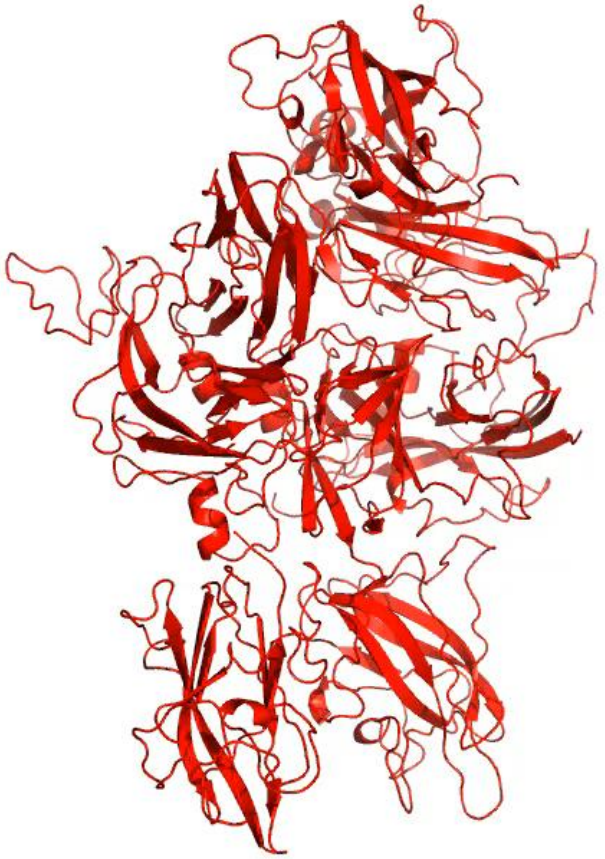
Structure Determination of a Bioengineered Human/Porcine Factor VIII for Hemophilia A Treatment,

and

Improvements to the Human Factor VIII Model



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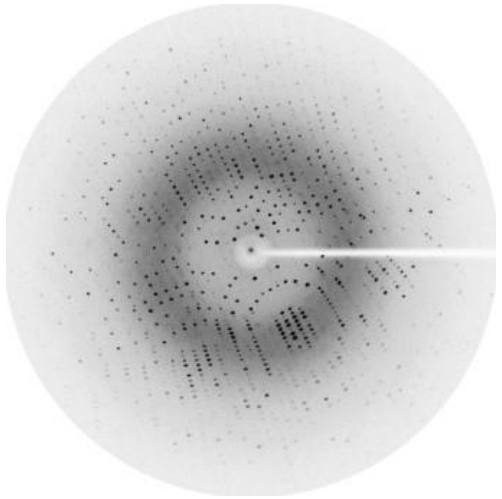
SPIEGEL LAB

2018 GRADUATE STUDENT SYMPOSIUM

WESTERN
WASHINGTON UNIVERSITY

This talk will:

- Introduce:
  - The bleeding disorder Hemophilia A
  - The Blood Coagulation Cascade
  - Blood Coagulation Factor VIII (FVIII) protein



- 3-D molecular structure of novel human/porcine chimeric FVIII for Hemophilia A therapy

