# Protein engineering and its therapeutic applications

Strategies to improve biotherapeutics

Giulia Pavani - 3 Maggio 2018

# Protein Engineering

Protein engineering can be defined as the modification of protein structure with recombinant DNA technology or chemical treatment to get a desirable function for better use in medicine, industry and agriculture.

*Can we improve nature and evolution?*

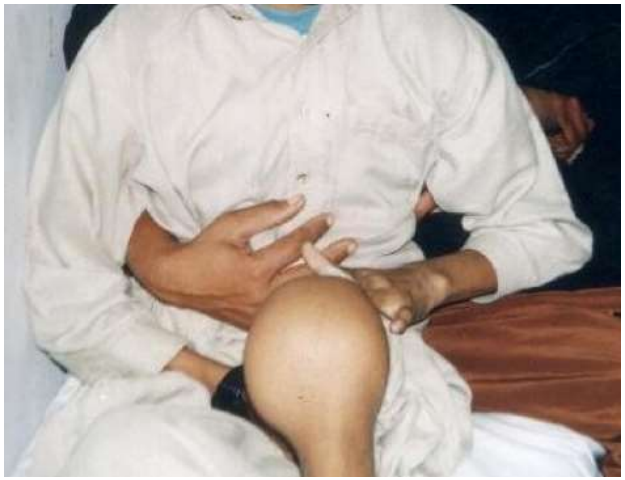
# Why should we do it?

- Improve recombinant production of protein-based drugs (monoclonal antibodies, enzymes, hormones…)
- Lowering therapeutic dose
- Increasing efficacy
- Reach different cell types (uptake)
- Extend therapeutic effect
- In gene therapy settings, compensate for delivery inefficiency

# Hemophilia A

- X-linked bleeding disorder caused by the absence of functional Factor VIII (1:5000 male birth)
- Patients experience spontaneous bleeding resulting in permanent joints damage and disabilities.

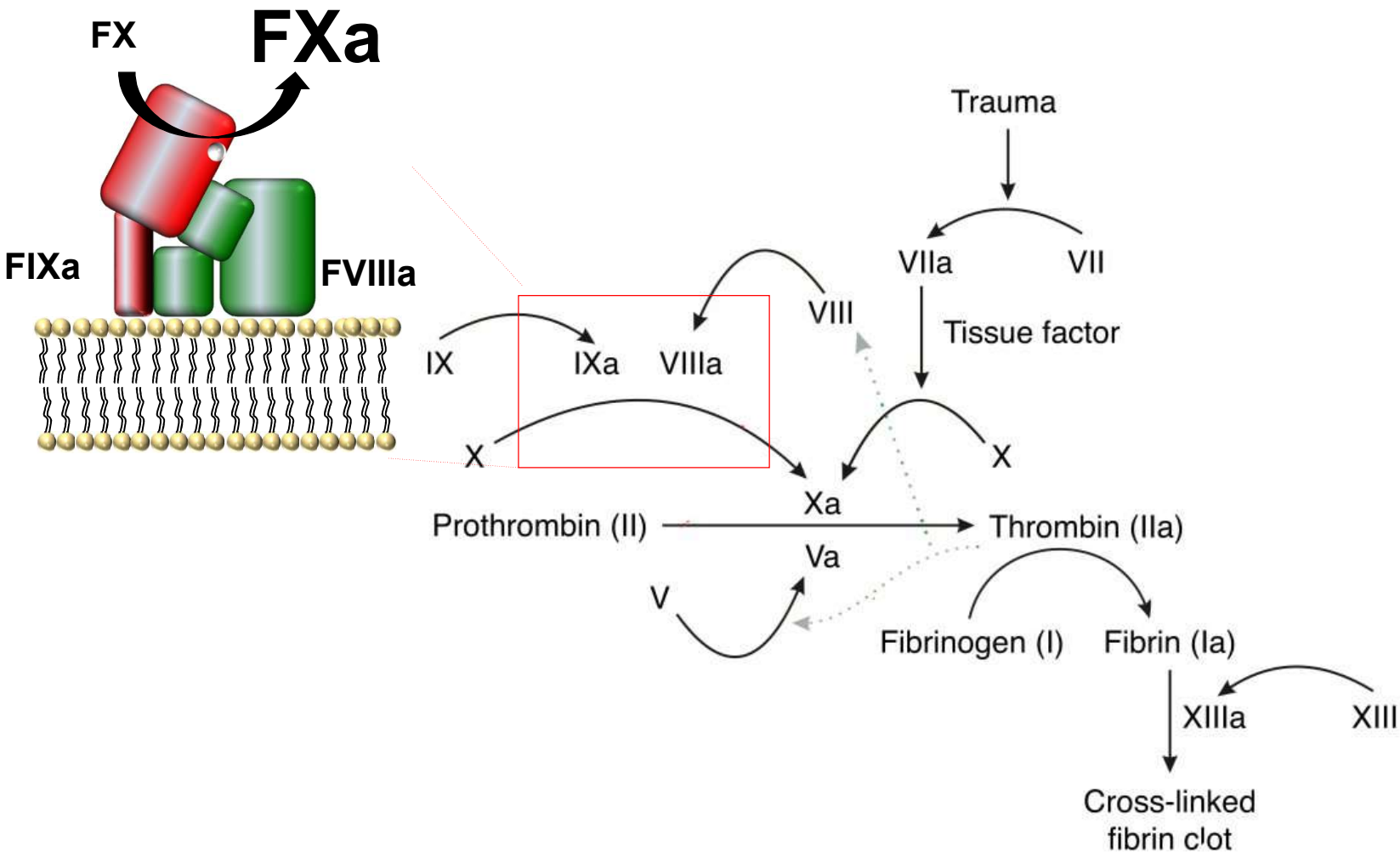
- Treatment by recombinant or plasma derived FVIII:
  - 3x week, ~468,000$/year
  - 30% of patient develops neutralizing antibodies



