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Letter to the editor

A model for the unique role of factor Va A2 domain extension in the human ternary thrombin-generating complex

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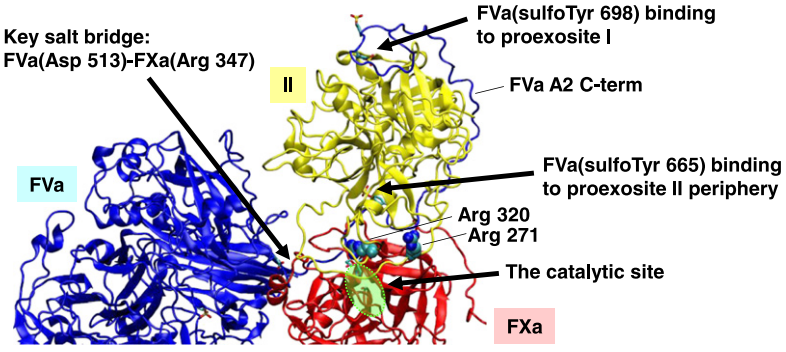
HIGHLIGHTS

- A complete human thrombin-generating model is developed.
- The factor Va A2 C-terminus with sulfotyrosines is crucial.
- The ternary model is solvent-equilibrated with molecular dynamics.

GRAPHICAL ABSTRACT



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ABSTRACT

An all-atom human ternary model for the prothrombinase–prothrombin complex, including metal ions and post-translationally modified residues, was constructed from existing X-ray crystal structures. The factor Xa–prothrombin interface was taken from an existing ternary model, which locates the active site of factor Xa in the vicinity of prothrombin cleavage positions. The three sulfotyrosine residues at the C-terminal sequence of factor Va A2 domain are accommodated by modelling rational interactions with positively charged patches on the surface of prothrombin. The entire model is then solvent-equilibrated with molecular dynamics. This ternary model for the thrombin-generating complex provides an estimate as to the role of the C-terminus of the factor Va A2 domain: to establish an interface between FXa and prothrombin and to stabilize the orientation of this interface.

Dear Editor,

We have developed an all-atom, solvent-equilibrated model for the human thrombin-forming ternary complex. In this complex, factor Xa (FXa) is the enzyme, factor Va (FVa) is the cofactor and prothrombin (II) is the substrate. FVa consists of five domains: A1–A2 and A3–C1–C2. FXa consists of four domains: Gla–EGF1–EGF2–SP. II also consists of four domains: Gla–K1–K2–SP. Cleavage at arginine 320 and arginine 271 of II by FVa/FXa (prothrombinase) produces thrombin (IIa), an enzyme itself that exhibits a number of activities in the blood coagulation cascade. For the human ternary complex, the C1–C2 and Gla domains are thought to interact with negatively charged components of specific membranes on platelets or cell surfaces, the K units in II are kringle

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domains, which are connected by a 25–amino acid linker, the EGF units in FXa are epidermal growth factor domains connected by a short linker and the SP units are serine protease domains, which act on proteins downstream in the cascade.

The essential building blocks of our model of the human ternary complex of FVa/FXa/II are the X-ray crystal structures of the FVa/FXa (SP domain) complex from *Pseudonaja textilis* [1], which provided molecular descriptions at the FVa/FXa interface, and the K2-SP model of II taken from our prior modelling efforts of the human ternary complex [2], in which the 320 loop in II was modelled from an X-ray crystal structure of prethrombin-1 [3] to position the catalytic site of FXa in prothrombinase, so as to facilitate competing arginine 320 and arginine 271 cleavages. Significant fragments of the current human FVa/FXa binary structure have been previously modelled based on the *P. textilis* structures [4]; our goal is to provide an alternate prothrombinase binary model that can be extended to the elusive ternary structure that accounts for the involvement of FVa with both FXa and prothrombin.