- Implications for future research

Central Dogma of Biology

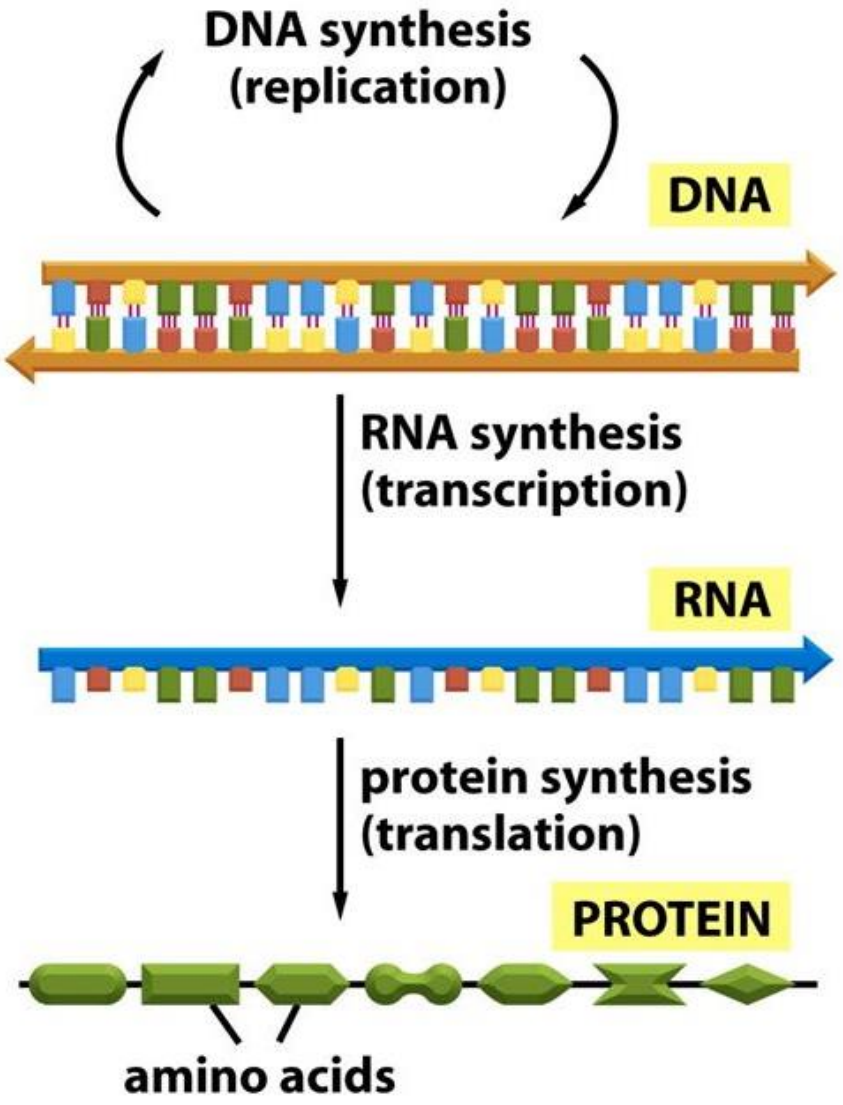
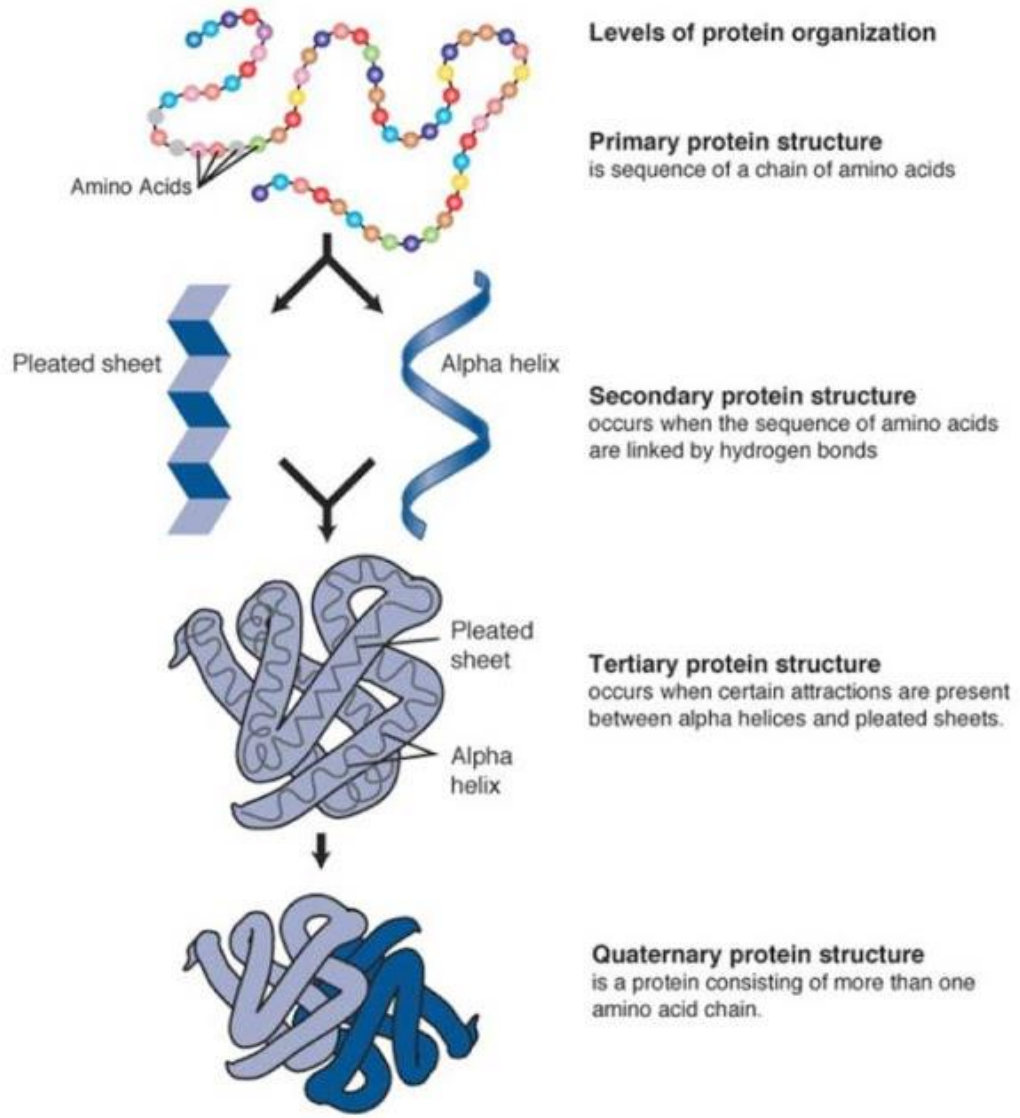
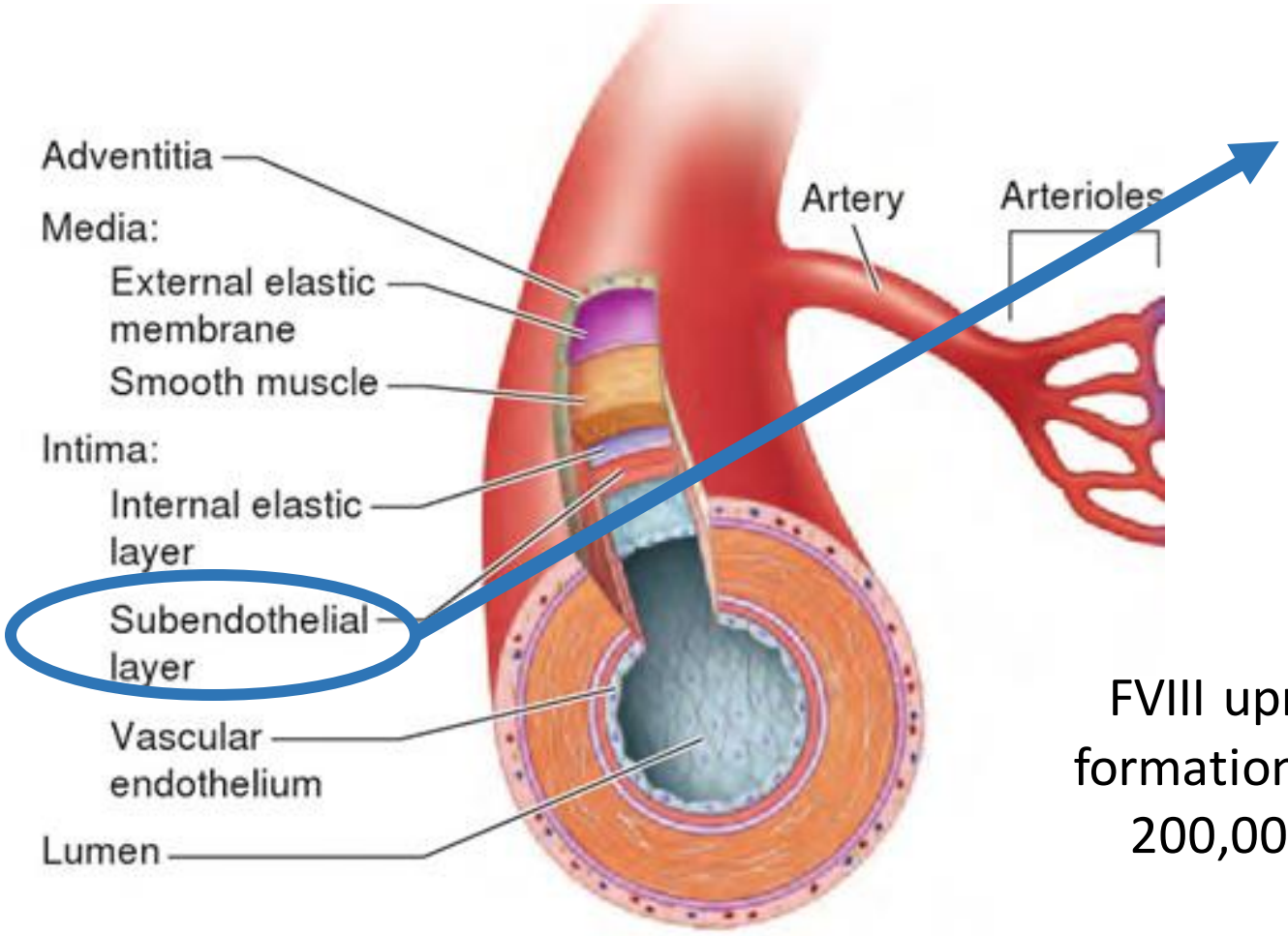


Figure 1-4 *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)