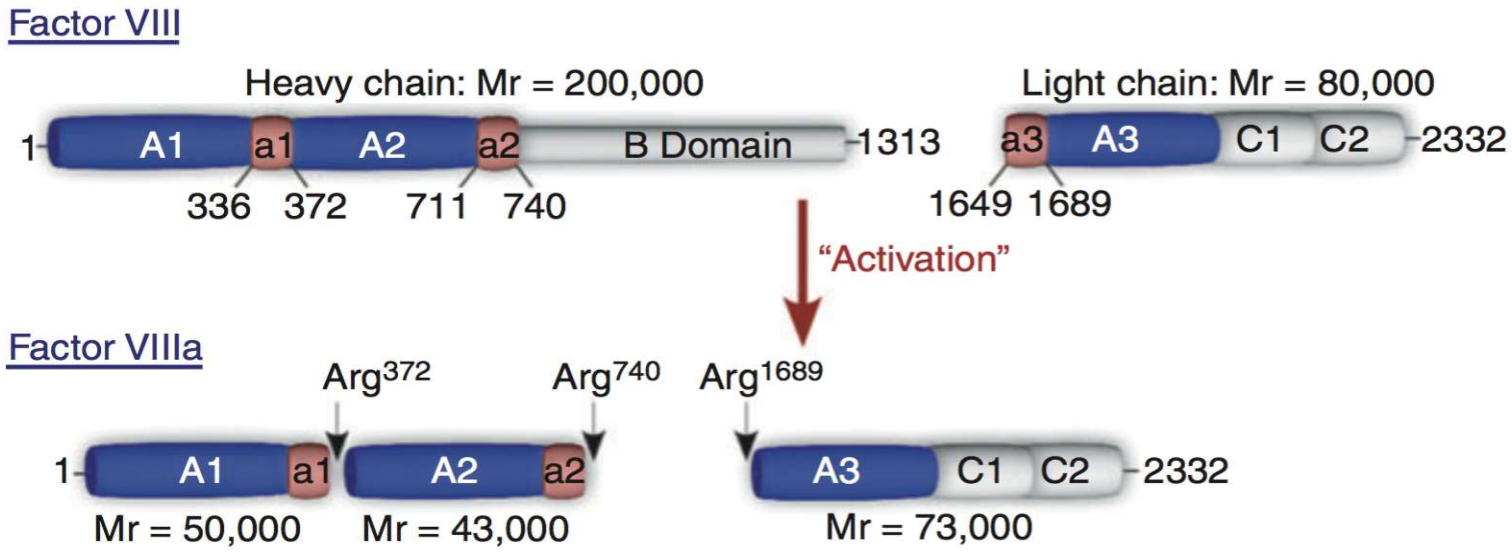
Severe knee bleed

- *Low increases (>1%) can ameliorate disease phenotype*

# FVIII mechanism of action



# Factor VIII

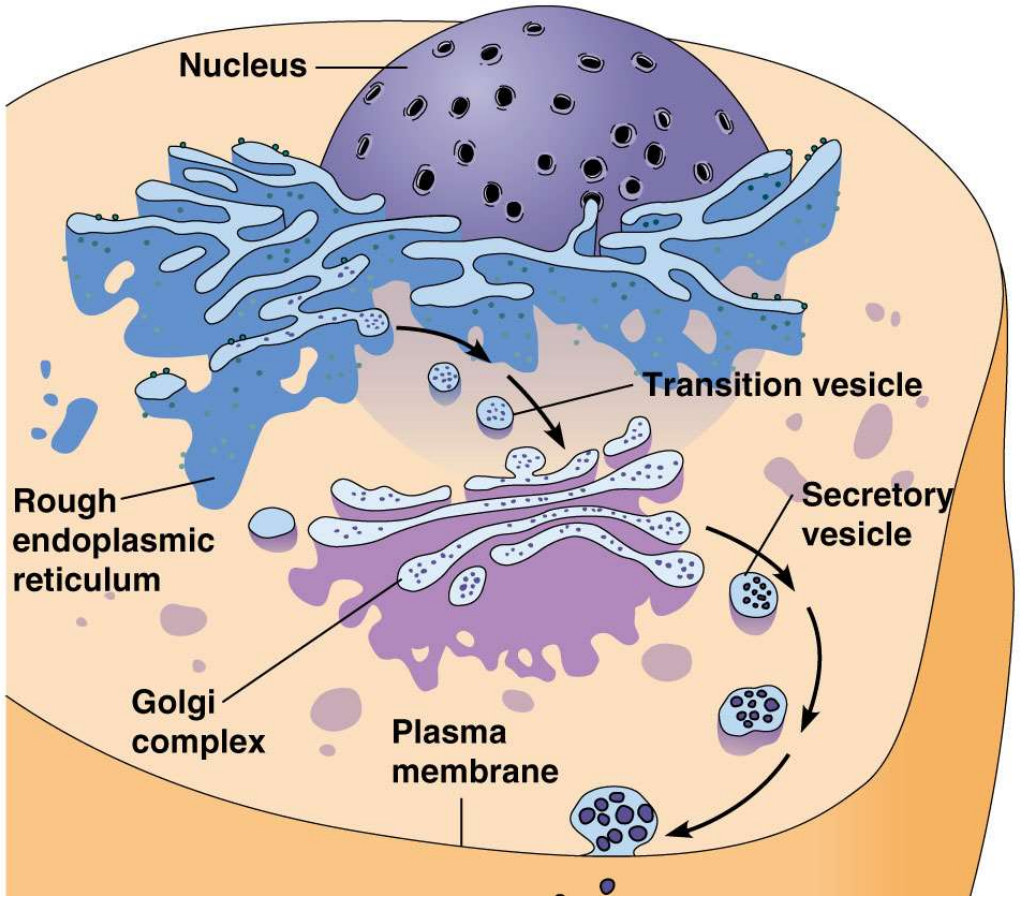

Factor VIII
Heavy chain: Mr = 200,000
Light chain: Mr = 80,000
1
A1
a1
A2
a2
B Domain
1313
a3
A3
C1
C2
2332
336
372
711
740
1649
1689
"Activation"
Factor VIIIa
Arg372
Arg740
Arg1689
1
A1
a1
A2
a2
A3
C1
C2
2332
Mr = 50,000
Mr = 43,000
Mr = 73,000

