

Tissue Factor Pathway Inhibitor: New insights in an old inhibitor

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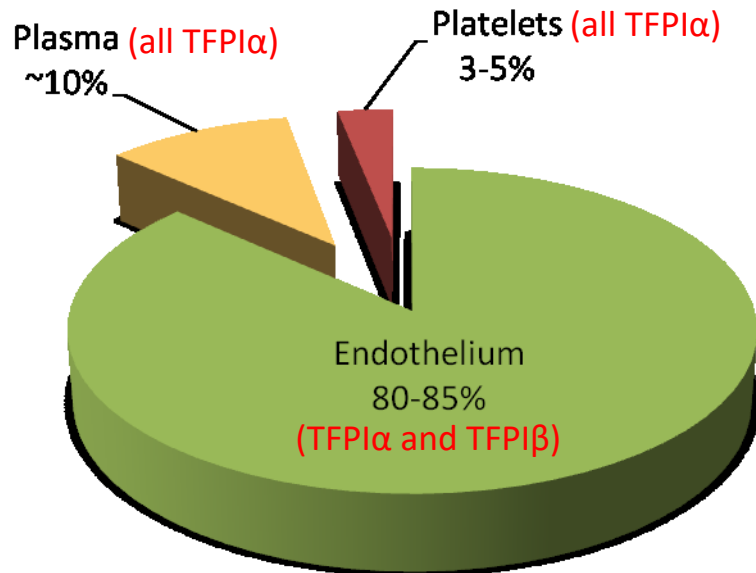
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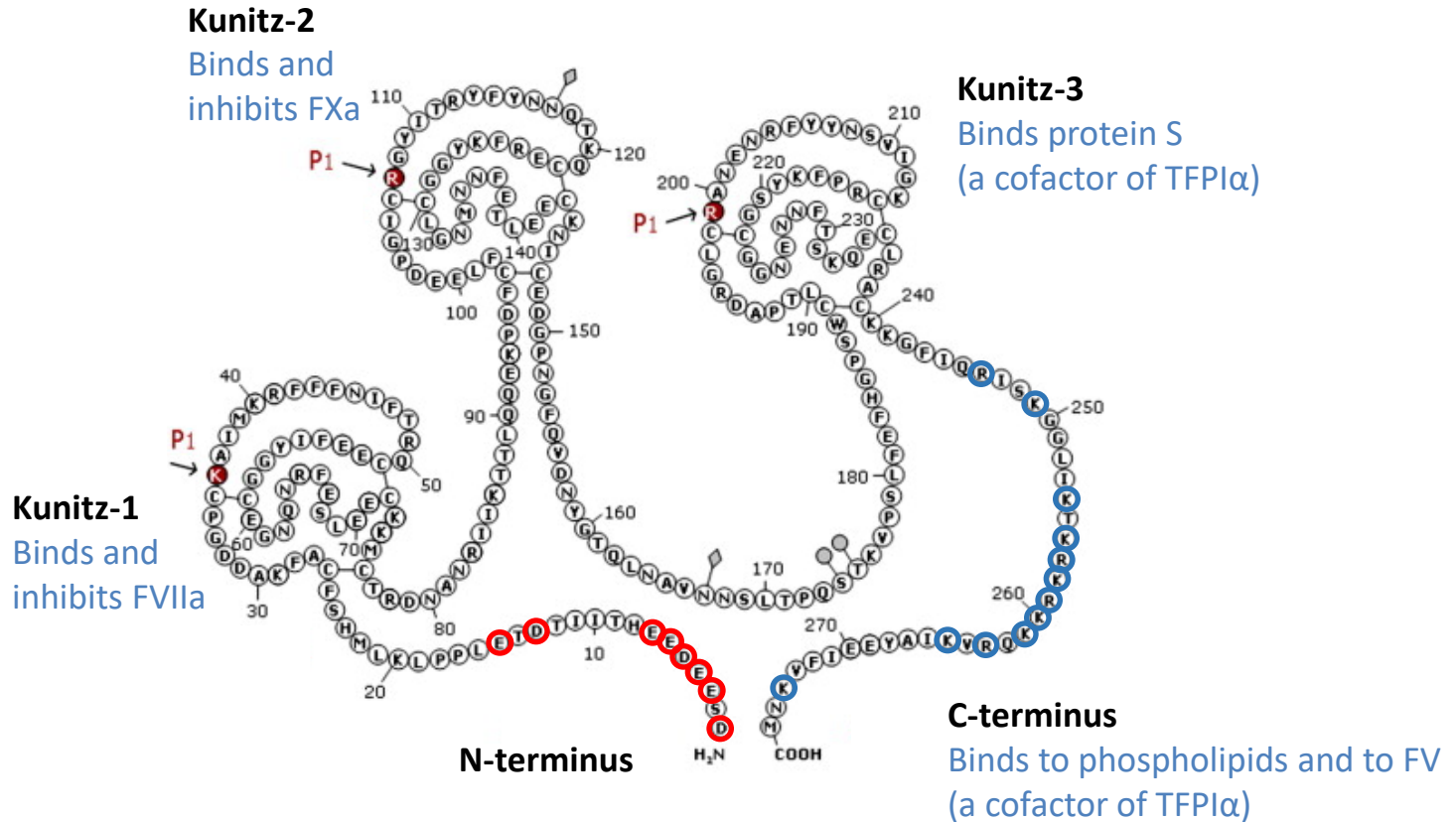
Leiden, 10th November 2016