The model building process involved the following steps:

1. **Modelling human FVa (A1–A2 (res. 1–669**) from a combination of several X-ray crystal structures using MODELLER ver. 9.13 [5]. The X-ray crystal structures of the FVa/FXa (SP domain) complex from *P. textilis* [1] served as the main template for the construction of our model of human FVa/FXa complex.
   We used dual templates to construct the human A1 and A3 domains of FVa by MODELLER. Thus, we superposed the A1 and A3 domains of the X-ray crystal structure of bovine FVa (pdb 1SDD) [6] to the A1 and A3 domains of the X-ray crystal structure of the *P. textilis* FVa/FXa complex (pdb 4BXS) [1]. Similarly, we modelled human FVa A2 (res. 1–669) from the A2 domain of the X-ray crystal structures of the *P. textilis* FVa/FXa complex (pdb 4BXS, pdb 4BXW) [1] using MODELLER. Since the C2 domain of FVa in the X-ray crystal structure of the *P. textilis* FVa/FXa complex (pdb 4BXS) [1] showed a 30 degree rotation when compared with human fVIII (pdb 3CDZ) [7] (see supplemental Fig. 6B in ref. 1), we constructed the C1–C2 domains of human FVa from the X-ray crystal structure of bovine FVa (pdb 1SDD) [6] after superposing it to the X-ray crystal structures of the *P. textilis* FVa/FXa complex (pdb 4BXS) [1]. FVa modelling also involved adding 2 Ca ions and a Cu ion to FVa as well as a phosphothreonine (res. 612) at the A2–A3 interface and a sulfotyrosine (res. 1565) in the A3 domain.
2. **Modelling human FVa (A1–A2 (res. 1–669) and A3–C1–C2)/FXa (EGF1–EGF2-SP).** We superposed the X-ray crystal structure of human FXa (EGF1–EGF2–SP) (pdb 1XKA) [8] to the X-ray crystal structure of the FVa/FXa (SP domain) complex from *P. textilis* [1] to construct the human FXa EGF1–EGF2–SP domains. At this point, we overlaid the model from step 1 with the human ternary complex [2] with respect to FXa (SP domain). This was done to extract and orient II (K2–SP) from the human ternary complex [2] and to thereby construct FVa (A2 term. 1–669)/FX (EGF1–EGF2–SP)/II (K2–SP). The fragment of the FVa A2 domain (res. 664–669) served without change as a new, complex interface between the SP domains of FXa and II. The fact that a single strand of FVa meshes well independently with both FXa and II serves as a measure of validation for the procedure.
3. **Completing the FXa and II models. FXa**: The human FXa EGF1–EGF2-SP domains from step 1 were superposed to the X-ray crystal structures of porcine FIXa Gla–EGF1–EGF2 (pdb 1PFX) [9] with respect to EGF1–EGF2. Then, the human FXa Gla domain was constructed by superposing Gla of the X-ray structure of human FXa (pdb 1IOD) [10] to Gla of the X-ray crystal structures of porcine FIXa Gla-EGF1-EGF2 (pdb 1PFX) [9] with respect to EGF1. **II**: The Gla–K1 domains of II were modelled by the protein–protein docking program ZDOCK ver. 3.0 [11], while the K1–K2 linker was constructed by MODELLER. The Gla-K1 domains were positioned to extend downward from the K2 domain toward a putative membrane. A target was to have the omega loops of both FXa and II reach a plane roughly defined by the loops of the C1 and C2 domains of FVa, which are thought to interact with negatively charged membrane surfaces.
4. **Completing the FVa A2 domain (res. 670–709)**. This sequence involves three sulfotyrosines: res. 665, res. 696 and res. 698 at the FVa A2 C-terminal region. The first (res. 665) was present in a binary complex model for FVa (A2 C-terminal ends at res. 669) [4] and was chosen in that model to interact with FXa (arginine 424 and lysine 427). In our current ternary model, we chose this residue to interact with II (the positively charged residues in proexosite II periphery arginine 174, arginine 443 (arginine 126) and arginine 565 (arginine 233)). (The chymotrypsin numbering is noted for some prothrombin residues of particular interest by parentheses.) The second and third sulfotyrosines were chosen to interact with II (the positively charged residues of proexosite I arginine 340 (arginine 35) and lysine 372 (lysine 60F)). To locate sulfotyrosine 696 and sulfotyrosine 698, we adopted the structure of a homologous sulfotyrosine 276 and sulfotyrosine 278 sequence bound to exosite I on α-thrombin (pdb 3PMH) [12]. Finally MODELLER was employed to complete the loop between the actual A2 terminus (which includes sulfotyrosines 696 and 698) and the sequence containing sulfotyrosine 695. The result gave a full model of human FVa/FXa/II.