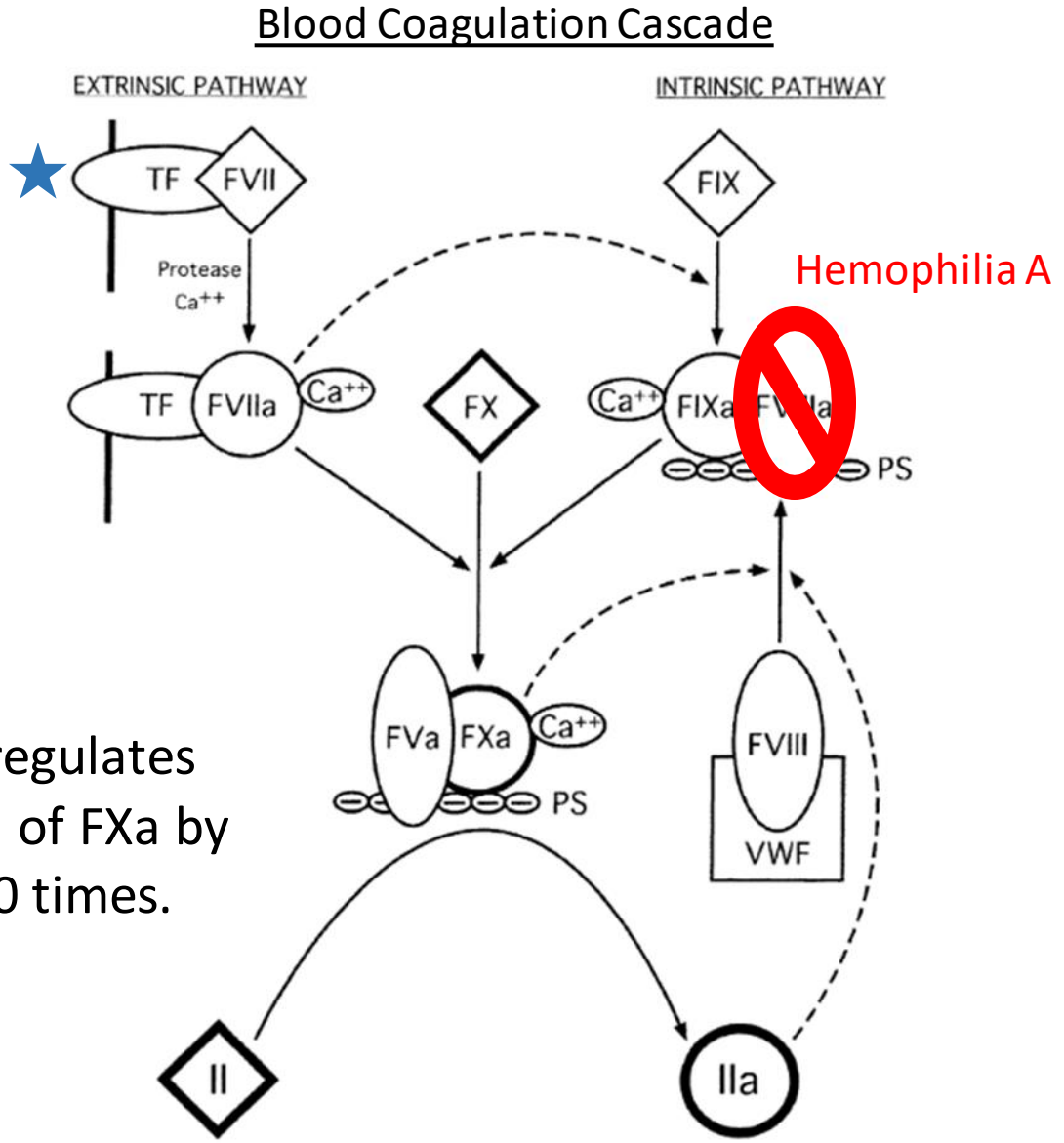
National Human Genome Research Institute (NHGRI)

Hemostasis



Moore, Keith L., et al. *Clinically Oriented Anatomy*. Lippincott Williams & Wilkins, 2014.

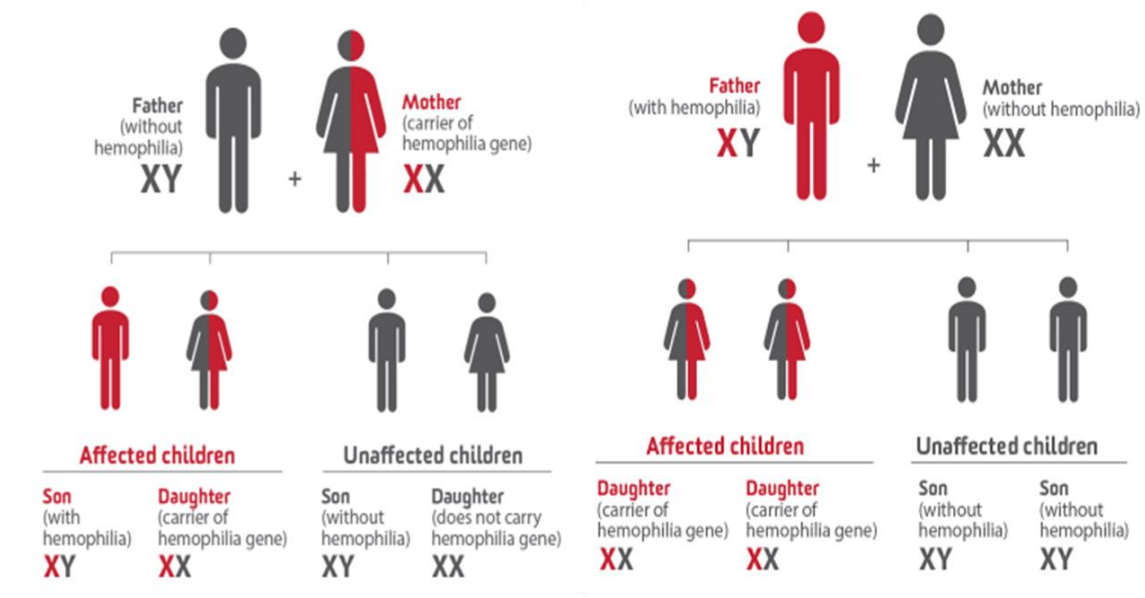
FVIII upregulates formation of FXa by 200,000 times.



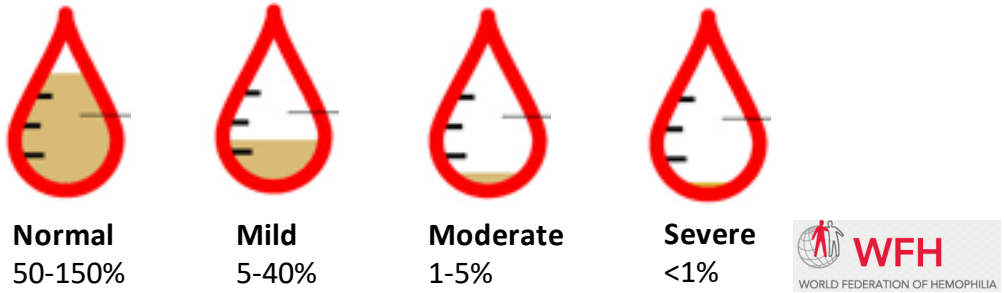
Spiegel et. al, British Journal of Haematology, 2002, 119, 310–322

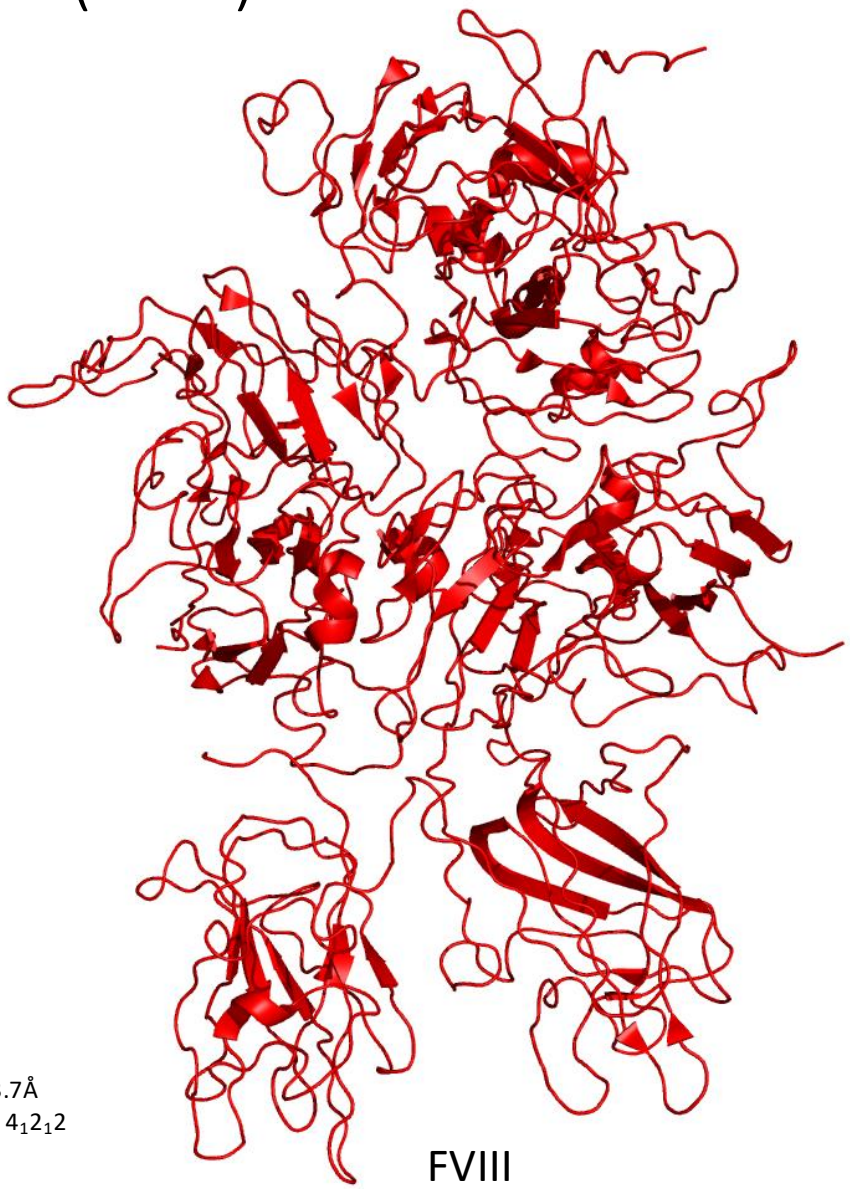
Hemophilia A and Factor VIII (FVIII)

