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COMMENTARY

# Factor XIII: What does it look like?

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Coagulation factor XIII (FXIII) is a stepchild among clotting factors. As opposed to all other zymogenic clotting factors, it is not the precursor of a proteolytic enzyme but of a transglutaminase. It is of tetrameric structure consisting of two types of subunits (FXIII-$A_2B_2$). In the final step of the coagulation cascade the A subunit (FXIII-A) is transformed into an active enzyme by the concerted action of thrombin and $Ca^{2+}$. Thrombin cleaves off an activation peptide (AP-FXIII) of 37 amino acids from the N-terminal end of FXIII-A. Subsequently, in the presence of $Ca^{2+}$, the protective/inhibitory B subunits (FXIII-B) dissociate from the cleaved A′ subunits, which then assume an active configuration (FXIII-A*). Activated FXIII is a transglutaminase, the main function of which is the cross-linking of fibrin $\alpha$ and fibrin $\gamma$ chains and the attachment of $\alpha_2$-plasmin inhibitor to fibrin through $\varepsilon(\gamma$-glutamyl) lysyl isopeptide bonds. This way FXIII is involved in mechanically strengthening the fibrin clot and protecting it from fibrinolytic degradation. A dimer of FXIII-A lacking the B subunits is also expressed in several cell types, including platelets, monocytes, macrophages, osteoblasts, chondrocytes, and preadipocytes in which it exerts diverse functions. The intracellular activation of FXIII-$A_2$ does not need limited proteolytic involvement. The elevation of the $Ca^{2+}$ level in the cytoplasm is sufficient to convert FXIII-$A_2$ to an active configuration (FXIII-A°). (For comprehensive reviews see references [1–5].)

X-ray crystallography revealed the atomic resolution structure of FXIII-$A_2$ and demonstrated that the FXIII-A monomer contains four domains ($\beta$-sandwich, catalytic core, $\beta$-barrel 1, and $\beta$-barrel 2) and AP-FXIII [6,7]. The crystallographic structure of FXIII-B is not available. This protein is of mosaic structure, it consists of 10 short tandem repeats (sushi domains) each containing about 60 amino acids and held together by a pair of disulfide bonds [8]. Our knowledge on the structure of FXIII and on structural changes during FXIII activation was enriched by important studies using electron microscopy, size exclusion chromatography, analytical ultracentrifugation, H-D exchange, mass spectrometry, and molecular modeling [2,9–13]. The use of high-resolution single-molecule atomic force microscopy (AFM) in the study of Protopopova *et al.*, published in this issue of *JTH*, provides a new dimension to this extensive line of research [14]. This technique visualizes the surface topology/contour of the molecule rather than the protein backbone, and this way an additional aspect of structural information is revealed. The authors investigated the morphology of the tetrameric FXIII-$A_2B_2$ complex and the individual subunits. The structural changes induced by thrombin and $Ca^{2+}$ were also demonstrated.

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## The structure of FXIII and its subunits

Based on early electronmicroscopic studies it was suggested that two FXIII-B subunits are wrapped around the FXIII-A dimers [9]. This hypothesis was supported by the finding that the presence of FXIII-B protects the A subunits from proteolytic inactivation [10,14]. The AFM pictures produced by Protopopova *et al.* provide a remarkably novel insight into the structure of FXIII-$A_2B_2$. In the published study it is clearly demonstrated that within the tetrameric structure, FXIII-$A_2$ is represented by a globular part of moderate ovality. In the majority of cases two strands corresponding to monomeric FXIII-B extend from the globular part; however, there were also minor structural variants with only one or no thin flexible extensions. This structural diversity very likely reflects the equilibrium between association and dissociation of FXIII-B subunits from the A2 dimer. According to the AFM data, 26% of the B subunits dissociate from the globular core, while 74% are connected to the globules, which is in excellent agreement with the values calculated from the actual FXIII concentration

(~6.3 nM) and the Kd ($4.17 \times 10^{-10}$ M) [15]. Earlier reports suggested that the first two N-terminal sushi domains of FXIII-B are involved in the association to FXIII-$A_2$ [10,15], which was confirmed by the AFM pictures. As expected, the individual FXIII-A monomers in the complex are not resolved by AFM. The location of FXIII-B in the complex is somewhat surprising. As discussed later, the individual free FXIII-B molecules form dimers, and it is not clear how the FXIII-B dimer falls apart into monomers to be able to combine with an individual FXIII-A molecule. The binding site for FXIII-B on FXIII-A also needs to be specified to explain how the two FXIII-B monomers enter the compact/closed structure of FXIII-$A_2$. Such investigations might be relevant for FXIII-A-deficient patients receiving recombinant FXIII-$A_2$ for prophylaxis: in these patients the binding of recombinant FXIII-$A_2$ to the patient's FXIII-B is essential for the formation of heterotetramers, which in contrast to uncomplexed FXIII-$A_2$ have a long half-life in the plasma.