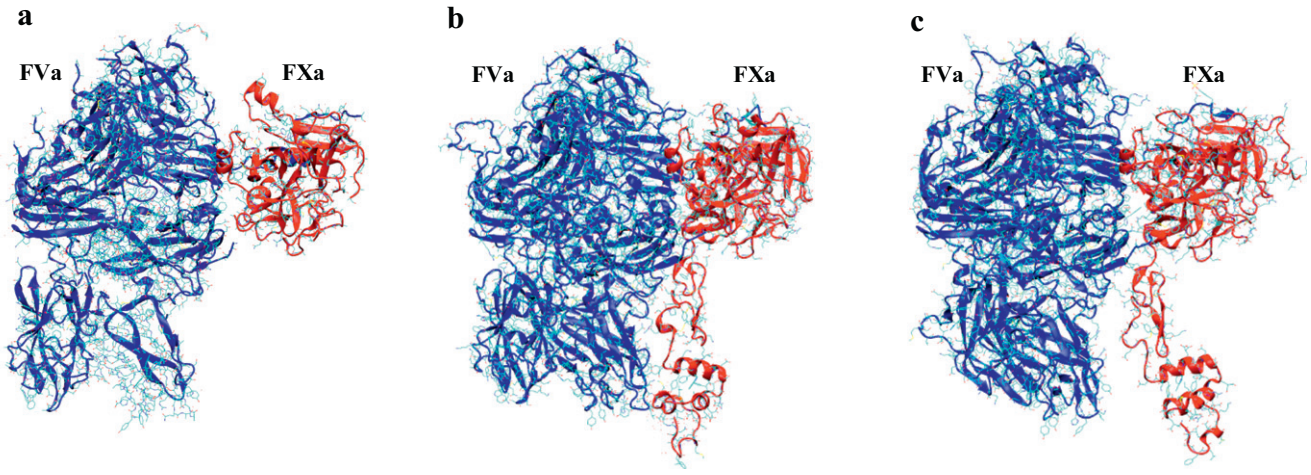

Fig. 1. a. The X-ray crystal structure of P. textilis FVa(A2 C-term. = 683)/FXa(SP) from pdb 4BXS [1]. b. The human homology model of FVa(A2 C-term. = 669)/FXa from Pomowski et al. [4]. c. Our final human model for FVa(A2 C-term. = 669)/FXa after geometry optimization. Proteins shown in line mode are colored by atom type: carbon, cyan; nitrogen, blue; oxygen, red; sulfur, yellow; phosphorous, tan. Proteins are also shown in cartoon representation (FVa in blue and FXa in red) to show secondary structures (α-helices and β-sheets).