- X-linked disease that affects 1 in 5000 males worldwide



Hemophilia Degrees of Severity (% FVIII or FIX)





Resolution: 3.7Å
SpaceGroup: 4_12_12

FVIII

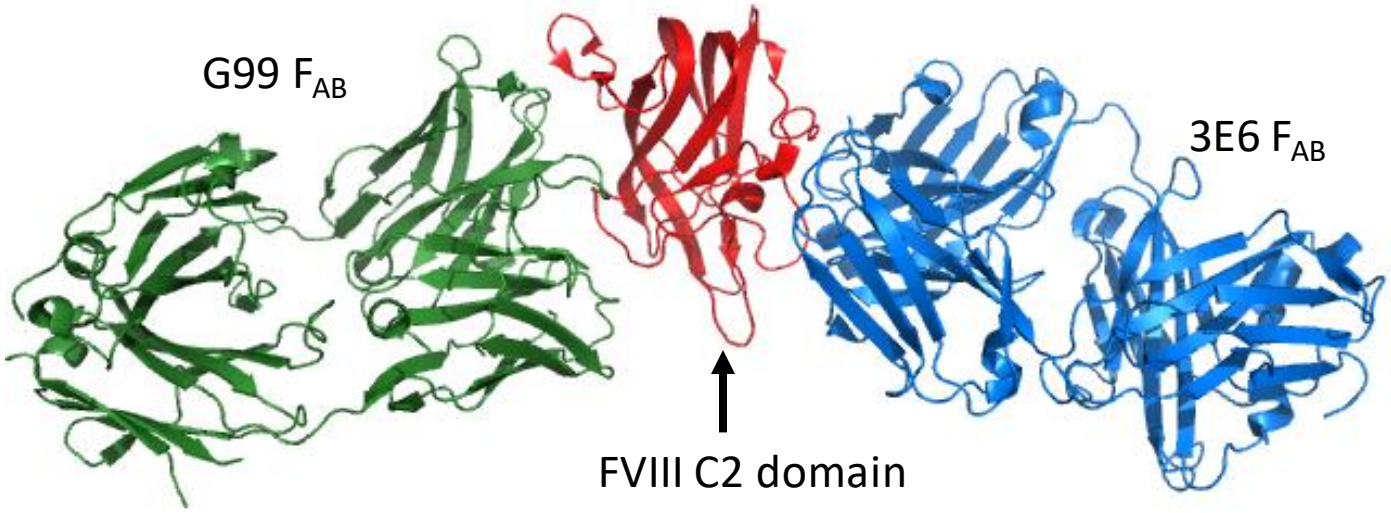
- Hemophilia A replacement therapy with recombinant FVIII

Shen et. al, *Blood*, 2008, 111, 1240-1257

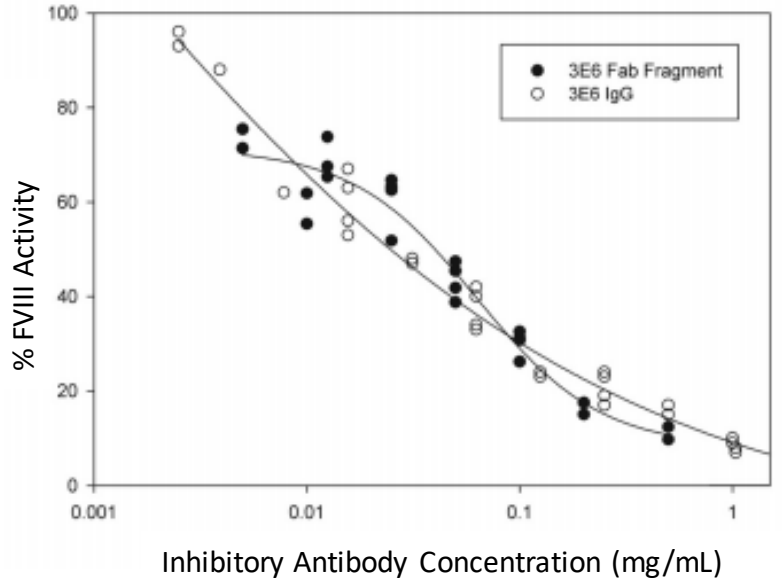
Treatment Complication

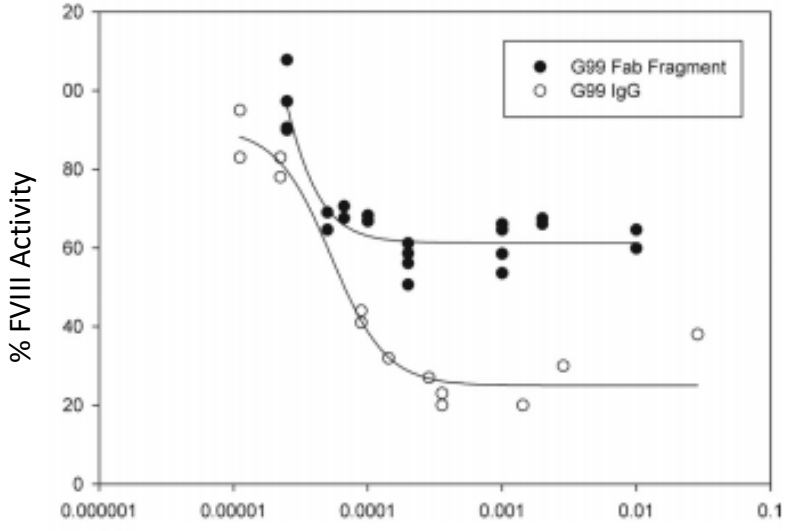
- ~30% patients receiving FVIII concentrate replacement therapy acquire an inhibitory alloantibody.

