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Solving the molecular structure of hybrid human-porcine factor VIII through X-ray crystallization

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Abstract:

Factor VIII (fVIII) is a protein that is involved in the coagulation cascade, a collection of reactions that is activated by injury and leads to the formation of blood clots. Deficiencies in fVIII lead to the bleeding disorder hemophilia A, a condition that occurs in 1 in 5000 births. The current treatment for hemophilia A is inefficient and costly; however, there is potential through the use of recombinant hybrid human-porcine fVIII. Hybrid fVIII shows up to 12-fold higher coagulant activity than human fVIII, and can retain its activity even in the presence of inhibitory antibodies. The primary objective of our study is to determine the molecular structure of full-length hybrid human-porcine fVIII through X-ray crystallography. This allows us to see how the protein functions on a molecular level, along with the details of its interactions with binding partners. Thus far we have produced crystals of fVIII in complex with IgG antibodies 3E6 F_{AB} and G99 F_{AB}, along with the TIL'E' domain of binding partner von Willebrand factor. These crystals have exhibited limited diffraction, and we are working towards optimizing crystals to increase the resolution of the diffraction pattern. The structure of hybrid fVIII has not yet been studied in detail, and this information could demonstrate the its viability, bringing us one step closer to a long-term, effective, and economical treatment of hemophilia.

Background: fVIII and Hemophilia A

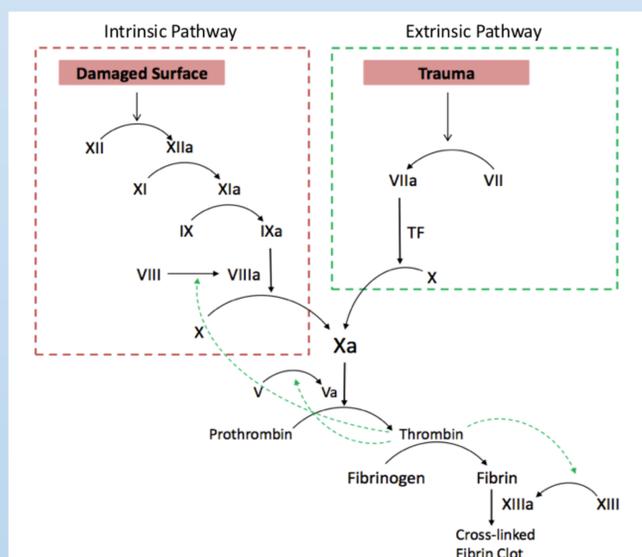


Figure 1. The Coagulation Cascade.¹ When tissue damage occurs, the intrinsic pathway of the coagulation cascade is activated. fVIII circulates in its inactive form bound to vWF. Upon activation, vWF is cleaved and fVIIIa acts as a cofactor for fIX.

- fVIII is a heterodimer made up of a heavy chain (with domains A1-A2-B) and a light chain (A3-C1-C2).²
- Individuals with hemophilia A have little or no ability to produce fVIII, and as a result cannot form clots.
- About one half of diagnosed hemophilia A patients have the severe form of the disease, with less than 1% of normal fVIII.³
- Treatment for hemophilia averages \$468,000 per year.⁴
- Recently, potential has been shown through the use of hybrid human-porcine fVIII.⁵
- Hybrid fVIII shows up to 12-fold higher coagulation activity than human fVIII, and retains activity in presence of inhibitory antibodies that affect human fVIII.⁵
- The structure of hybrid fVIII has not yet been studied in detail.

Acknowledgements:

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Methods:

Hybrid fVIII expression and preparation:

- Hybrid fVIII was prepared by Dr. Lollar's lab at Emory University
- To increase expression, the B-domain was removed and porcine sequences were added to the A1 and ap-A3 domain (Figure 3).⁶
- To decrease immune response, porcine sequences were added to the C1 domain and substitutions were made in A2 (Figure 3).⁶
- fVIII complex consists of hybrid fVIII bound to combinations of antibodies (3E6 F_{AB} and/or G99 F_{AB}) along with the TIL'E' domain of the D'D3 subunit of vWF (Figure 2).