Tissue factor pathway inhibitor (TFPI)

- Multivalent Kunitz-type serine-protease inhibitor
- Synthesised in endothelial cells and megakaryocytes
- Three pools: endothelium, plasma and platelets
- Two splicing variants: TFPI α (~80%) and TFPI β (~20%)



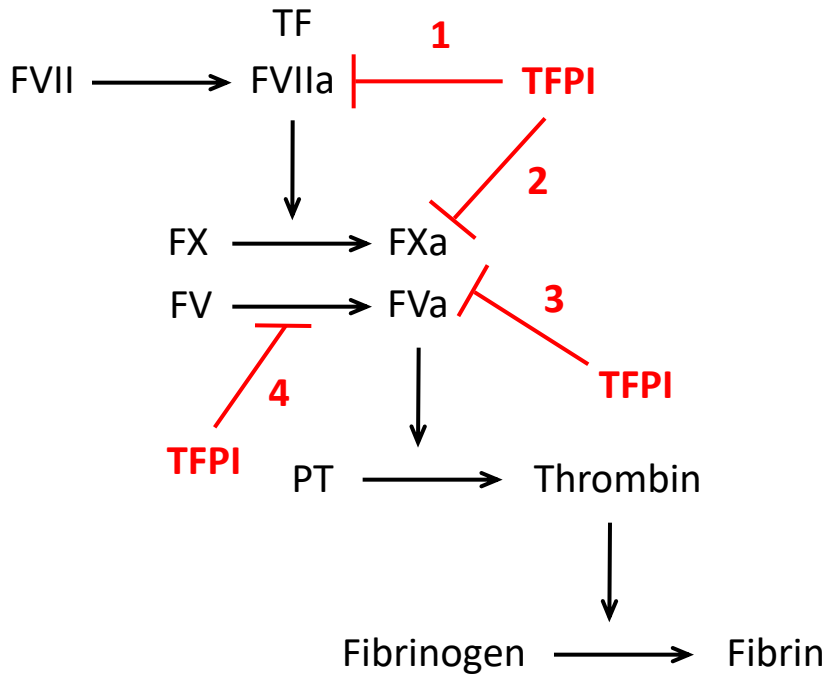
Structure of TFPI α



Mature protein of 276 amino acids (~43 kDa including glycosylation)