Bray et al, *Blood,* 1994, 83 (9), 2428-2435



Walter et al. *Blood*, 2013, 122 (26), 4270-4278





Walter et al., *J. Biol. Chem*, 2013, 228, 9905-9914

Immune Tolerance Induction (ITI)

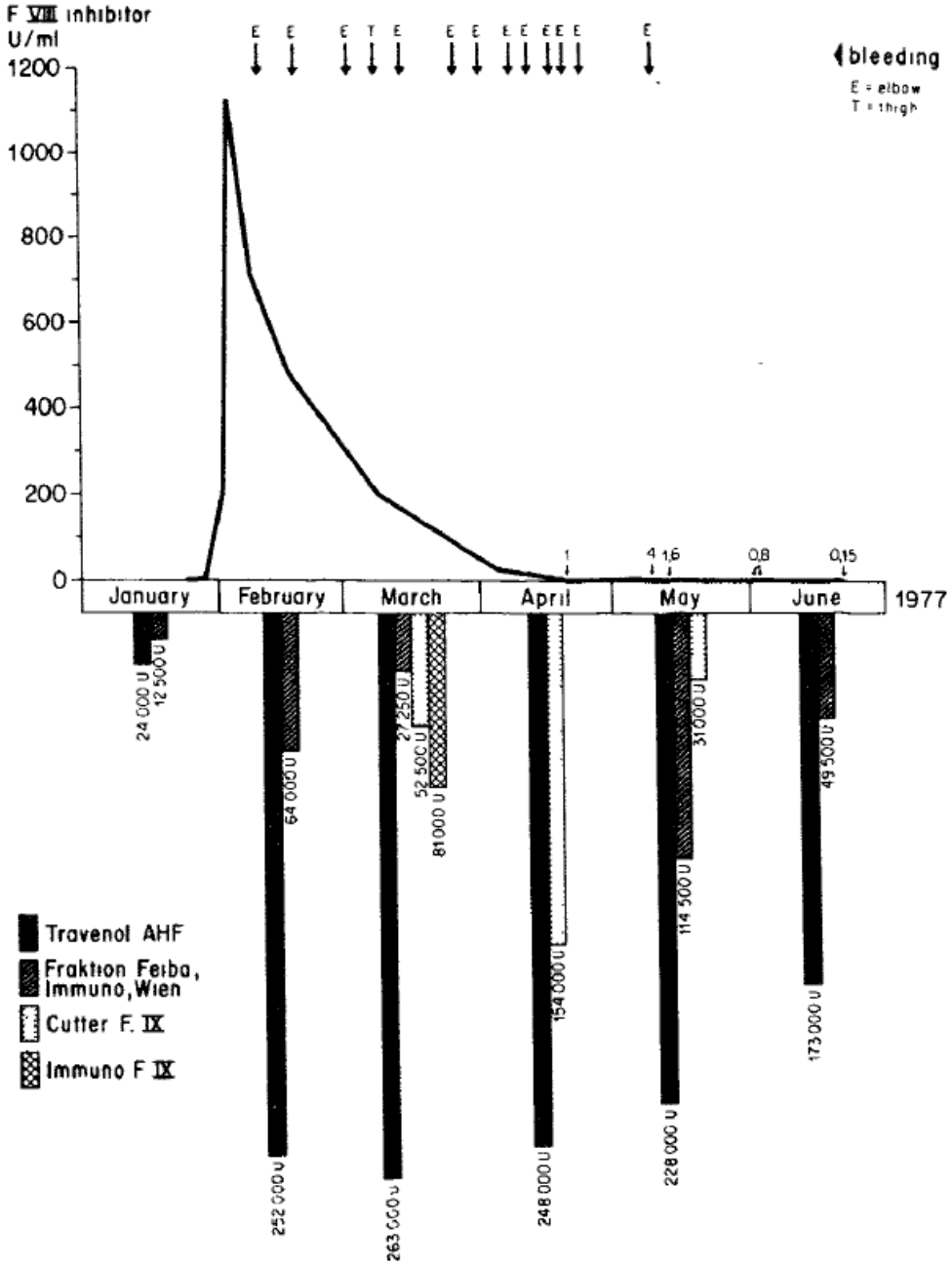


Fig.—Factor VIII inhibitor during treatment.

Brackmann, H.h., and J. Gormsen. *The Lancet*, 1977, 310, p.933.

Table 1
Predictors of success following immune tolerance induction (ITI): data from the International Immune Tolerance Registry (IITR) and North American Immune Tolerance Registry (NAITR) [5,11].

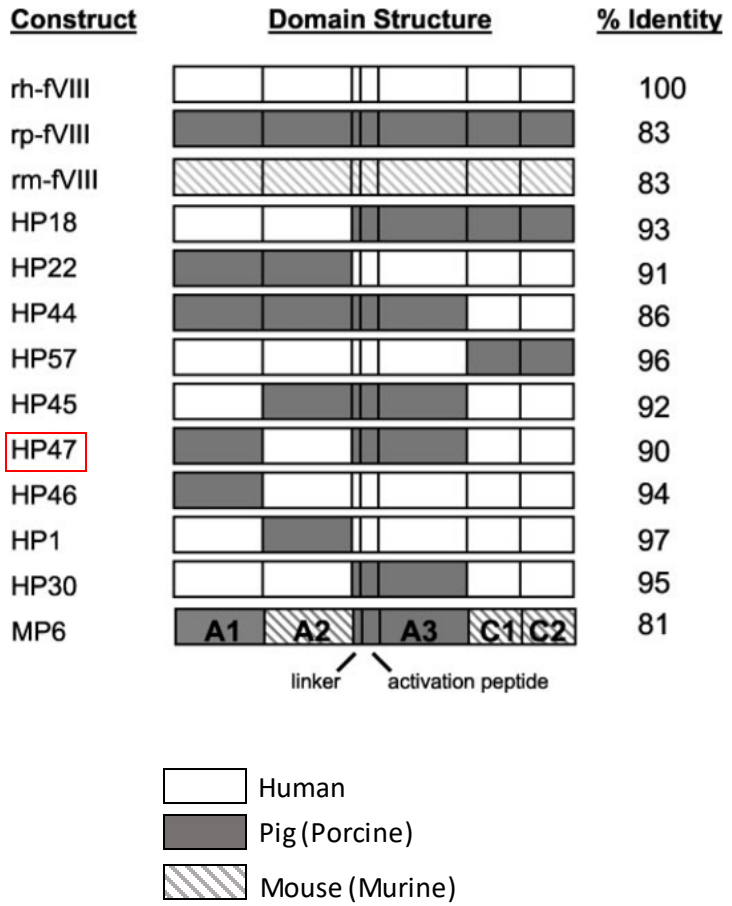
Variable	Success rate (%)		Cutoff for 'good risk'
	IITR	NAITR	
Age (years)			
< 20	70–78		< 20 years
> 20	40		
Peak titer (BU/mL)			
< 20	85–89		< 20 BU/mL
> 20	44–58		
Pre-ITI titer (BU/mL)			
< 10	78–79	83	< 10 BU/mL
> 10	41–65	40	
Dose (IU/kg/day)			
< 200	48–66	72–83	> 200 IU/kg/day
> 200	86	41	

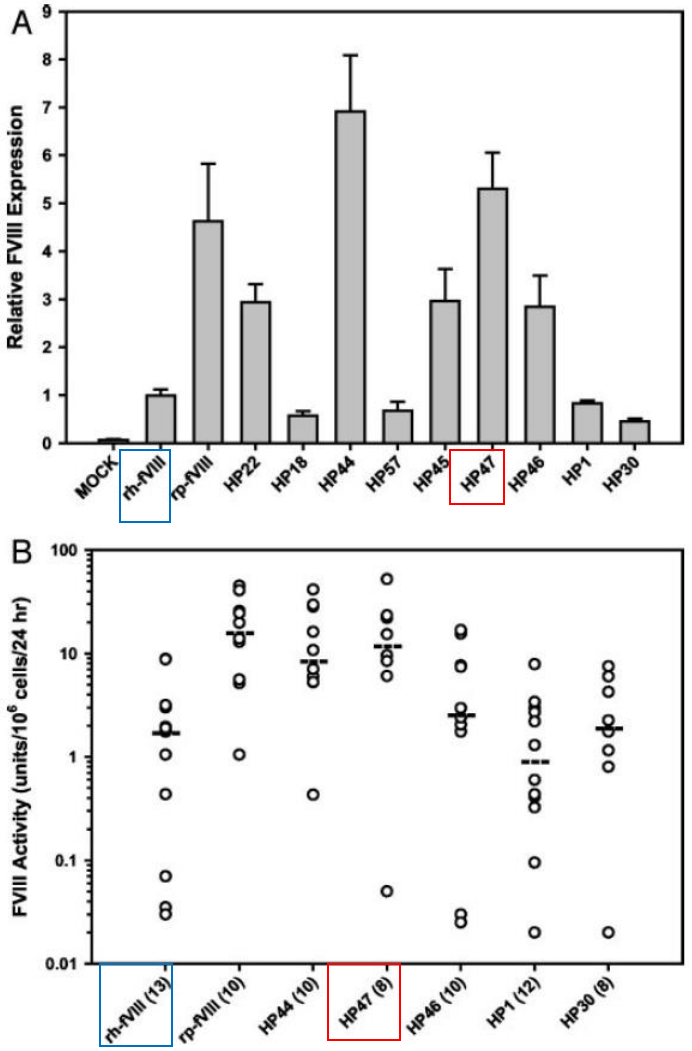
BU, Bethesda unit.