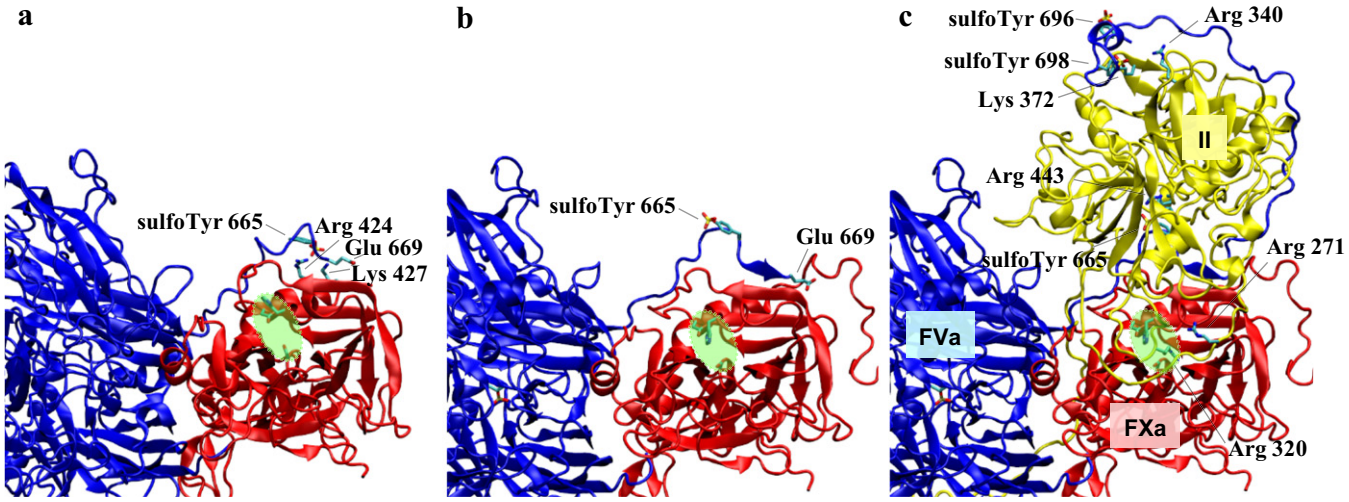

Fig. 2. Close-up of the FVa(A2 terminal region)/FXa(SP) interaction region. a. The human homology model of FVa (res. 1–669)/FXa from Pomowski et al. [4], for which sulfotyrosine 665 of FVa A2 C-terminal was modelled to interact with FXa (arginine 424 and lysine 427). b. Our final derived model for this region FVa (res. 1–669)/FXa (SP), in which sulfotyrosine 665 was modelled to interact with II (arginine 174, arginine 443 (arginine 126)), after geometry optimization. c. Our ternary complex model of FVa (res. 1–709)/FXa (SP)/II (K2-SP), where sulfotyrosines 696 and 698 were modelled to interact with the positively charged residues of proexosite I (arginine 340 (arginine 35) and lysine 372 (lysine 60F)). Coloring schemes are the same as in Fig. 1. The catalytic triad (histidine 236, aspartic acid 282 and serine 379) of FXa and arginine 271 and arginine 320 of II, the target cleavage sites by FVa/FXa for the production of IIa, are also represented (green dotted circle). Only side chains of residues (sticks) are shown for clarity.

5. **Equilibration in ionic solvent**. The model from step four was solvated in a large water box containing charge-neutralizing counterions using a procedure outlined in the Supplementary Data. The result is our FVa/FXa/II (human model). This model is freely available at the journal homepage or from the authors.

Fig. 1a shows the X-ray crystal structure (pdb 4BXS) of *P. textilis* FVa (A2 C-term. = 683)/FXa (SP) [1], Fig. 1b shows a human homology model of FVa (A2 C-term. = 669)/FXa [4], and Fig. 1c shows our final human model for FVa (A2 C-term. = 669)/FXa (SP) before solvent equilibration. Fig. 2a shows the structure of FVa (res. 1–669)/FXa (SP) from Pomowski et al. [4], Fig. 2b shows our final derived model for this region FVa (res. 1-669)/FXa (SP) before solvent equilibration, and Fig. 2c shows our ternary complex model of FVa (res. 1–709)/FXa (SP)/II (K2-SP), for which II (K2-SP) was extracted from the model of human FVa/FXa/II [2]. Fig. 3 shows our final goal—the solvent-equilibrated structure of the ternary complex model of human FVa/FXa/II structurally converged as indicated by the RMSD plot (Supplementary Fig. S1). The previously modelled fragment of human prothrombinase [4] is shown overlaid onto our model in Supplementary Fig. S2. As shown in Supplementary Table S1, RMSDs of the current human FVa (res. 1–709)/FXa, compared with the structure of FVa (res. 1-669)/FXa (SP) from Pomowski et al. [4], indicate that our FVa/FXa structure is, overall, similar in structure. However, after solvent equilibration, our structure shows some noticeable differences from the structure of FVa/FXa from Pomowski et al. [4], especially in FVa. Details of the key intermolecular interactions of FVa–FXa, FVa–II and FXa–II in the current human ternary model of FVa/FXa/II complex, measured by