Anticoagulant functions of TFPI α

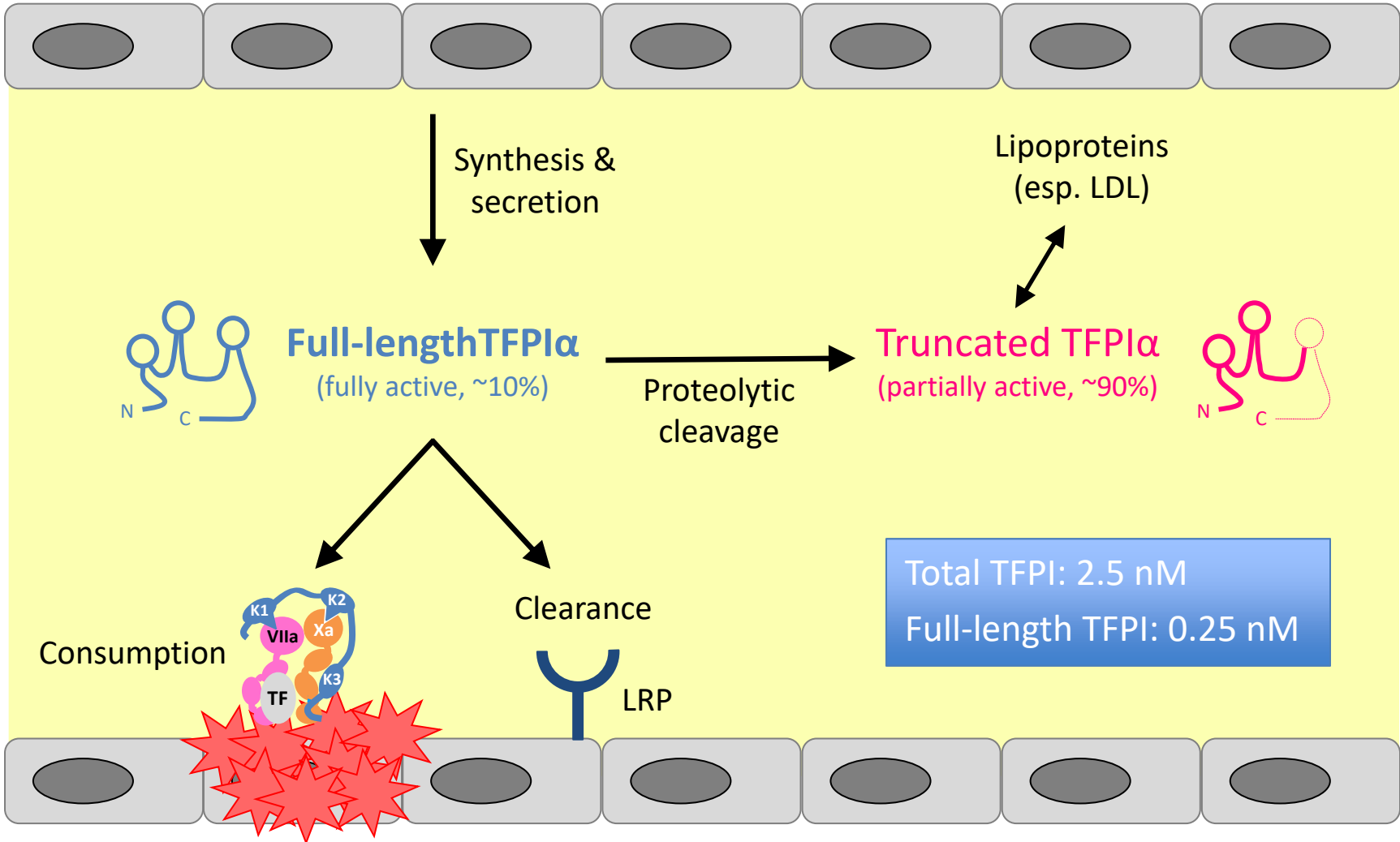
Extrinsic pathway of coagulation



TFPI efficiently inhibits the initiation of coagulation by four mechanisms:

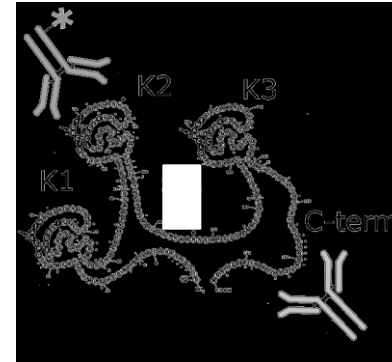
- 1) Inhibition of TF/FVIIa (K1)
- 2) Inhibition of FXa (K2)
- 3) Inhibition of prothrombinase *via* interactions with FV(a) (C-term)
- 4) Inhibition of FV activation (C-term)

Plasma TFPI

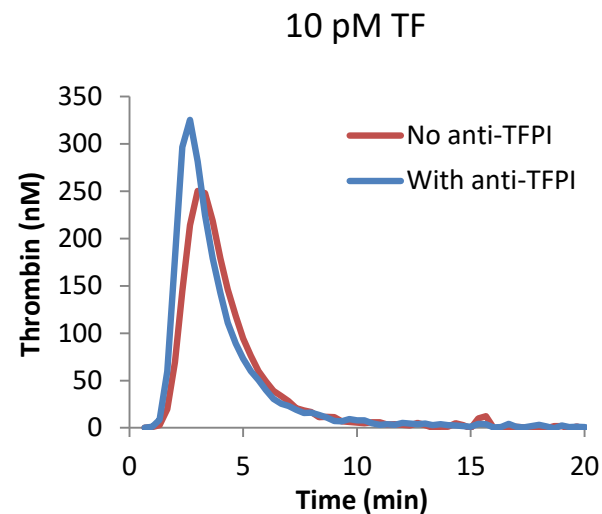
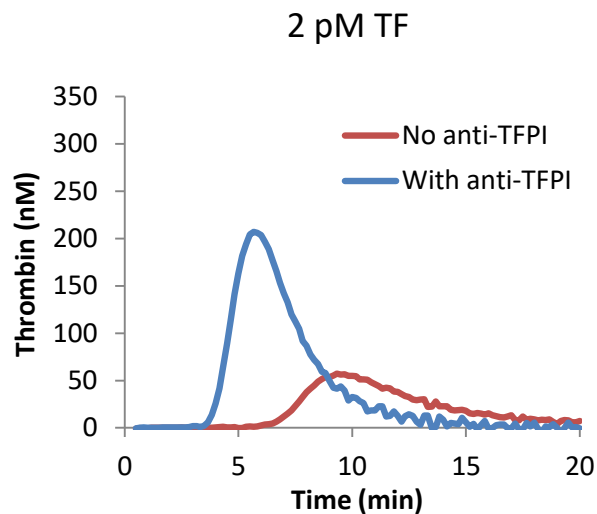


Measurement of plasma TFPI levels

- TFPI antigen assays:
 - Total TFPI ELISA (Asserachrom)
 - Free TFPI ELISA (Asserachrom)
 - Full-length TFPI ELISA (in-house)



- Thrombin generation-based TFPI functional assay (in-house):



Plasma TFPI levels and VTE risk

- Complete TFPI deficiency (KO mice) not compatible with life
- No reports of genetic TFPI deficiencies in humans
- Large inter-individual variation in plasma TFPI levels, due to:
 - age and sex
 - acquired conditions (*e.g.* oral contraceptive use)
 - common genetic variation ($h^2 = 27\text{-}52\%$)
- Low TFPI levels are a mild risk factor for venous thrombosis

Levels	Total TFPI	Free TFPI	TFPI activity
<10 th percentile	1.5 (0.98 - 2.3)	1.7 (1.1 - 2.6)	1.1 (0.73 - 1.8)
<5 th percentile	2.1 (1.1 - 4.1)	2.1 (1.1 - 4.1)	1.6 (0.87 - 2.8)
<2 nd percentile	3.0 (1.3 - 7.2)	2.2 (0.89 - 5.3)	2.4 (1.1 - 5.1)