Astermark, *Thrombosis Research*, 2011, 127, S6-S9

Human / Porcine Chimeric FVIII (HP47)

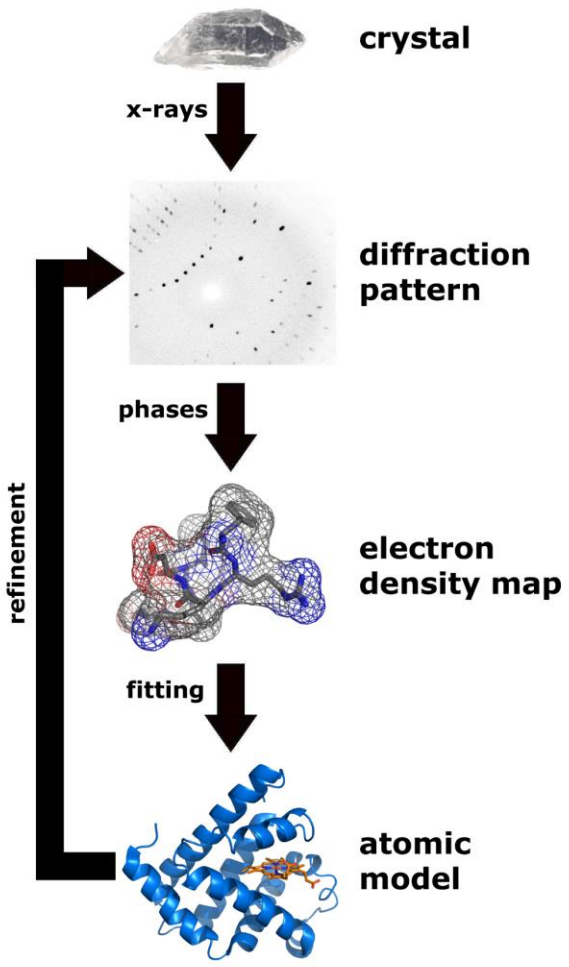
- hFVIII expresses poorly. [Serum] = ~0.5nM
- Recombinant porcine FVIII displays increased cellular secretion
- Human/Porcine Chimera as high output protein therapeutic
- HP47 demonstrates higher expression and comparable activity to hFVIII





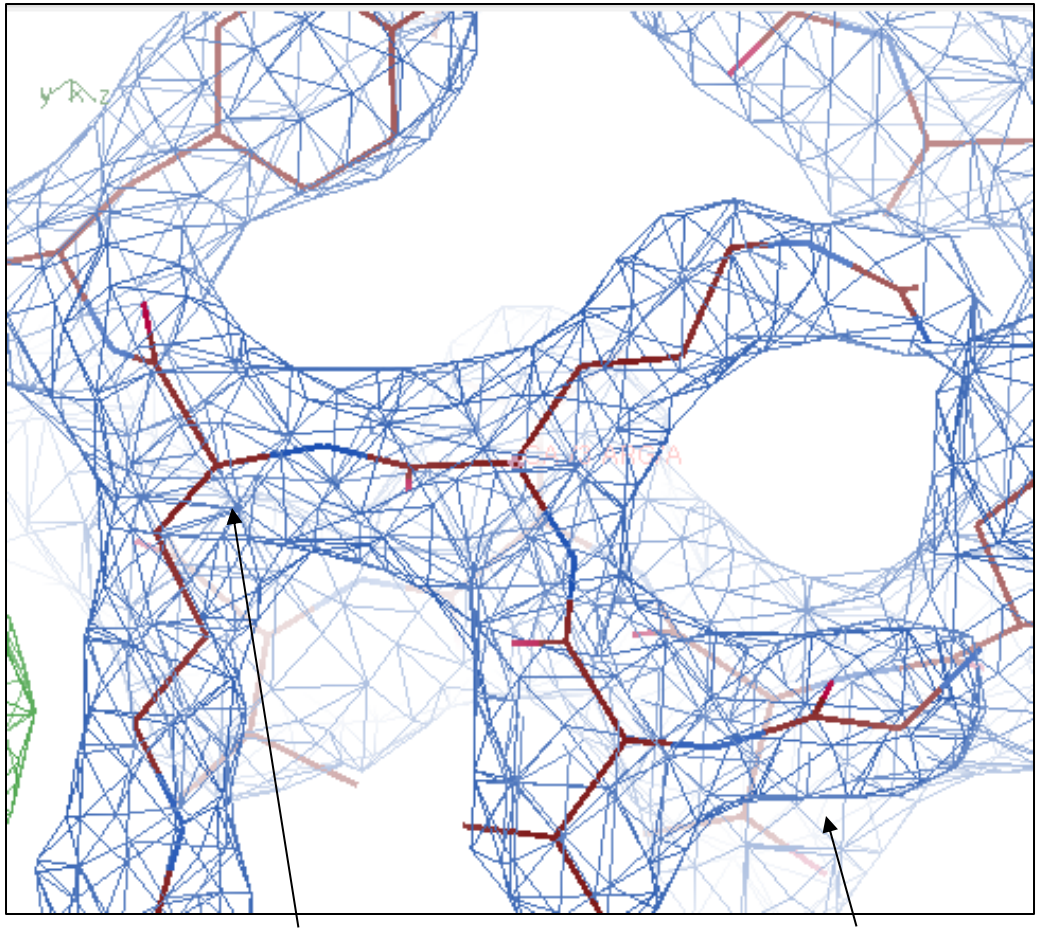
Doering et al., *J. Biol. Chem.*, 279 (8), 2003, 6546–6552.

