Article:
Duval, C orcid.org/0000-0002-4870-6542 and Ariëns, RAS orcid.org/0000-0002-6310-5745 (2017) Fibrinogen splice variation and cross-linking: Effects on fibrin structure/function and role of fibrinogen γ' as thrombomobulin II. Matrix Biology, 60-61. pp. 8-15. ISSN 0945-053X

https://doi.org/10.1016/j.matbio.2016.09.010

Accepted Manuscript

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PII: S0945-053X(16)30229-3
DOI: doi:10.1016/j.matbio.2016.09.010
Reference: MATBIO 1299

To appear in: *Matrix Biology*

Received date: 12 September 2016
Accepted date: 30 September 2016

Please cite this article as: Duval, Cédric, Ariëns, Robert A.S., Fibrinogen splice variation and cross-linking: Effects on fibrin structure/function and role of fibrinogen γ' as thrombomobulin II, *Matrix Biology* (2016), doi:10.1016/j.matbio.2016.09.010

Fibrinogen splice variation and cross-linking: Effects on fibrin structure/function and role of fibrinogen γ' as thrombomobulin II

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Abstract

Fibrin is an important matrix protein that provides the backbone to the blood clot, promoting tissue repair and wound healing. Its precursor fibrinogen is one of the most heterogenous proteins, with an estimated 1 million different forms due to alterations in glycosylation, oxidation, single nucleotide polymorphisms, splice variation and other variations. Furthermore, ligation by transglutamimase factor XIII (cross-linking) adds to the complexity of the fibrin network. The structure and function of the fibrin network is in part determined by this natural variation in the fibrinogen molecule, with major effects from slice variation and cross-linking. This mini-review will discuss the direct effects of fibrinogen αEC and fibrinogen γ' splice variation on clot structure and function and also discuss the additional role of fibrinogen γ' as thrombomodulin II. Furthermore, the effects of cross-linking on clot function will be described. Splice variation and cross-linking are major determinants of the structure and function of fibrin and may therefore impact on diseases affecting bleeding, thrombosis and tissue repair.

Fibrinogen structure

Fibrinogen is one of the most heterogeneous proteins due to alterations in glycosylation, oxidation, splice variation, single nucleotide polymorphisms amongst other variations. Henschen estimated that there could be up to 1 million different forms of fibrinogen in the blood circulation[1]. While many of the minor alterations and polymorphisms are neutral and do not influence the structure and function of fibrinogen or its multimeric form fibrin, some of the larger alterations such as splice variation and particular single nucleotide polymorphisms such as AαThr312Ala and BβArg448Lys have been shown to influence structure and function of the fibrin clot[2].

Fibrinogen is a dimer of 6 polypeptides ($A\alpha_2B\beta_2\gamma_2$) which are arranged in a long flexible protein with three regions (D-E-D) that are connected by coiled coil segments (Fig. 6 from Litvinov & Weisel review article in this issue[3]).

Splice variations of fibrinogen

Fibrinogen αEC

By far the largest splice variation occurring in fibrinogen involves the α-chain of the protein. In around 1% of all fibrinogen, the normal α-polypeptide which is 610 residues long is extended by another 236 residues at the C-terminus, leading to an extended αEC chain of 846 residues. This large extension is due to splice variation occurring at the exon V/intron E boundary of the Aα transcript[4]. The additional polypeptide sequence of the αEC variant contains 3 glycosylation sites, further adding to the size and molecular weight of the protein. The αEC splice variant has also been called fibrinogen-420 as compared with the normal fibrinogen-340, due to its increase in molecular mass. Interestingly, the αEC sequence is highly conserved among vertebrates with 93.2% homology in rats, 70.3% in *Xenopus* and 52.2% in lamprey fish compared with humans [5]. Its structure has been elucidated through crystallography[6], but its functional characteristics have been poorly described. The αEC region is susceptible to early proteolysis by plasmin[7] and can act as a ligand for neutrophils and monocytes via $\alpha_M\beta_2$ and $\alpha_X\beta_2$ integrins[8]. Fibrinogen αEC has been shown to produce clots with thinner, more branched fibres with increased maximum amplitude in thromboelastometry, which is an indication of increased clot stiffness[9].

Fibrinogen γ' and clot structure

While αEC is the largest fibrinogen splice variant, by far the most studied fibrinogen splice variation occurs in the γ-chain. This splice-variation, called γ' (also γB or γ57.5), occurs at the γ-chain C-terminus, where the last AGDV sequence of the regular γA chain is substituted with VRPEHPAETEYDSLYPEDDL of the γ' chain[10]. The γ' splice variation occurs in around 10-15% of transcripts, and the majority of γ' in circulation occurs as a γA/γ' heterodimer, whereas γ'/γ' homodimers are less than 1% of total fibrinogen. The average γA/γ' concentration is 12%, but levels can drop to 3% or increase up to 40%[11]. The γ' extension confers increased negative charge to the γ-chain C-terminus through a high proportion of aspartic and glutamic acid residues and two sulphated tyrosines.