# Factor VIII

- The coding region of full length FVIII is approximately 7.0 kb. FVIII protein is a 2,351 aa single-chain glycoprotein of 280 kDa
- Predominately synthesized and secreted by sinusoidal endothelial cells in the liver
- mRNA is very unstable compared to similar sized proteins
- Heavily glycosylated and processed
- Short half-life

# Optimizing cellular processes

- We can improve every step of protein production



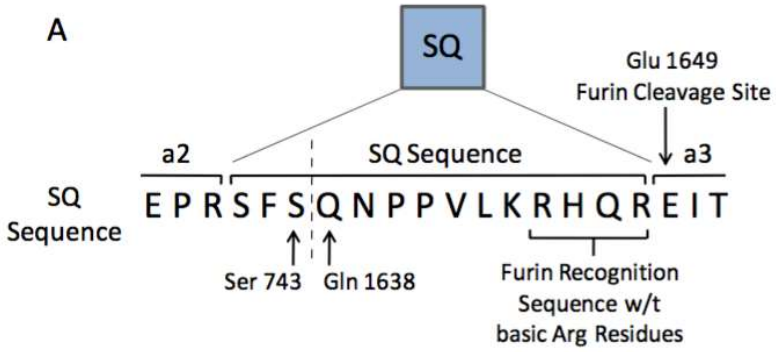
# Improve transcription/translation

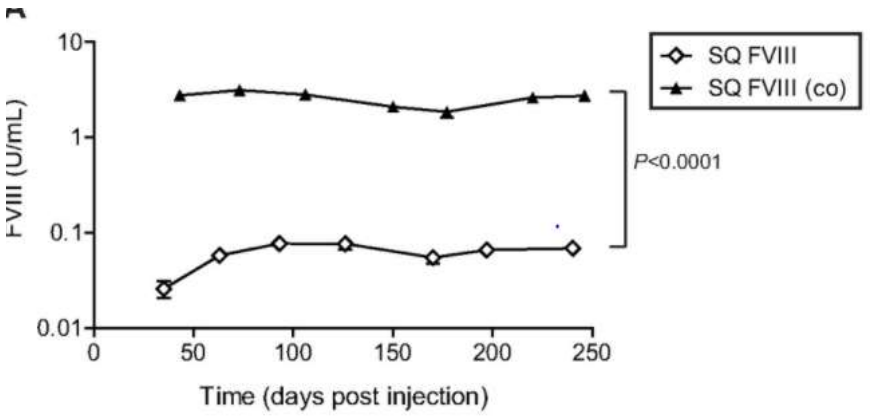
- FVIII mRNA is unstable
- Codon optimization can improve protein synthesis by:
  - Increasing transcriptional efficacy: GC content, CpG dinucleotides content, cryptic splicing sites, negative CpG islands, Shine-Dalgarno sequence codon-context, TATA boxes and terminal signals
  - Enhancing the translation efficiency: codon usage bias

# Improve transcription/translation

- Deletion of the entire B domain leads to a 17-fold increase in mRNA and primary translation product