Earlier reports concerning the dimeric or monomeric state of free FXIII-B were controversial. Sedimentation analysis and low-resolution electronmicroscopic images suggested free FXIII-B to be a flexible and kinked monomeric structure [9], while gel filtration experiments suggested the protein to be a dimeric structure [10,16]. In the present study the dimeric nature of free FXIII-B was clearly confirmed. It has been shown that the measured length of FXIII-B dimer corresponded to a previously suggested model, in which the two FXIII-B subunits have an antiparallel orientation, which is ensured by the interaction of sushi domains 4 and 9 [10]. Accepting this model, further experiments are needed to explain the mechanism of complex formation considering that the two N-terminal sushi domains are positioned at the opposite ends of the dimeric strand. One possibility is that the two opposite ends of FXIII-$B_2$ bind to FXIII-A molecules present in distinct FXIII-A dimers. The other possibility is that the binding of a FXIII-B subunit to FXIII-$A_2$ induces the dissociation of the FXIII-$B_2$ and the released FXIII-B monomer becomes available for combining with FXIII-$A_2$. Obviously such speculations and others call for experimental support.

## Structural changes upon FXIII activation

Earlier reports suggested that after activation of FXIII-$A_2B_2$ and FXIII-$A_2$ by thrombin and $Ca^{2+}$ the two active subunits stick together (FXIII-$A_2$*) and express transglutaminase activity. Most recently this paradigm has been challenged. The dissociation of FXIII-$A_2$ into FXIII-A* monomers upon both proteolytic and non-proteolytic activation was demonstrated by analytical ultracentrifugation, and FXIII-A* also remained monomeric upon binding of substrate analogs [11]. Protopopova *et al.* provided direct evidence on the monomeric form of FXIII-A* by AFM [14]. Using volume calculations the FXIII-$A_2$ dimer was found to be 2.3-fold larger than FXIII-A*. Molecular modeling also supported a monomeric form for FXIII-A* [13,17]. The monomeric structure of all other active transglutaminases is in line with these findings [2,18]. Based on the results of Protopopova *et al.* and on earlier findings a schematic representation of FXIII activation is proposed in Fig. 1. The transformation of FXIII-A does not induce drastic conformational changes within a particular domain. However, the changes in the position of individual domains indicate considerable structural transitions, usually termed as transition from closed/compact to open/extended structure (reviewed in references [2,19]). The resolution of single-molecule AFM does not seem sufficient to capture such a difference.

The introduction of high-resolution single-molecule AFM opens new perspectives in FXIII structural research. It helps in evaluating controversial issues and might confirm or contradict existing paradigms. New findings by this technique contribute to our better understanding of the structural-functional relationship of this

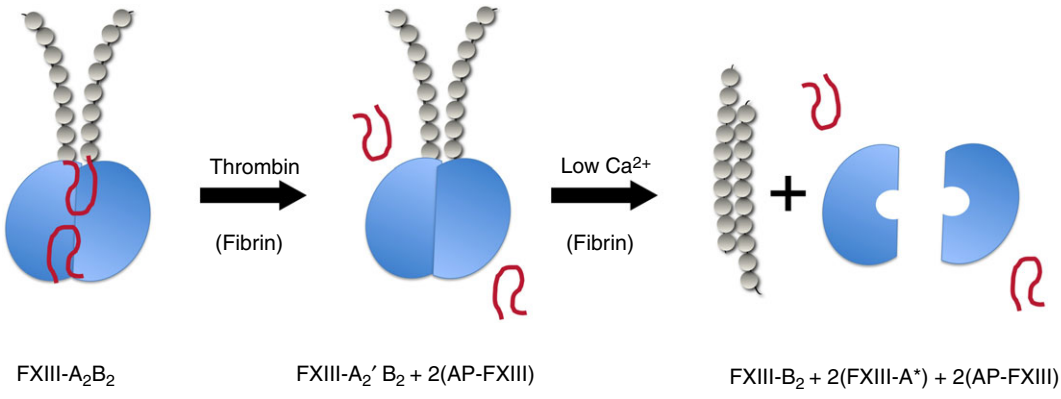


**FIGURE 1.** Schematic representation of proteolytic FXIII activation. The A subunit of (FXIII-A) is depicted in blue. Elongated strands consisting of gray pearls represent the FXIII B subunit (FXIII-B) consisting of sushi domains. Activation peptides (AP-FXIII) are shown as red loops. FXIII-A′, FXIII-A cleaved by thrombin; FXIII-A*, FXIII activated by thrombin; $Ca^{2+}$ (active transglutaminase).

unique coagulation factor and its role in the hemostatic mechanism.