Fibrinogen γ' has been shown to modulate clot structure in a direct manner, independently of thrombin[12]. Fibers made with fibrinogen γ' are thinner, tightly interknit and the network structure is heterogeneous. This effect on clot structure is caused by direct interference of the negatively charged γ' extension with fibrin polymerisation and protofibril formation. Gersh *et al.* showed increased ends or "capping" of γ' fibers[13]. The γ' fibers pack significantly less protofibrils and are less stiff than γA fibers[14]. While γ' clots are mechanically impaired, they are more resistant to proteolysis by plasmin than γA clots due to impaired conversion of plasminogen to plasmin by tissue plasminogen activator on γ' clots[15]. The significance of these effects for health and disease is less clear since increased levels of fibrinogen γ' have been associated with coronary artery disease and myocardial infarction on one hand, while decreased levels have been associated with venous thrombosis on the other[16].

Fibrinogen γ' as thrombomodulin II

The fibrinogen γ' chain contains a high affinity binding site for thrombin exosite II[17]. The K_D of this interaction is around 100 nM, which compares with a K_D of 1.5 μM for the lower affinity interaction of thrombin exosite I with the fibrinogen E-region, where the fibrinopeptides reside that thrombin cleaves during the conversion to fibrin. In view of the plasma concentration of fibrinogen γ' (around 100 nM) and the amount of thrombin generated in tissue factor triggered clotting of blood plasma (up to 200-300 nM at its peak[18]), the thrombin-γ' binding reaches saturation during the peak of blood coagulation activity. Fibrinogen γ' was originally described as antithrombin I after its initial discovery[19]. This antithrombin activity is caused by the high affinity binding of thrombin

to the γ' chain in the fibrin clot, which therefore acts as a "sink" for thrombin, and effectively reduces the amount of thrombin available for other substrates in the supernatant plasma. However, recent studies indicate that the effects of γ' on thrombin activity is far more complex, with γ' directing thrombin towards certain target substrates while inhibiting others.

At this point, we would like to introduce the term "thrombomodulin II" for fibrinogen γ' in this mini-review (Fig. 1). In analogy with thrombomodulin, which binds exosite I on thrombin and directs thrombin away from exosite I driven reactions (such as the conversion of fibrinogen to fibrin) to exosite II driven reactions (particularly the activation of protein C), fibrinogen γ' also shows thrombomodulating activity. Fibrinogen γ' competitively inhibits the binding of heparin or glycosaminoglycans to thrombin as their binding site closely overlap on exosite II[17]. Recent studies by Kremer *et al.* showed that γ' protects thrombin from degradation by antithrombin and α_2-macroglobulin, thereby increasing plasma thrombin generation[20]. Other studies show that fibrinogen γ' inhibit the activation of a number of substrates that require interaction with thrombin exosite II. Fibrinogen γ' has been shown to inhibit the activation of factor VIII[21] and factor V[22] by thrombin. Moreover, Omarova *et al.* showed that fibrinogen γ' increases the sensitivity of plasma to the inhibition of clotting to activated protein C[23]. So while γ' on one hand protects thrombin against degradation and increases thrombin generation in plasma, it inhibits exosite II driven procoagulant reactions on the other hand. Exosite I driven reactions such as the cleavage of fibrinopeptide A by thrombin remain unaffected[24]. Some of these functions are not compatible with antithrombin activity, but highlight a modulating activity of thrombin activity by γ' consistent with the inhibition of exosite II directed reactions, hence the term thrombomodulin II to more accurately describe the interactions between γ' and thrombin. Further studies are required to study the effect of γ' on other thrombin-mediated reactions that have hitherto not yet been analysed.

Crosslinking of fibrin

Factor XIII, isopeptide bond formation and fibrin cross-linking

Cross-linking, resulting from the formation of isopeptide bonds, is catalysed by the family of transglutaminases. Unlike the other eight members of this family, which are located within tissues, factor XIII (FXIII) is a plasma-specific endo-γ-glutamine:ε-lysine transferase[25].

Isopeptide γ-glutamyl-ε-lysyl bond formation results from the removal of the ammonia from a glutamine residue, leading to the formation of an acyl-enzyme intermediate (Fig. 2A), and its replacement with an ε-amine group from a lysine residue (Fig. 2B). This bond is then irreversible and can only be broken down by proteolysis[25]. Factor XIII is capable of cross-linking the fibrin α- and γ-chains (Fig. 3A), as well as other substrates onto fibrin (Fig. 3B), which affect the overall structure and stability of the fibrin mesh[25]. While factor XIII is able to cross-link both fibrin α- and γ-chains, the kinetics is different between the two chains, with the γ-chains being cross-linked at a faster rate than the α-chains[26, 27].