- Codon optimization of FVIII led to a 29-to 44-fold increase in expression

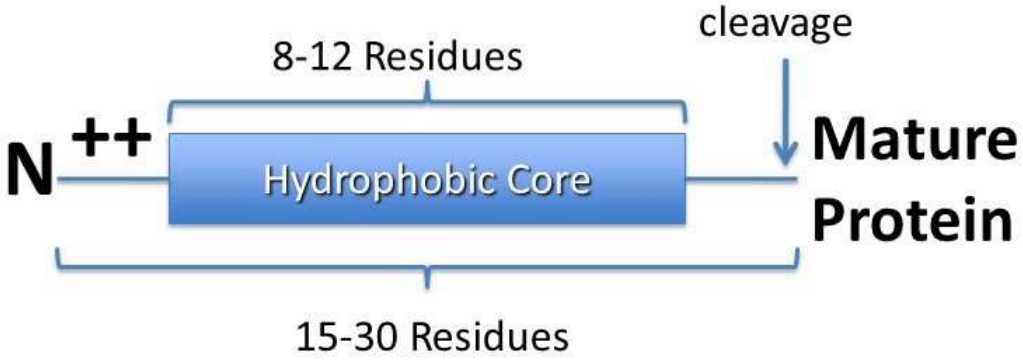


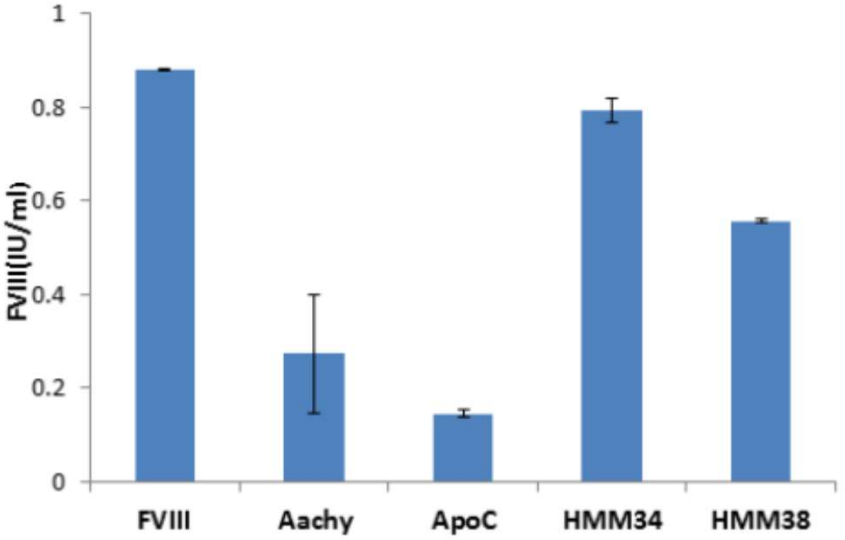

Ward et al, Blood 2010

# Enhance secretion

- Secretory signal peptides play a critical role in mediating eukaryotic protein secretion.
- The hidden Markov model (HMM) provides a protocol to describe and predict relative strengths of secretory signals.
- HMM score for FVIII is low compared to other proteins



# Enhance secretion

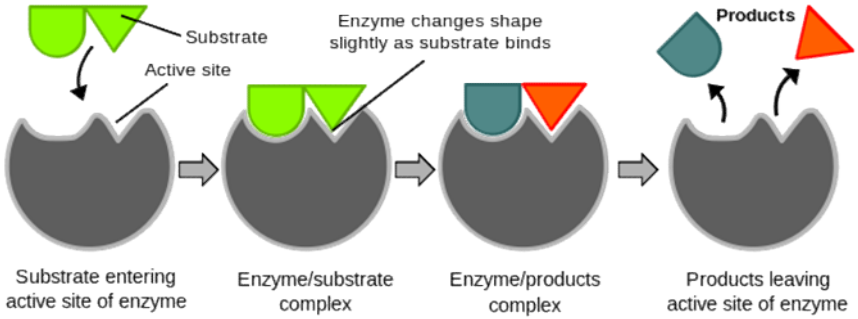


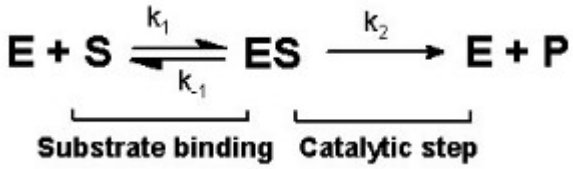

| Signal Peptide Name | Amino Acid Sequence | SP HMM bit Scores |
|---|---|---|
| Factor VIII | MQIELSTCFFLCLLRFCFS | 12.5 |
| α-1-antichymotrypsin (Aachy) | MDKLLLLVTLVCGTQA | 19.1 |
| Apolipoprotein C-III (ApoC) | MQPRVLLVVALLALLASRASEA | 20.9 |
| HMM34 | MRPTWAWWLFLVLLLALWAPARG | 34 |
| HMM38 | MWWRLWWLLLLLLLLWPMVWA | 38 |