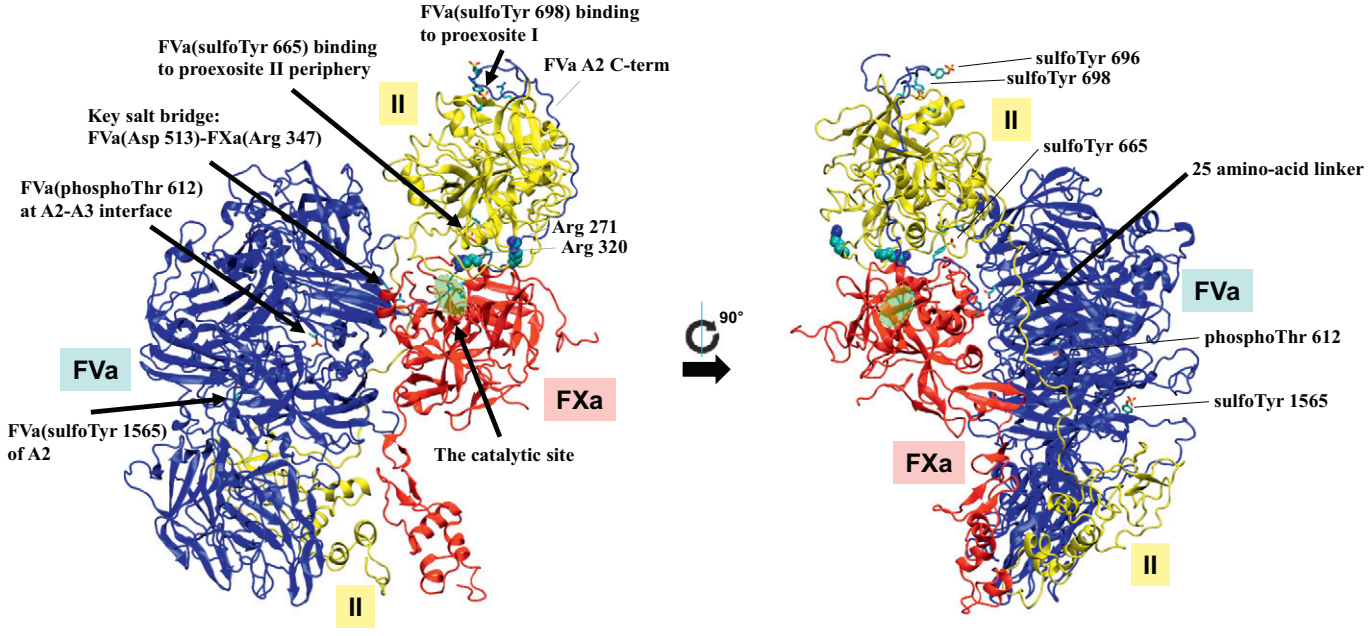

Fig. 3. Our final goal—the solvent-equilibrated structure of the ternary complex model of human FVa/FXa/II. Color coding: FVa, blue; FXa, red; and II, yellow. The catalytic site on FXa are represented by the green dotted circle.

salt bridges at the interfaces, are represented in Supplementary Figs. S3, S4 and S5, respectively.