Fibrin α-α chains cross-linking

Fibrin α-chains are cross-linked to each other by FXIII via glutamine residues 221, 237, 328 and 366, and lysine residues 208, 219, 224 with an additional 10 lysine residues in the αC domain[25]. The effects of fibrin α-chain cross-linking (Fig. 3A) on clot structure have been studied in detail using two different fibrinogen mutants.

The first, fibrinogen α-251, described in 1998, contained a deletion of α-chain amino acids 251-610 where most of cross-linking site are located[28]. Collet *et al.* recently showed that in the presence of FXIII, clots made of fibrin α-251 were denser, with the pore size decreasing by 1.8-fold, compared to wild-type fibrin[29]. The viscoelastic properties of the clots were also analysed using a torsion pendulum, and in the absence of FXIII, clots made of the wild-type fibrin were 2.3-fold stiffer than those made of fibrin α-251. In the presence of FXIII, the increase in stiffness was 1.6-fold for fibrin α-251 compared to 2.3-fold for wild-type fibrin, indicating that fibrin α-chain cross-linking play a role in increasing fibrin clot stiffness[29].

A more recent study also showed that cross-linking of the fibrin α-chains affects clot structure in several ways. Using a fibrinogen mutant (γ-3X) unable to generate γ-γ cross-links[30] and studying clot microrheology using magnetic tweezers, fibrin α-chain cross-linking was shown to significantly increase clot stiffness (storage modulus, G') by 1.4-fold and significantly decrease clot deformation (loss modulus, G'') by 1.4-fold compared to uncross-linked clots[31]. This was suggested to be most likely be due to increased fibre tautness observed by electron microscopy in clot formed in the presence of FXIII[31]. Fibrin α-chain cross-linking was also shown to increase fibre appearance time by 1.3-fold in confocal microscopy experiments. Using a turbidity set-up, the time to maximum absorbency was significantly lengthened for both the wild-type (2.7-fold) and γ-3X fibrin

(2.2-fold) in the presence of FXIII, indicating that once polymerisation is complete, α-chain cross-linking increases fibre thickness over time[31].

Fibrinogen γ-3X, alongside the previously mentioned fibrinogen α-251, were used by Byrnes *et al.* to study the effects of α- and γ-chains cross-linking on red blood cell retention within the clot[32]. Inhibition of FXIII cross-linking did not affect the loss of red blood cells in clots made of fibrin α-251, whereas clots made of fibrin γ-3X and wild-type both showed a reduction of clot weight by around 60%. These data indicate that FXIII-dependent retention of red blood cells is mediated by the cross-linking of fibrin α-chains[32].

Fibrin γ-γ chains cross-linking

Fibrin γ-chains are cross-linked to each other by FXIII via glutamine residues 398 and 399, and lysine residue 406[25]. Using confocal microscopy, fibrin γ-3X described in the previous section (Fig. 3A) was shown to significantly increase fibre density within the clot[31]. Whilst cross-linking of wild-type fibrin by FXIII induced a 1.3-fold increase in fibre density, no changes were observed in the presence of fibrin γ-3X, indicating a specific role for the γ-chain cross-linking in determining clot fibre density.

Two earlier studies using this mutant showed a contribution of γ-chain cross-linking in increasing stiffness of the clot. These studies used whole clot rheology by torsion pendulum[30] and single fibre measurement by atomic microscopy[33], and showed an increase in fibre and clot stiffness of around 40% due to γ-chain cross-linking.

Fibrinolysis inhibitors cross-linking to fibrin

Fibrinolysis inhibitor α_2-antiplamin has been shown to be the main protein responsible for inhibiting proteolysis of fibrin clots by plasmin, and is cross-linked by FXIII to lysine residue 303 of fibrin α-chain via its glutamine residue 2[25] (Fig. 3B). Cross-linking of fibrin by FXIII has been shown to increase the clot lysis time, but this was found to be exclusively mediated by cross-linking of α_2-antiplamin within the clot[34]. It has recently been proposed by Rijken *et al.* that cross-linking of α_2-antiplamin to fibrin by FXIII prevents it from being expelled from the clot during the process of clot contraction[35]. As such, α_2-antiplamin density within the fibrin fibres increases during the contraction process, rendering the whole clot more resistant to fibrinolysis. There are no studies up to date that have investigated the effect(s) of the cross-linking of α_2-antiplamin to fibrin on the overall structure of the clot.