Roberts SA, J Genet Syndr Gene Ther 2011

# Enhance specific activity

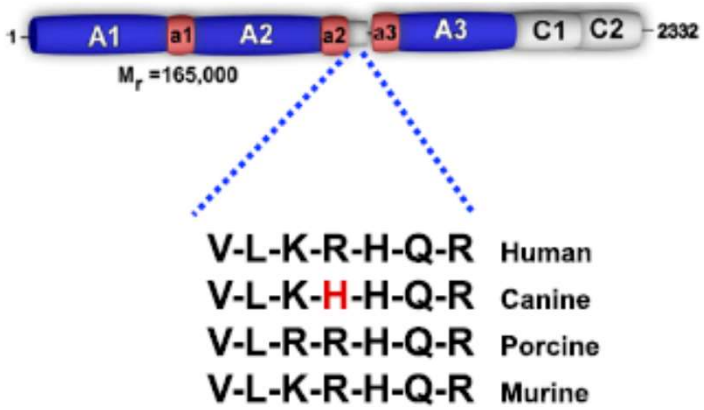
- Enzymes are molecules that catalyse chemical reactions
- Their catalytic activity is linked to their aminoacid sequence (structure)
- We can intervene at every step
  - Substrate recognition
  - Catalytic reaction
  - Product release
  - Exosites
  - Inhibitor binding
- FVIII is a inactive cofactor (not an enzyme)

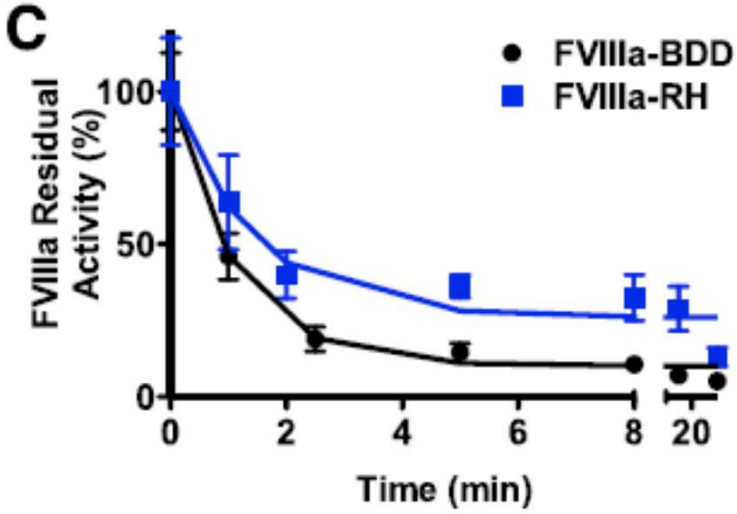


# Improve Stability

- Canine FVIII has longer half life compared to human
- Disruption of a (PACE)/furin cleavage sequence arginine-x-x-arginine (RxxR)
- FVIII secreted a single chain