Protein Crystallography



T. Splettstoesser

Building the FVIII molecular model



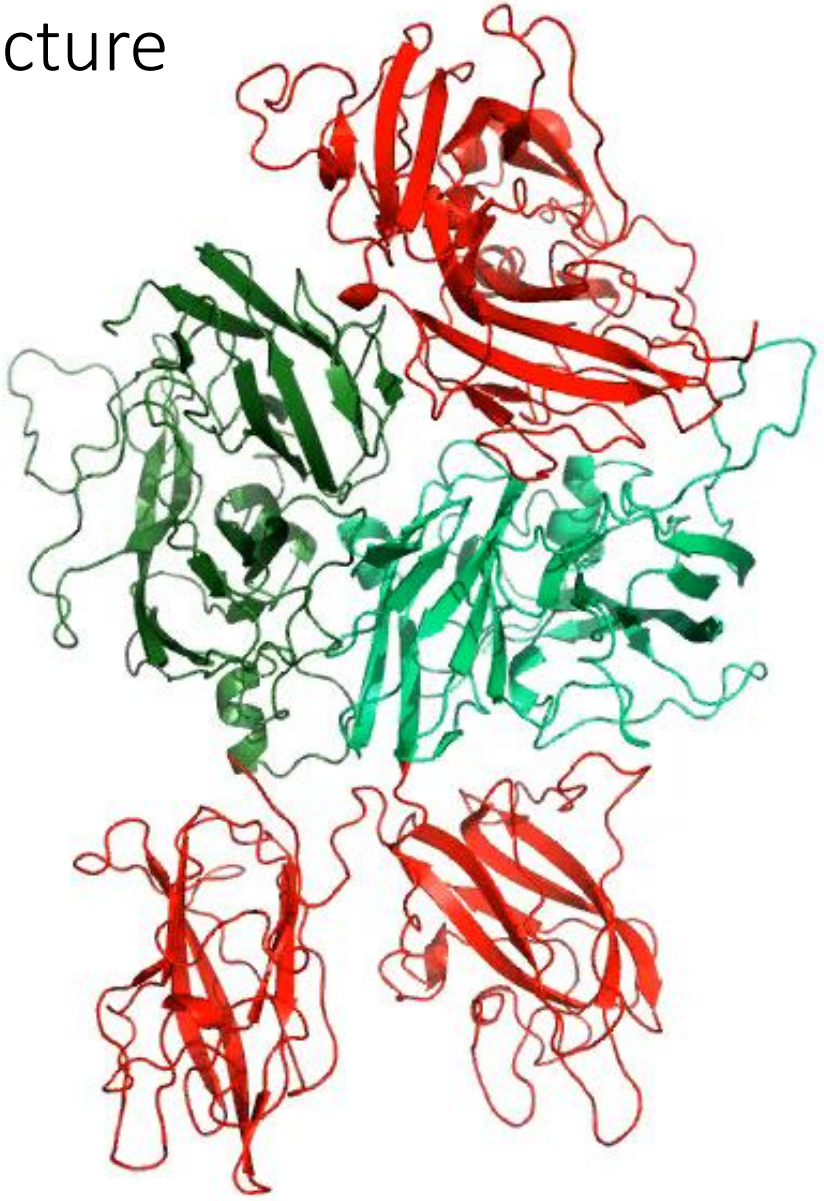
FVIII amino acids (red)

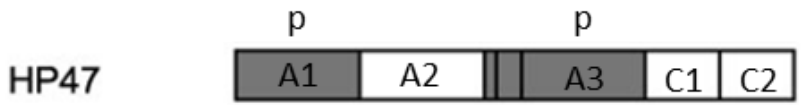
Electron density (blue wire mesh)

- Assess model agreement to electron density with R factors
  - R_{work} and R_{free}
- Scale of 0.0 (perfect fit) - 0.6 (random/poor fit)

HP47 Crystal Structure

- At 3.2Å, highest resolution FVIII model to date
  - More confidently rebuild sections





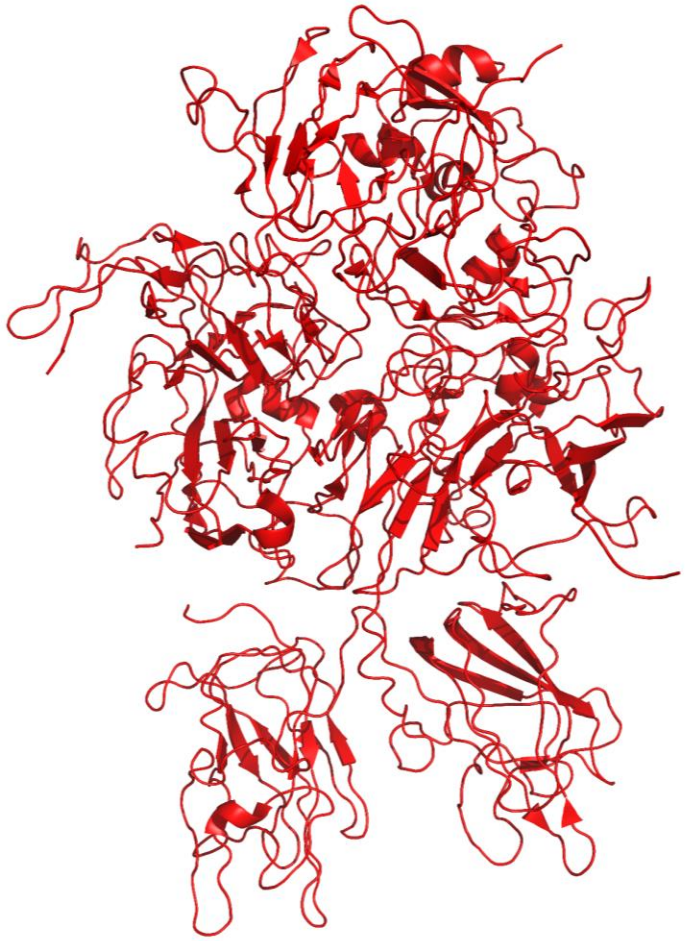
HP47 Model Overview

- ~85% of the 1467aa HP47 sequence built into model

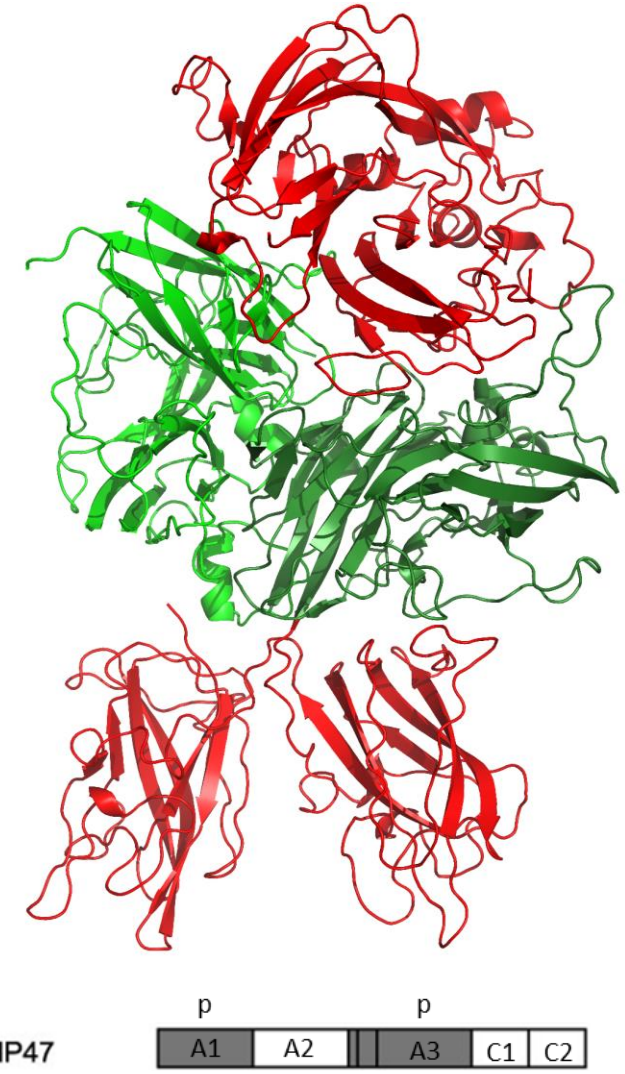
Quality of the current model

- R_{work}: 0.1972
- R_{free}: 0.2863
- Ramachandran outliers: 10.40%
- Space Group: $P2_1$