Other fibrinolysis inhibitors (Fig. 3B) cross-linked to fibrin are plasminogen-activator inhibitor 2 (PAI-2) and thrombin activatable fibrinolysis inhibitor (TAFI). PAI-2 is cross-linked by FXIII to lysine residues 148, 176, 183, 230, 413 and 457 of fibrin α-chain via its glutamine residues 83 and 86[25]. TAFI is cross-linked to fibrin via its glutamine residues 2, 5 and 292[25] to fibrin α-chain lysine residues 77, 79 and 212[36]. Neither PAI-2 nor TAFI are yet to be shown to have an influence on the fibrin clot structure itself.

Other cross-linking to fibrin

Numerous other substrates are cross-linked to fibrin by FXIII[25], but only a few have be shown to affect the clot structure.

Fibronectin, an extracellular matrix protein involved in cell adhesion and migration, is cross-linked to fibrin α-chains by FXIIIa, via its glutamine residues 3, 4 and 16[37], although no lysine residue donors have yet been identified in fibrin. Cross-linking of fibronectin to fibrin has been shown to have no effect on clot rigidity, however those clots were denser, with smaller pores[37]. A more recent study by Ramanathan *et al.*, in a purified system in the absence of FXIII, showed that fibronectin increases the initial rate of fibrin polymerisation, leading to the formation of denser clots[38], thought to be more resistant to fibrinolysis[39].

Thrombospondin-1, an extracellular matrix protein involved in cell adhesion and motility, is cross-linked to fibrin α-chains by FXIII, via residues that remain to be determined[40]. Ligation of thrombospondin-1 within the fibrin clot was found to reduce the lag-time and lead to the formation of denser clots containing thinner fibres, in a dose-dependent manner[41], rendering them more resistant to fibrinolysis[39].

Summary

Fibrinogen, the precursor of the clot matrix protein fibrin, is a highly variable protein that is subject to modification by splice-variation and ligation by transglutaminase factor XIII. These modifications have major implications for fibrin clot structure and function, impacting on diseases of thrombosis and bleeding. Examples include fibrinogen αEC and γ', the latter of which has a number of effects on clotting: γ' inhibits protofibril formation thereby altering clot structure, γ' binds thrombin and it influences thrombin behaviour towards substrates, acting as thrombomodulin II. Another example is the cross-linking of fibrin by factor XIII, which has major effects on the mechanical properties of fibrin polymer fibres and matrix. These protein modifications serve to diversify the function of a single matrix

protein such as fibrinogen or fibrin and could play major roles in aspects of health and disease involving these functional variations.

Reference List

[1] A.H. Henschen, Human fibrinogen--structural variants and functional sites, Thrombosis and haemostasis, 70 (1993) 42-47.

[2] A. Undas, R.A. Ariens, Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases, Arteriosclerosis, thrombosis, and vascular biology, 31 (2011) e88-99.

[3] R.I. Litvinov, J.W. Weisel, Fibrin mechanical properties and their structural origins, Matrix Biol, (2016).

[4] G. Grieninger, Contribution of the alpha EC domain to the structure and function of fibrinogen-420, Annals of the New York Academy of Sciences, 936 (2001) 44-64.

[5] Y. Fu, Y. Cao, K.M. Hertzberg, G. Grieninger, Fibrinogen alpha genes: conservation of bipartite transcripts and carboxy-terminal-extended alpha subunits in vertebrates, Genomics, 30 (1995) 71-76.

[6] G. Spraggon, D. Applegate, S.J. Everse, J.Z. Zhang, L. Veerapandian, C. Redman, R.F. Doolittle, G. Grieninger, Crystal structure of a recombinant alphaEC domain from human fibrinogen-420, Proc Natl Acad Sci U S A, 95 (1998) 9099-9104.

[7] D. Applegate, L.S. Steben, K.M. Hertzberg, G. Grieninger, The alpha(E)C domain of human fibrinogen-420 is a stable and early plasmin cleavage product, Blood, 95 (2000) 2297-2303.

[8] V.K. Lishko, V.P. Yakubenko, K.M. Hertzberg, G. Grieninger, T.P. Ugarova, The alternatively spliced alpha(E)C domain of human fibrinogen-420 is a novel ligand for leukocyte integrins alpha(M)beta(2) and alpha(X)beta(2), Blood, 98 (2001) 2448-2455.

[9] M.W. Mosesson, J.P. DiOrio, I. Hernandez, J.F. Hainfeld, J.S. Wall, G. Grieninger, The ultrastructure of fibrinogen-420 and the fibrin-420 clot, Biophys Chem, 112 (2004) 209-214.

[10] F.L. Macrae, M.M. Domingues, A. Casini, R.A. Ariens, The (Patho)physiology of Fibrinogen gamma', Seminars in thrombosis and hemostasis, (2016).