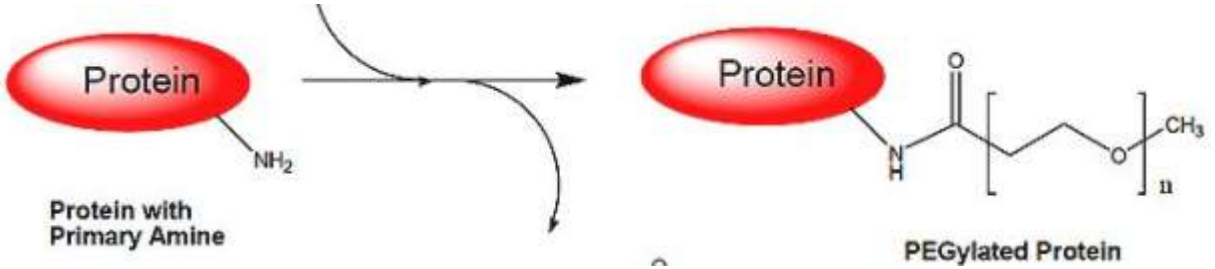



*J. Siner, Blood 2013*

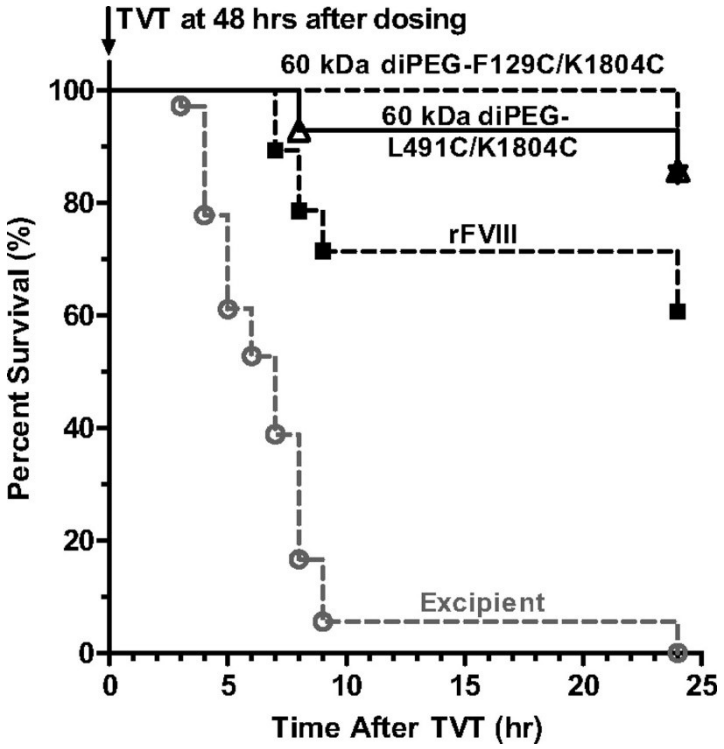
# Improve Stability II: PEGylation

- PEGylation is the process of covalent attachment of Poly Ethylene Glycol (PEG) polymer chains to another molecule, normally a drug or therapeutic protein.
- PEGylation is routinely achieved by incubation of a reactive derivative of PEG with the target molecule.
- The covalent attachment of PEG to a protein can "mask" the agent from the host's immune system and increase the hydrodynamic size of the agent which prolongs its circulatory time by reducing renal clearance.



# Improve Stability II: PEGylation

- Pegylation of FVIII extended protein half-life



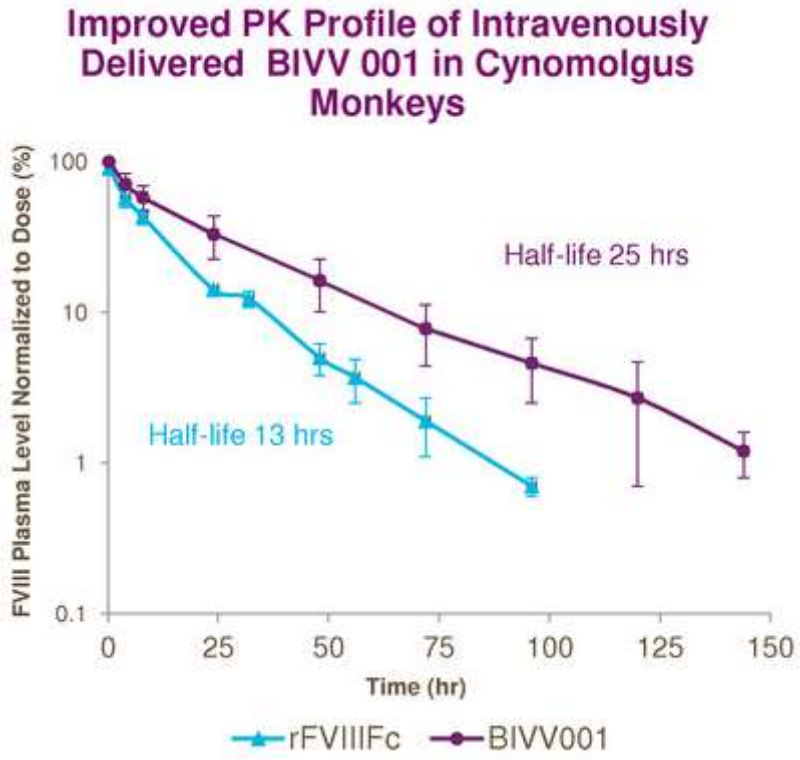

*B. Mai, Blood 2010*

ADYNOVATE
[Antihemophilic Factor
(Recombinant), PEGylated]