A key interaction at the FVa–FXa interface seen in the X-ray crystal structure of *P. textilis* FVa/FXa [1] is a salt bridge formed between aspartic acid 514 of FVa and arginine 330 of FXa. The corresponding interaction (aspartic acid 513 of FVa and arginine 347 of FXa) is also conserved in our model of the human ternary model of FVa/FXa/II (Fig. 3, Supplementary Table S2 and Supplementary Figure S3). Phosphothreonine 612 at the A2–A3 interface of FVa and sulfotyrosine 1565 of the A3 domain may be important for stabilizing intramolecular interactions of FVa (Fig. 3). The sulfotyrosines at the FVa A2 C-terminal region show a tight intermolecular interaction with prothrombin. Thus, sulfotyrosine 665 interacts with positively charged arginine 443 (arginine 126) at the prothrombin proexosite II periphery, a site distinct from the heparin binding site [13], and sulfotyrosine 698 interacts with lysine 474 (149E), arginine 340 (arginine 35) and lysine 372 (lysine 60F) of prothrombin proexosite I. Our model of the FVa A2 domain extension is largely in agreement with a large body of experimental studies [14–16], which showed the important role of the FVa A2 C-terminal region in the catalytic activity of prothrombinase. In the current human ternary model of the FVa/FXa/II complex, intermolecular, charged interactions are quite extensive (Supplementary Table S2 and Supplementary Figs. 3 to 5), while intermolecular hydrophobic interactions are rather scarce (Supplementary Table S3).

Our model has the II cleavage positions of the two loops containing arginine 271 and arginine 320 in the vicinity of the active site of FXa (Figs. 2c and 3). In the final water-equilibrated model of the ternary complex of FVa/FXa/II (Fig. 3), the distances between the side chain oxygen atom of serine 379 and the backbone Cα atoms of arginine 320 and arginine 271 in the FXa catalytic site are 12.5 Å and 17.4 Å, respectively. Both cleavages are necessary to reach full activity of IIa—a central purpose of the blood cascade—but the order of cleavage depends on the details of the environment [17–19]. If arginine 271 is cleaved first, there is probably little change in the conformation of the proenzyme or the substrate; if arginine 320 is cleaved first, there is probably a local conformational change in the substrate (II) that enables the final cleavage. The model also provides a possible role for the three terminal sulfotyrosines in the FVa A2 sequence: to snare the SP domain of II from solution and to secure the SP domain cleavage positions of II near the active site of FXa. Once both cleavages have occurred, the IIa molecule is released due to the reversibility of the ionic sulfotyrosine–II interactions in ionic solution. Our model shows that the three sulfotyrosines in the FVa A2 sequence are exposed to solvent, particularly sulfotyrosines 696 and 698, suggesting that these interactions with prothrombin may not be strong. For example, it has been observed that cleavage of the FVa C-terminal region after res. 678 (the deleted fragment contains two sulfotyrosines, 696 and 698) does not have a dramatic effect on prothrombin activation [16]. The present ternary complex model of human FVa/FXa/II represents the first modelled structure in which the FVa A2 C-terminal region embraces the SP domain of II (Figs. 2c and 3), providing new insight into how FVa contributes to a 3,000-fold increase in the catalytic efficiency of FXa for prothrombin activation [20].

Although the ternary model presented is based largely on partial X-ray crystal structures, caution must be exercised, as there remain interesting experimental observations to possibly be incorporated into a fully functional model. For instance, it has been shown [17] that when the Gla domain of prothrombin has only glutamic acid residues rather than γ-carboxyglutamic acid residues, the prothrombin activity is reduced by only a factor of five (although the cleavage pathway changes from initial cleavage at arginine 320 to arginine 271). Further, it has also been shown very recently [19,21] that when twenty-two residues are deleted from the linker between the two kringle domains of prothrombin, which would presumably negate the binding of the Gla domain to a membrane surface as shown in our current (and past [2]) model(s) of the ternary complex, the activity of prothrombin in the presence of prothrombinase is reduced by only a third, although the pathway still involves initial cleavage of arginine 320. The latter work [19,21] is especially interesting in that the structure of the prothrombin deletion mutant, which was also determined, has the K2 and K1 domains interacting and parallel along the base of the SP domain and opposed to the position of arginine 320. If this arrangement is compared to our present model by overlay of the K2 and SP domains, the Gla domain would be located some distance from our model's putative membrane surface. Perhaps the role of the Gla domain of prothrombin is to increase the concentration of prothrombin (3D → 2D enhancement) near the membrane bound prothrombinase complex, but once the location of the SP domain of prothrombin has been fixed by the SP domain of FXa and the A2 domain of FVa, its importance is lessened [17,19,21]. It is likely that these issues will be resolved in the next several years!

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bpc.2015.02.003.

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