[11] M. Pieters, R.C. Kotze, J.C. Jerling, A. Kruger, R.A. Ariens, Evidence that fibrinogen gamma' regulates plasma clot structure and lysis and relationship to cardiovascular risk factors in black Africans, Blood, 121 (2013) 3254-3260.

[12] P. Allan, S. Uitte de Willige, R.H. Abou-Saleh, S.D. Connell, R.A. Ariens, Evidence that fibrinogen gamma' directly interferes with protofibril growth: implications for fibrin structure and clot stiffness, Journal of thrombosis and haemostasis : JTH, 10 (2012) 1072-1080.

[13] K.C. Gersh, C. Nagaswami, J.W. Weisel, S.T. Lord, The presence of gamma' chain impairs fibrin polymerization, Thrombosis research, 124 (2009) 356-363.

[14] M.M. Domingues, F.L. Macrae, C. Duval, H.R. McPherson, K.I. Bridge, R.A. Ajjan, V.C. Ridger, S.D. Connell, H. Philippou, R.A. Ariens, Thrombin and fibrinogen gamma' impact clot structure by marked effects on intrafibrillar structure and protofibril packing, Blood, 127 (2016) 487-495.

[15] P.Y. Kim, T.T. Vu, B.A. Leslie, A.R. Stafford, J.C. Fredenburgh, J.I. Weitz, Reduced plasminogen binding and delayed activation render gamma'-fibrin more resistant to lysis than gammaA-fibrin, The Journal of biological chemistry, 289 (2014) 27494-27503.

[16] R.A. Ariens, Fibrin(ogen) and thrombotic disease, Journal of thrombosis and haemostasis : JTH, 11 Suppl 1 (2013) 294-305.

[17] A.O. Pineda, Z.W. Chen, F. Marino, F.S. Mathews, M.W. Mosesson, E. Di Cera, Crystal structure of thrombin in complex with fibrinogen gamma' peptide, Biophys Chem, 125 (2007) 556-559.

[18] H.C. Hemker, P.L. Giesen, M. Ramjee, R. Wagenvoord, S. Beguin, The thrombogram: monitoring thrombin generation in platelet-rich plasma, Thrombosis and haemostasis, 83 (2000) 589-591.

[19] M.W. Mosesson, Update on antithrombin I (fibrin), Thrombosis and haemostasis, 98 (2007) 105-108.

[20] R.M. Kremers, R.J. Wagenvoord, H.C. Hemker, The effect of fibrin(ogen) on thrombin generation and decay, Thrombosis and haemostasis, 112 (2014) 486-494.

[21] R.S. Lovely, L.K. Boshkov, U.M. Marzec, S.R. Hanson, D.H. Farrell, Fibrinogen gamma' chain carboxy terminal peptide selectively inhibits the intrinsic coagulation pathway, Br J Haematol, 139 (2007) 494-503.

[22] F. Omarova, S. Uitte De Willige, R.A. Ariens, J. Rosing, R.M. Bertina, E. Castoldi, Inhibition of thrombin-mediated factor V activation contributes to the anticoagulant activity of fibrinogen gamma', Journal of thrombosis and haemostasis : JTH, 11 (2013) 1669-1678.

[23] F. Omarova, S. Uitte de Willige, P. Simioni, R.A. Ariens, R.M. Bertina, J. Rosing, E. Castoldi, Fibrinogen gamma' increases the sensitivity to activated protein C in normal and factor V Leiden plasma, Blood, 124 (2014) 1531-1538.

[24] A.V. Cooper, K.F. Standeven, R.A. Ariens, Fibrinogen gamma-chain splice variant gamma' alters fibrin formation and structure, Blood, 102 (2003) 535-540.

[25] C. Duval, H. Philippou, R.A. Ariens, Factor XIII, in: V.J. Marder, W.C. Aird, J.S. Bennett, S. Schulman, G.C. White (Eds.) Hemostasis and Thrombosis: Basic Principles and Clinical Practice, Lippincott Williams & Wilkins, Philadelphia, 2012, pp. 272-285.

[26] P.A. McKee, P. Mattock, R.L. Hill, Subunit structure of human fibrinogen, soluble fibrin, and cross-linked insoluble fibrin, Proc Natl Acad Sci U S A, 66 (1970) 738-744.

[27] C. Duval, M. Ali, W.W. Chaudhry, V.C. Ridger, R.A. Ariens, H. Philippou, Factor XIII A-Subunit V34L Variant Affects Thrombus Cross-Linking in a Murine Model of Thrombosis, Arterioscler Thromb Vasc Biol, 36 (2016) 308-316.