## Addendum

The authors were equally involved in writing and constructing the commentary.

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## Disclosure of Conflict of Interests

Neither author had any real or potential conflict of interest.

## References

1 Lorand L. Factor XIII: structure, activation, and interactions with fibrinogen and fibrin. *Ann NY Acad Sci* 2001; **936**: 291–311.
2 Komaromi I, Bagoly Z, Muszbek L. Factor XIII: novel structural and functional aspects. *J Thromb Haemost* 2011; **9**: 9–20.
3 Muszbek L, Bereczky Z, Bagoly Z, Komaromi I, Katona E. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. *Physiol Rev* 2011; **91**: 931–72.
4 Schroeder V, Kohler HP. Factor XIII: structure and function. *Semin Thromb Hemost* 2016; **42**: 422–8.
5 Mitchell JL, Mutch NJ. Let's cross-link: diverse functions of the promiscuous cellular transglutaminase factor XIII-A. *J Thromb Haemost* 2019; **17**: 19–30.
6 Yee VC, Pedersen LC, Le Trong I, Bishop PD, Stenkamp RE, Teller DC. Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. *Proc Natl Acad Sci USA* 1994; **91**: 7296–300.
7 Weiss MS, Metzner HJ, Hilgenfeld R. Two non-proline cis peptide bonds may be important for factor XIII function. *FEBS Lett* 1998; **423**: 291–6.
8 Ichinose A, McMullen BA, Fujikawa K, Davie EW. Amino acid sequence of the b subunit of human factor XIII, a protein composed of ten repetitive segments. *Biochemistry* 1986; **25**: 4633–8.
9 Carrell NA, Erickson HP, McDonagh J. Electron microscopy and hydrodynamic properties of factor XIII subunits. *J Biol Chem* 1989; **264**: 551–6.
10 Souri M, Kaetsu H, Ichinose A. Sushi domains in the B subunit of factor XIII responsible for oligomer assembly. *Biochemistry* 2008; **47**: 8656–64.
11 Anokhin BA, Stribinskis V, Dean WL, Maurer MC. Activation of factor XIII is accompanied by a change in oligomerization state. *FEBS J* 2017; **284**: 3849–61.
12 Woofter RT, Maurer MC. Role of calcium in the conformational dynamics of factor XIII activation examined by hydrogen-deuterium exchange coupled with MALDI-TOF MS. *Arch Biochem Biophys* 2011; **512**: 87–95.
13 Gupta S, Biswas A, Akhter MS, Krettler C, Reinhart C, Dodt J, Reuter A, Philippou H, Ivaskevicius V, Oldenburg J. Revisiting the mechanism of coagulation factor XIII activation and regulation from a structure/functional perspective. *Sci Rep* 2016; **6**: 30105.
14 Protopopova AD, Ramirez A, Klinov DV, Litvinov RI, Weisel JW. Factor XIII topology: organization of B subunits and changes with activation studied with single-molecule atomic force microscopy. *J Thromb Haemost* 2019; https://doi.org/10.1111/jth.14412 [Epub ahead of print].
15 Mary A, Achyuthan KE, Greenberg CS. b-chains prevent the proteolytic inactivation of the a-chains of plasma factor XIII. *Biochim Biophys Acta* 1988; **966**: 328–35.
16 Katona E, Penzes K, Csapo A, Fazakas F, Udvardy ML, Bagoly Z, Orosz ZZ, Muszbek L. Interaction of factor XIII subunits. *Blood* 2014; **123**: 1757–63.
17 Seelig GF, Folk JE. Noncatalytic subunits of human blood plasma coagulation factor XIII. Preparation and partial characterization of modified forms. *J Biol Chem* 1980; **255**: 8881–6.
18 Stieler M, Weber J, Hils M, Kolb P, Heine A, Buchold C, Pasternack R, Klebe G. Structure of active coagulation factor XIII triggered by calcium binding: basis for the design of next-generation anticoagulants. *Angew Chem Int Ed Engl* 2013; **52**: 11930–4.
19 Lorand L, Iismaa SE. Transglutaminase diseases: from biochemistry to the bedside. *FASEB J* 2019; **33**: 3–12.
20 Iismaa SE, Mearns BM, Lorand L, Graham RM. Transglutaminases and disease: lessons from genetically engineered mouse models and inherited disorders. *Physiol Rev* 2009; **89**: 991–1023.