# Improve Stability III

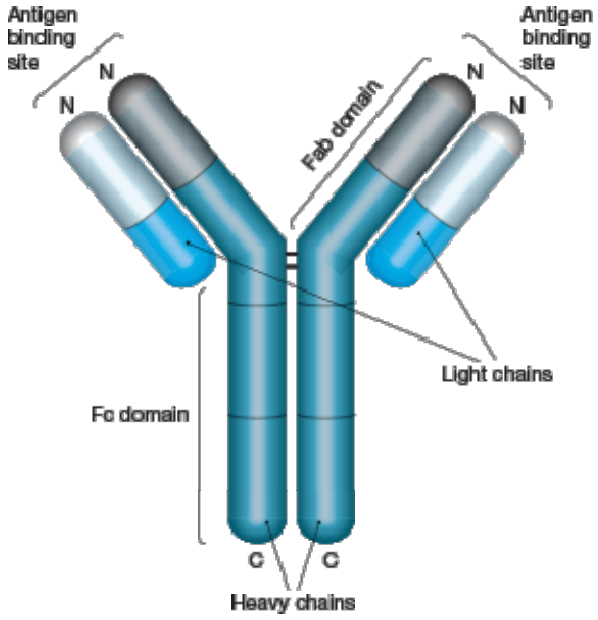
- Fusion protein with domains of long half-life proteins (e.g. IgG Fc 14 days, or Albumin 19 days)
  - binds to the neonatal Fc receptor (FcRn) on endothelial cells and it's recycled back
- CTP fusion: Half-life extension through charge
  - 31 aa, negatively charged, heavily sialylated CTP impairs renal clearance
- XTEN: recombinant polypeptide
  - The bulking effect of XTEN greatly reduces renal clearance of attached molecules, thus increasing their in vivo half-lives

# Improve Stability III



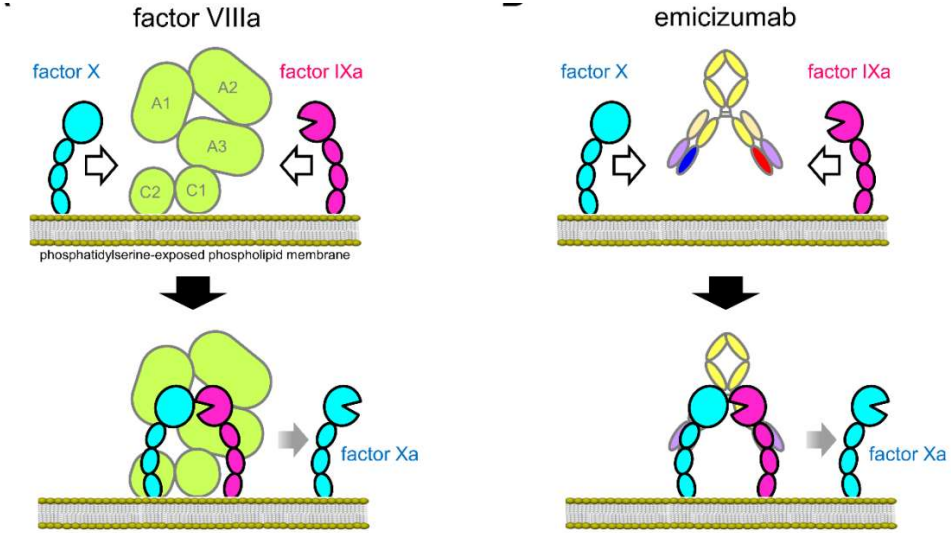
# Create a protein that will do the same job

- Antibodies are nature's favourite engineered protein
- Antibodies can be engineered to mimic the function of other proteins (even enzymes!!)



# Create a protein that will do the same job

- ACE910 is a recombinant humanized bispecific antibody that binds to activated FIX and FX and mimics the cofactor function of FVIII
- Long half-life
- Functions in the presence of anti FVIII antibodies



# Conclusion I

- Proteins are great, but we can make them better
- We can intervene at different cellular levels:
  - Transcription/translation (codon optimization)
  - Improving specific activity
  - Enhancing secretion
  - Increasing stability
- We can design an antibody that mimic a protein's function
- Always make sure that modifications don't impact function