[28] O.V. Gorkun, A.H. Henschen-Edman, L.F. Ping, S.T. Lord, Analysis of A alpha 251 fibrinogen: the alpha C domain has a role in polymerization, albeit more subtle than anticipated from the analogous proteolytic fragment X, Biochemistry, 37 (1998) 15434-15441.

[29] J.P. Collet, J.L. Moen, Y.I. Veklich, O.V. Gorkun, S.T. Lord, G. Montalescot, J.W. Weisel, The alphaC domains of fibrinogen affect the structure of the fibrin clot, its physical properties, and its susceptibility to fibrinolysis, Blood, 106 (2005) 3824-3830.

[30] K.F. Standeven, A.M. Carter, P.J. Grant, J.W. Weisel, I. Chernysh, L. Masova, S.T. Lord, R.A. Ariens, Functional analysis of fibrin {gamma}-chain cross-linking by activated factor XIII: determination of a cross-linking pattern that maximizes clot stiffness, Blood, 110 (2007) 902-907.

[31] C. Duval, P. Allan, S.D. Connell, V.C. Ridger, H. Philippou, R.A. Ariens, Roles of fibrin alpha- and gamma-chain specific cross-linking by FXIIIa in fibrin structure and function, Thromb Haemost, 111 (2014) 842-850.

[32] J.R. Byrnes, C. Duval, Y. Wang, C.E. Hansen, B. Ahn, M.J. Mooberry, M.A. Clark, J.M. Johnsen, S.T. Lord, W.A. Lam, J.C. Meijers, H. Ni, R.A. Ariens, A.S. Wolberg, Factor

XIIIa-dependent retention of red blood cells in clots is mediated by fibrin alpha-chain crosslinking, Blood, 126 (2015) 1940-1948.

[33] C.C. Helms, R.A. Ariens, S. Uitte de Willige, K.F. Standeven, M. Guthold, alpha-alpha Cross-links increase fibrin fiber elasticity and stiffness, Biophys J, 102 (2012) 168-175.

[34] S.R. Fraser, N.A. Booth, N.J. Mutch, The antifibrinolytic function of factor XIII is exclusively expressed through alpha(2)-antiplasmin cross-linking, Blood, 117 (2011) 6371-6374.

[35] D.C. Rijken, S. Abdul, J.J. Malfliet, F.W. Leebeek, S. Uitte de Willige, Compaction of fibrin clots reveals the antifibrinolytic effect of factor XIII, Journal of thrombosis and haemostasis : JTH, (2016).

[36] N.J. Mutch, N.R. Moore, E. Wang, N.A. Booth, TAFI is cross-linked by its activation peptide to sites on fibrin(ogen) distinct from other inhibitors of fibrinolysis, Journal of thrombosis and haemostasis : JTH, 1 (2003) OC064.

[37] V.R. Richardson, P. Cordell, K.F. Standeven, A.M. Carter, Substrates of Factor XIII-A: roles in thrombosis and wound healing, Clin Sci (Lond), 124 (2013) 123-137.

[38] A. Ramanathan, N. Karuri, Fibronectin alters the rate of formation and structure of the fibrin matrix, Biochemical and biophysical research communications, 443 (2014) 395-399.

[39] J.P. Collet, D. Park, C. Lesty, J. Soria, C. Soria, G. Montalescot, J.W. Weisel, Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy, Arteriosclerosis, thrombosis, and vascular biology, 20 (2000) 1354-1361.

[40] M.D. Bale, D.F. Mosher, Thrombospondin is a substrate for blood coagulation factor XIIIa, Biochemistry, 25 (1986) 5667-5673.

[41] M.D. Bale, D.F. Mosher, Effects of thrombospondin on fibrin polymerization and structure, The Journal of biological chemistry, 261 (1986) 862-868.

Figure Legends

Figure 1: Fibrinogen γ' as thrombomodulin II. Both fibrinogen γA (green) and γ' (orange) bind thrombin exosite I via a low affinity interaction, but fibrinogen γ' contains an additional high-affinity binding site for thrombin exosite II. Through its interaction with thrombin exosite II, fibrinogen γ' gains a number of functional features, including higher affinity thrombin binding, inhibition of factor V (FV) activation, inhibition of factor VIII (FVIII) activation and increased sensitivity of plasma for activated protein C (APC), which can be grouped as thrombomodulin II activity. In addition, fibrinogen γ' alters clot structure by direct interference with protofibril formation and acts as a thrombin "sponge", protecting thrombin against degradation by α2-macroglobulin and antithrombin.

Figure 2: Isopeptide bond formation catalysed by FXIIIa. The glutamine residue from a fibrin molecule is deaminated (A) and replaced by the ε-amino group from the lysine residue of an adjacent fibrin molecule (B), to form an isopeptide bond. **X**: fibrin molecule; **=**: isopeptide bond.