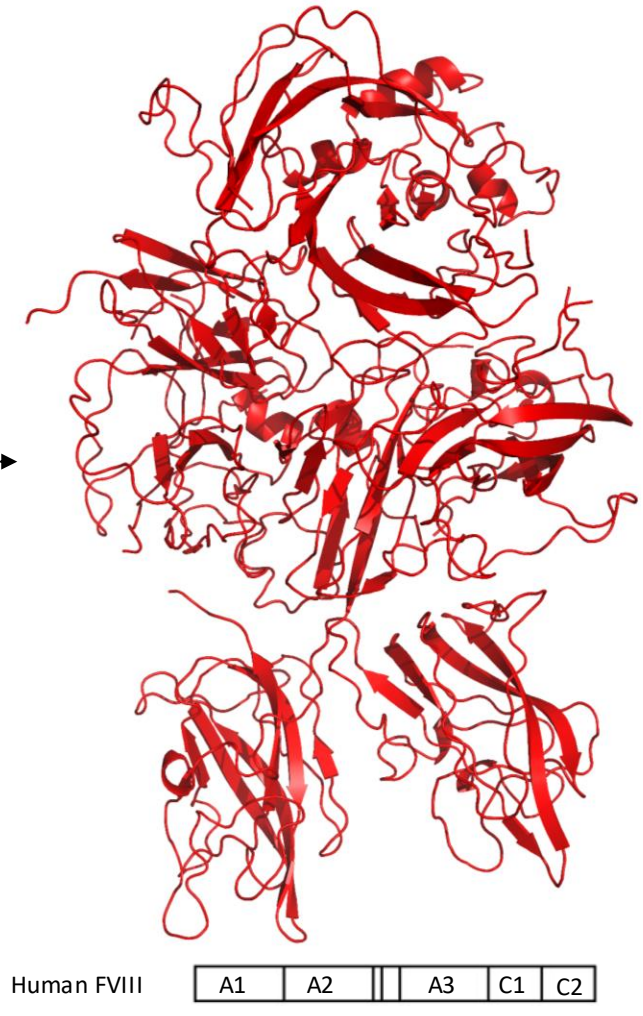
Improvements to the Human Model FVIII



2008 FVIII Structure (PDB: 2R7E)

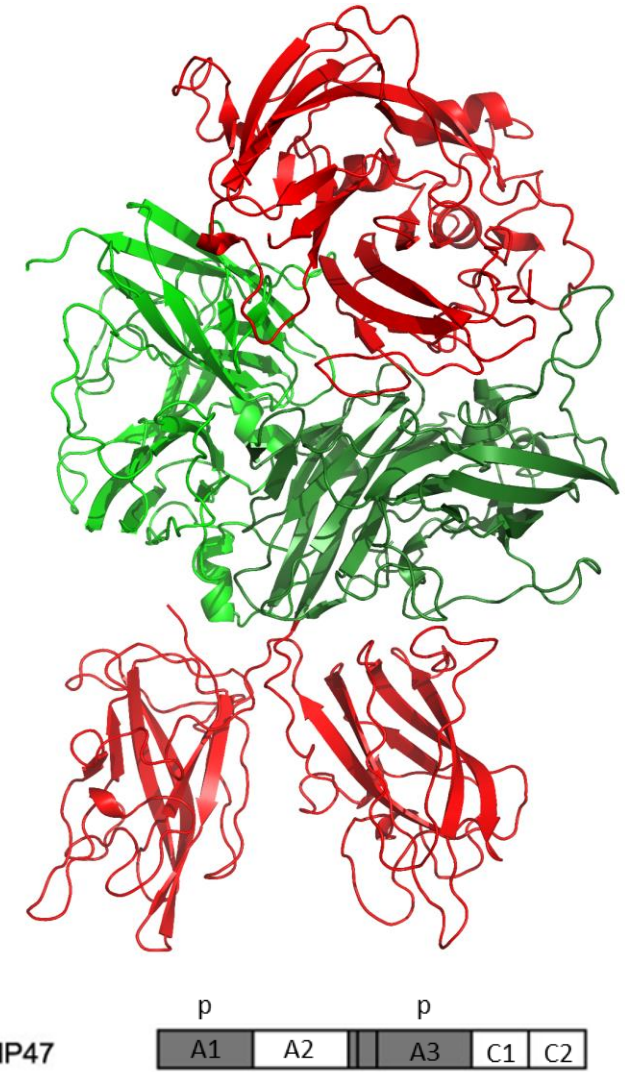


New human/porcine FVIII structure

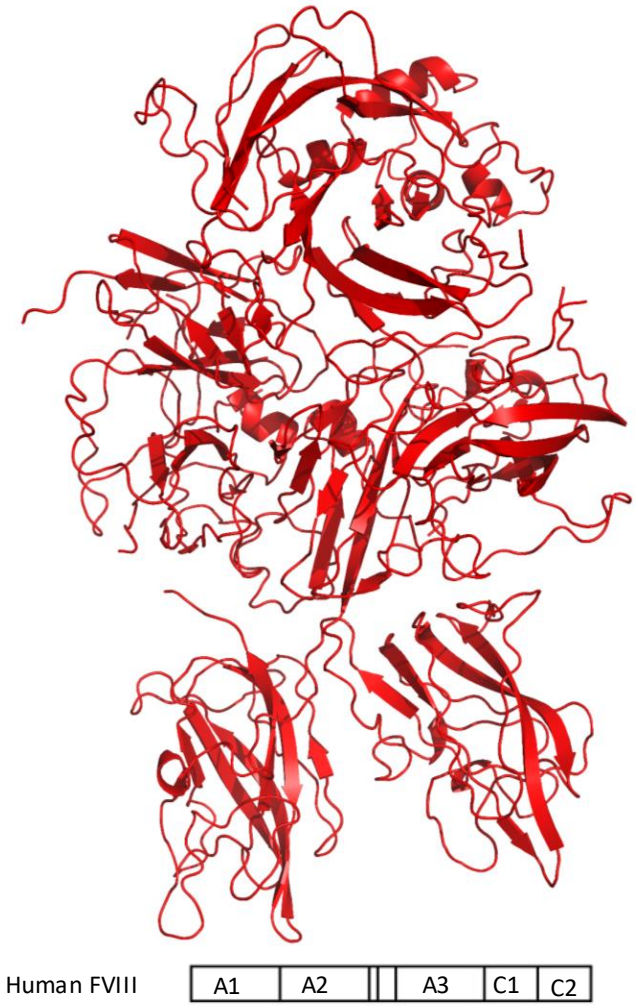


Newly refined FVIII Structure

Improvements to the Human Model FVIII

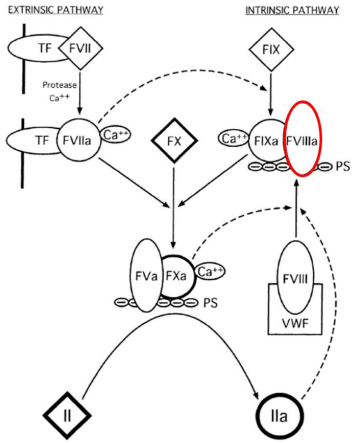


New human/porcine FVIII structure



Newly refined FVIII Structure

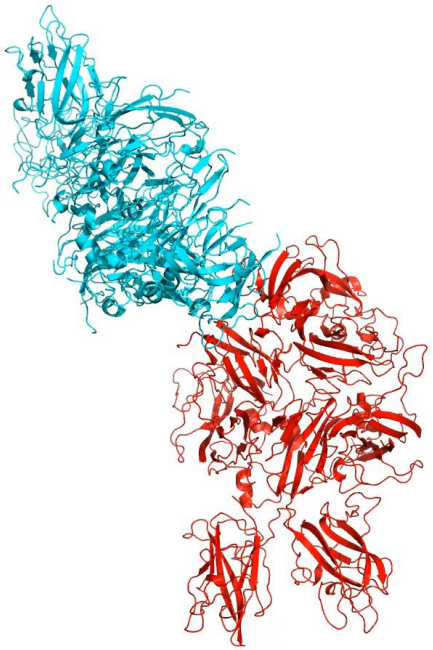
- Enhance basic understanding of FVIII's function in coagulation
- Develop more effective Hemophilia A therapeutics



Summary

- Hemophilia A is a X-linked disease caused by a lack of blood coagulation factor VIII (FVIII) protein.

- Patients who develop an immune response to FVIII rely on large quantities of improved therapeutics.



- Determined crystal structure of novel Human/Porcine Chimeric therapeutic. Improved human FVIII protein model.

Questions?

National Institutes of Health

NIH National Heart, Lung, and Blood Institute

Western Washington University