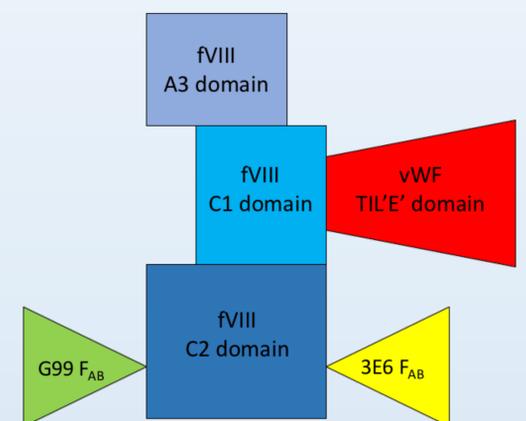


Figure 2. Schematic of the light-chain subunit of fVIII with TIL'E' and IgG antibodies G99 F_{AB} and 3E6 F_{AB} bound.

X-ray Crystallography:

- Crystals are produced through hanging drop vapor diffusion (Figure 4).
- Successful crystallization involves extensive modulation of buffers, ionic strengths, and precipitants.

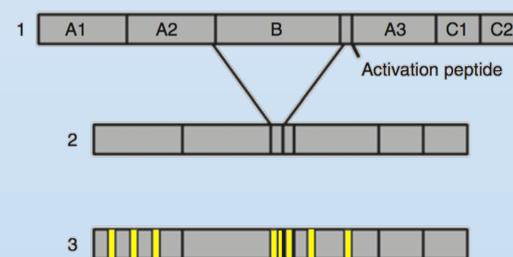


Figure 3. Hybrid fVIII gene construct.⁶ The B-domain of human fVIII was removed, and porcine sequences were inserted (shown in yellow).

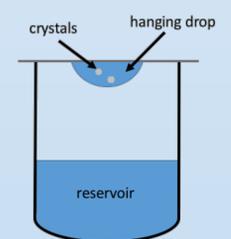


Figure 4. The hanging-drop vapor diffusion crystallization method.

Results and Future Work:

- Hybrid fVIII + G99 F_{AB} crystals have diffracted to a resolution of 4-8 Å (Figure 4a,b).
- Crystals of fVIII complex with various binding partners have been produced in several conditions.
- Current goals include modulation of conditions to produce crystals of fVIII+TIL'E'+3E6 F_{AB} and fVIII+TIL'E'+G99 F_{AB}.
- The ultimate goal is to obtain diffraction results with a resolution of 1-3 Å.

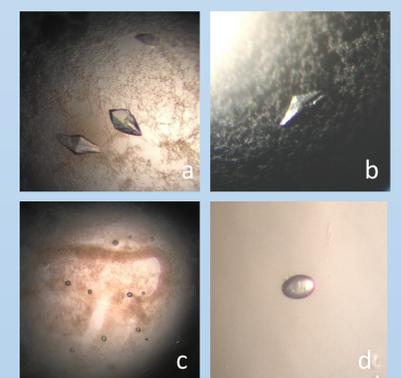


Figure 4. Hybrid fVIII complex crystallization results. a) fVIII+G99 F_{AB} in 0.050 M DL-Malic acid pH 7.0, 12% PEG 3350. b) fVIII+G99 F_{AB} in 0.050 M DL-Malic acid pH 6.8, 10% PEG 3350 c) fVIII+TIL'E'+3E6 F_{AB} in 0.1 M BIS-TRIS propane pH 8.0, 32% PEG 6000. d) fVIII+TIL'E'+G99 F_{AB} in 0.1 M MES monohydrate pH 6.0, 22% PEG 400

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