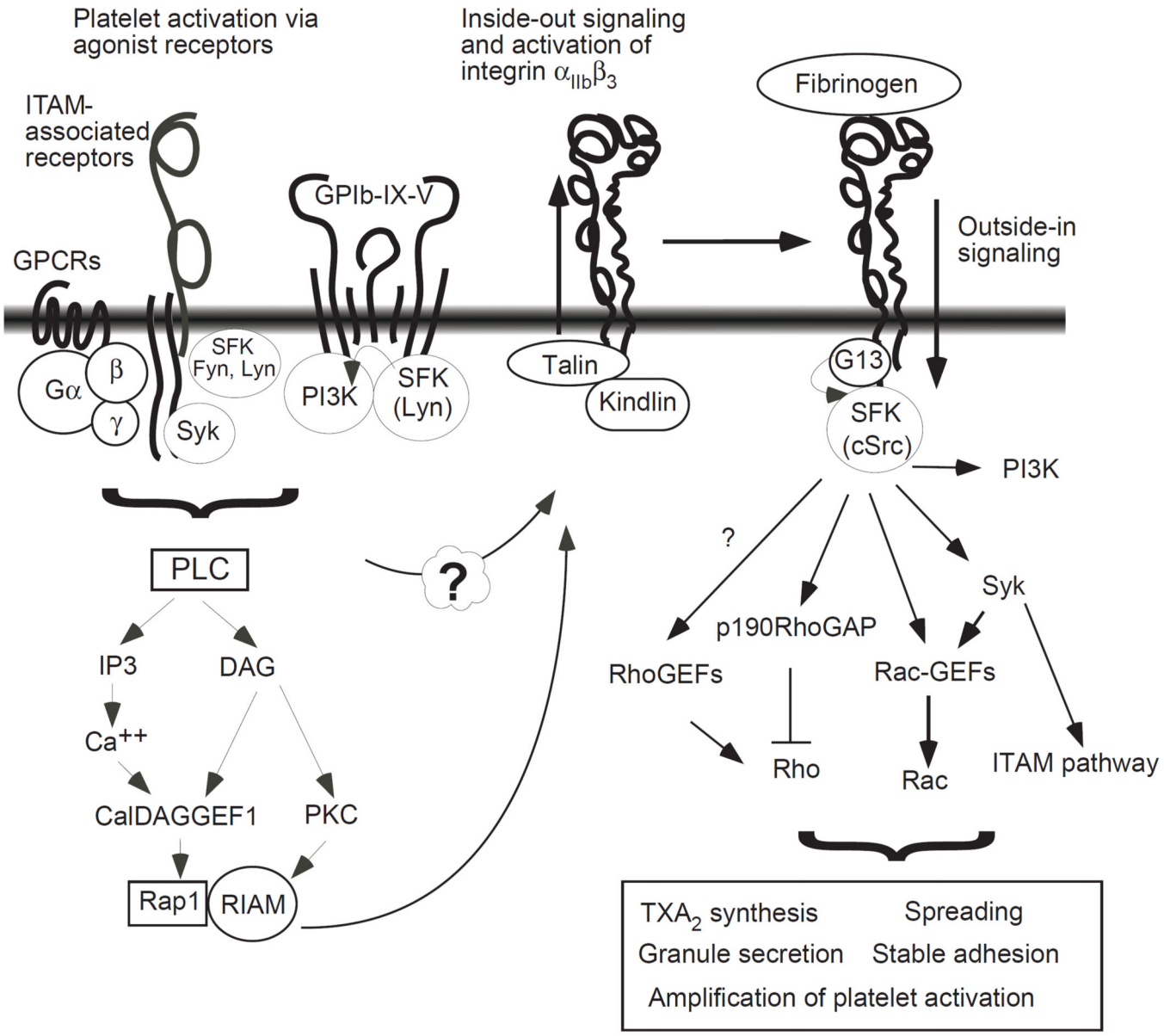


**Figure 2. Inside-out and Outside-in signaling of the platelet integrin $\alpha_{IIb}\beta_3$**

For a detailed description of individual agonist-induced platelet activation pathways, please see ref[58]. PLC, phospholipase C; PI3K, phosphoinositide 3-kinase; GPCRs, G-protein coupled receptors; IP3, inositol trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; Syk, spleen tyrosine kinase; SFK, src family kinase, CalDAG-GEF1, calcium and diacylglycerol-regulated guanine nucleotide exchange factor 1; RIAM, Rap1-GTP interacting adapter molecule; G13, guanosine nucleotide-binding protein alpha subunit 13; GEF, Guanine nucleotide exchange factor; GAP, GTPase-activating protein; ITAM, immunoreceptor tyrosine-based activation motif; $TXA_2$, thromboxane $A_2$.

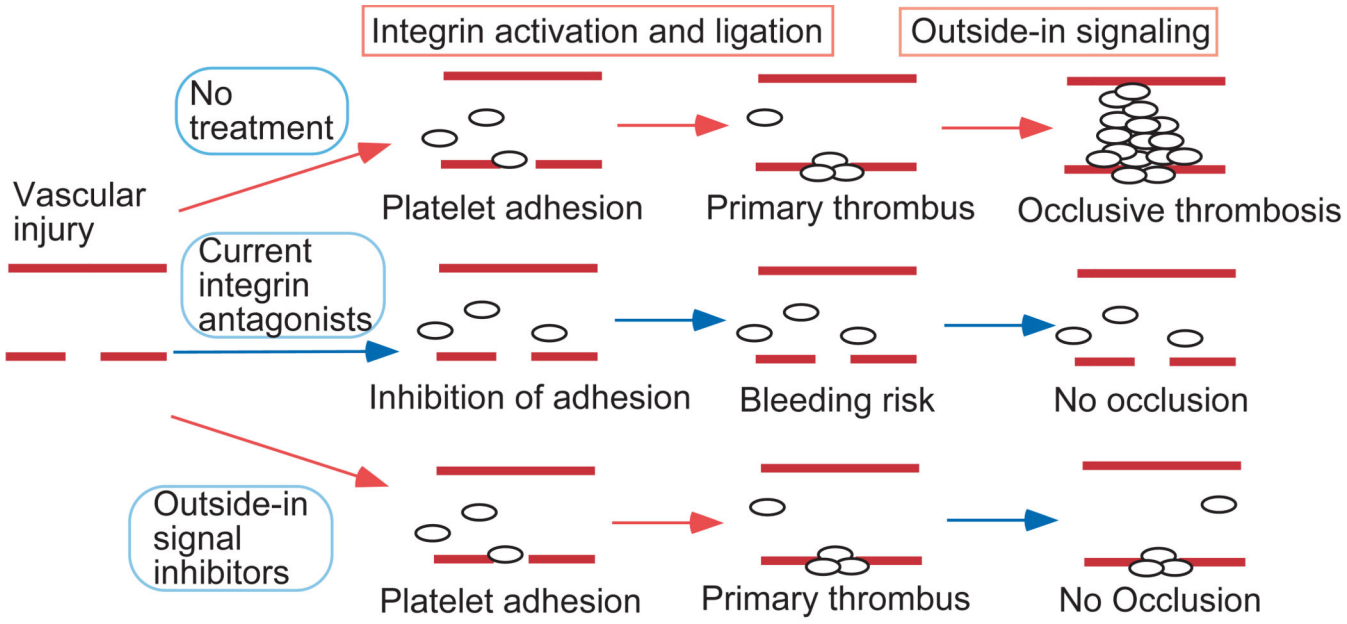


**Figure 3. A schematic showing the anti-thrombotic effect of selective inhibitors of outside-in signaling without causing hemorrhage**

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