Figure 3: Factor XIII cross-linking of fibrin α- and γ-chains, and fibrinolysis inhibitors to fibrin. A) Fibrin α-α- and γ-γ chains cross-linking play a role in clot formation, structure and stability. B) Fibrinolysis inhibitors cross-linking, such as α_2-antiplasmin, PAI-2 and TAFI, play a role in protecting the clot from proteolytic degradation by plasmin.

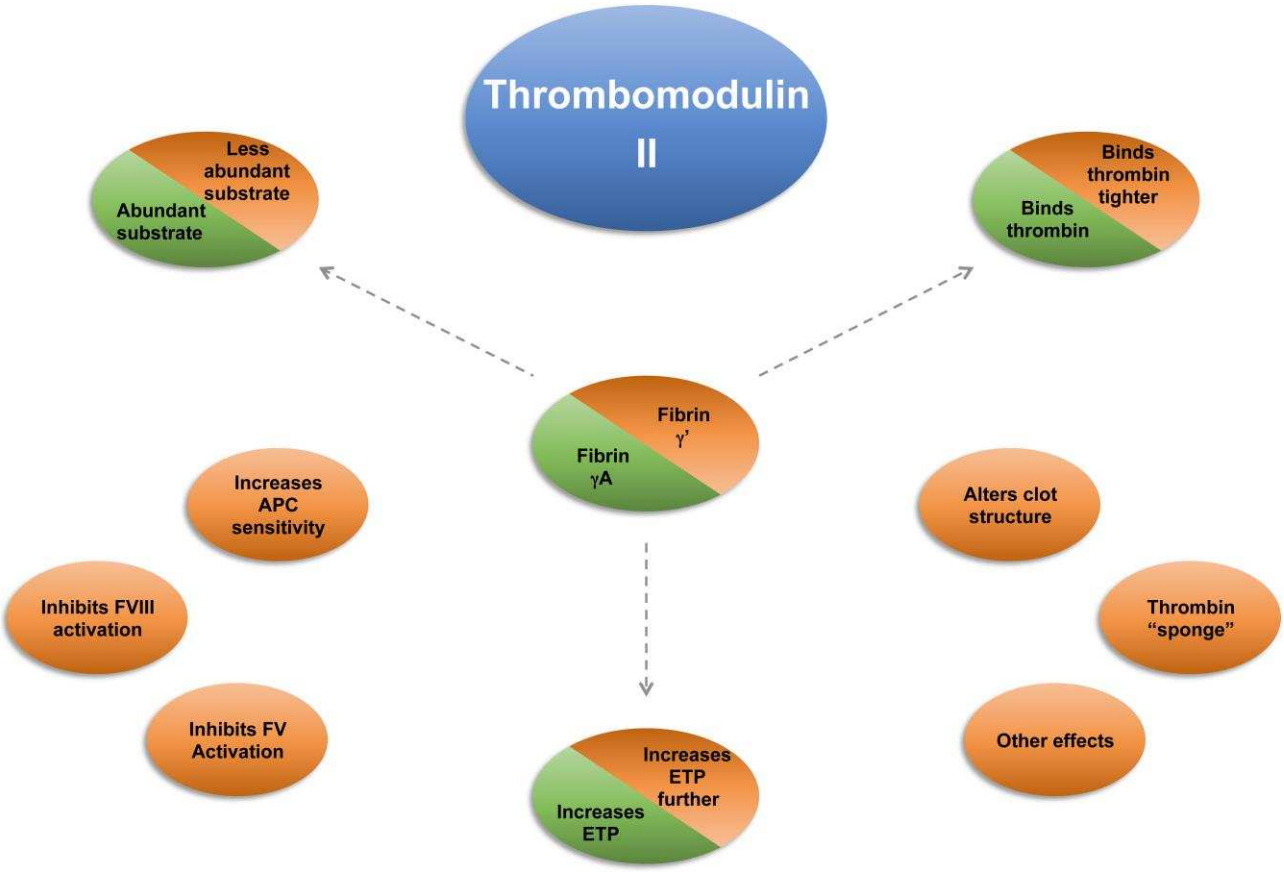

Figure 1

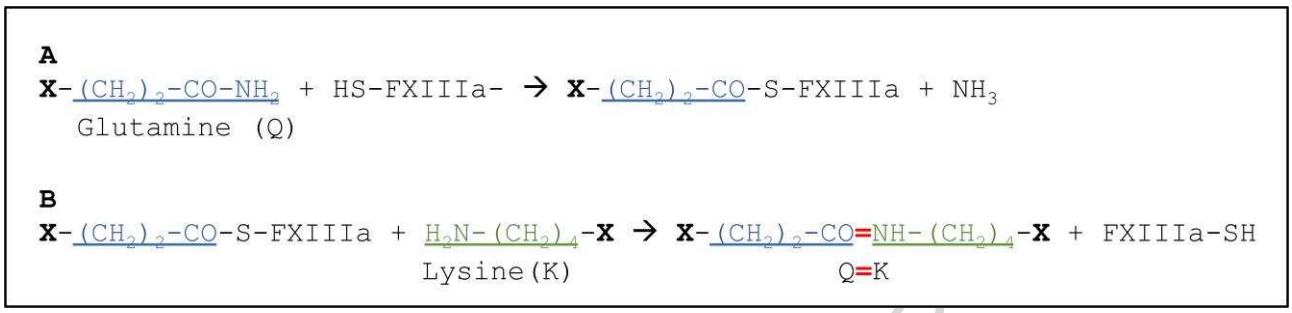

Figure 2

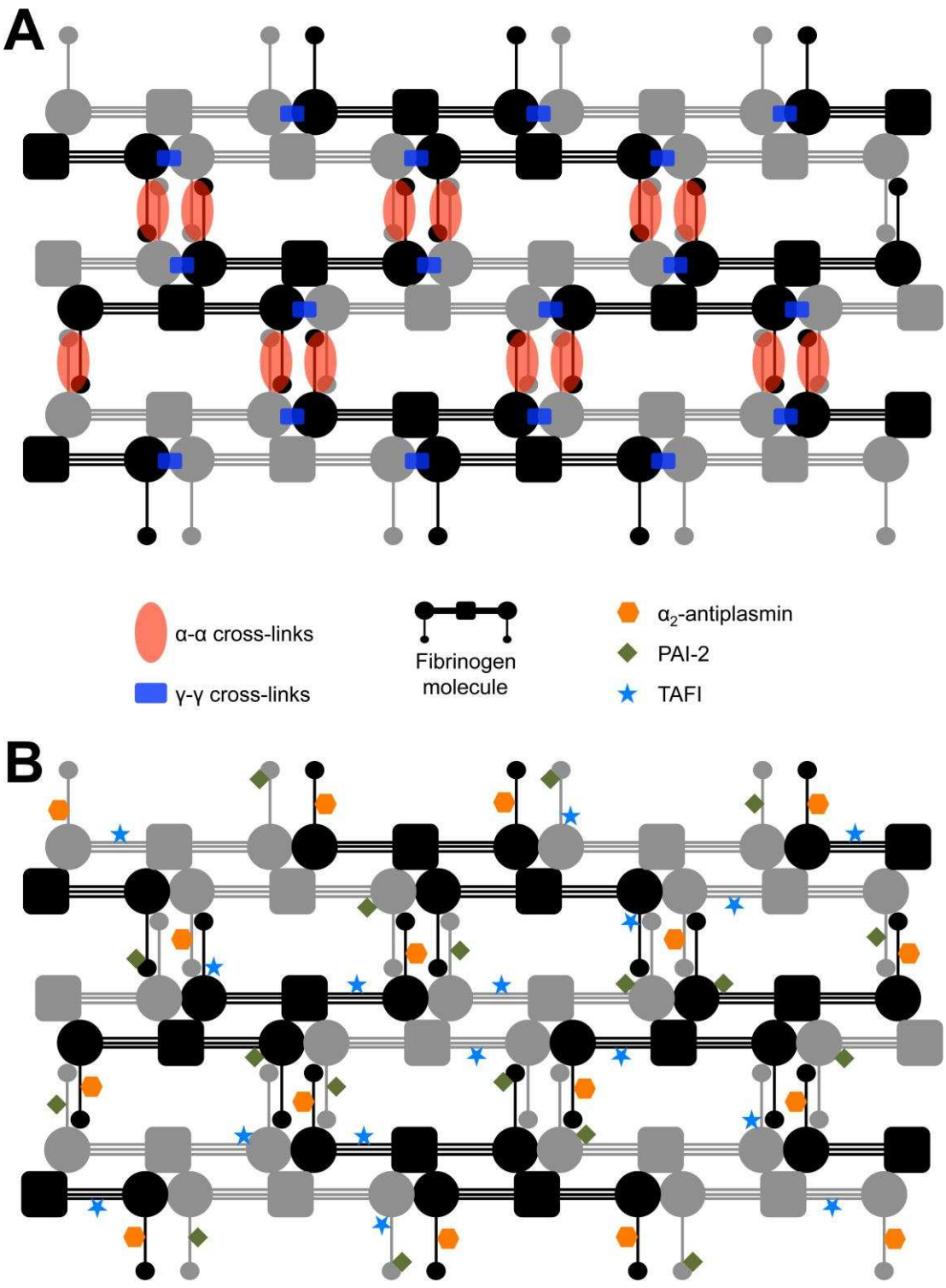

Figure 3