Comparison with AT, PC and PS deficiencies

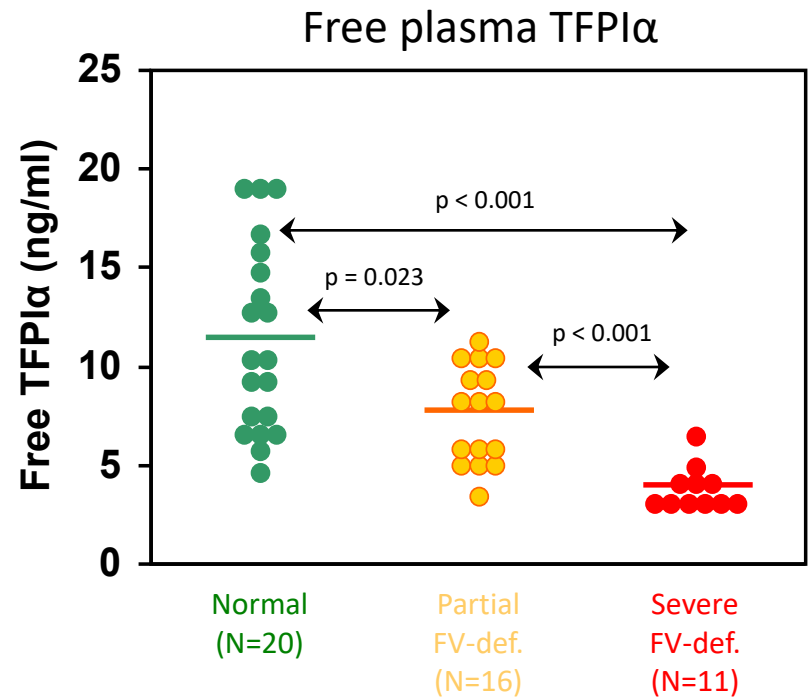
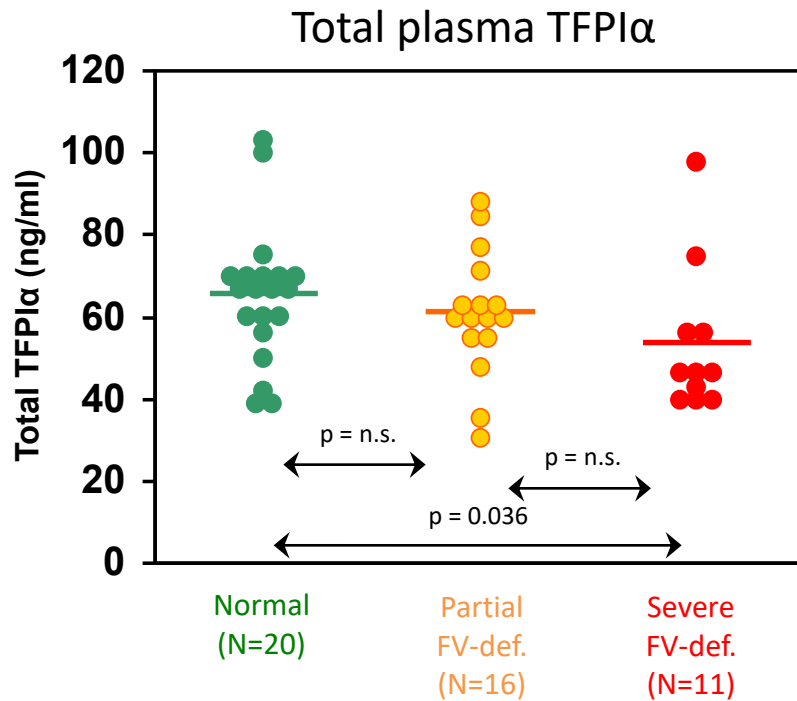
Risk factor	Protein level	Prevalence	VTE risk
(Heterozygous) AT deficiency	~50%	~0.03%	10-20
(Heterozygous) PC deficiency	~50%	~0.2%	10
(Heterozygous) PS deficiency	~50%	~0.2%	8
Low TFPI levels	<5 th precentile	5%	2 !!!

- Are plasma TFPI levels representative for the TFPI status?
- Which fraction of plasma TFPI should we measure?
- Are TFPI levels in the absence of a *TFPI* mutation low enough?
- What if the TFPI level is only transiently reduced?

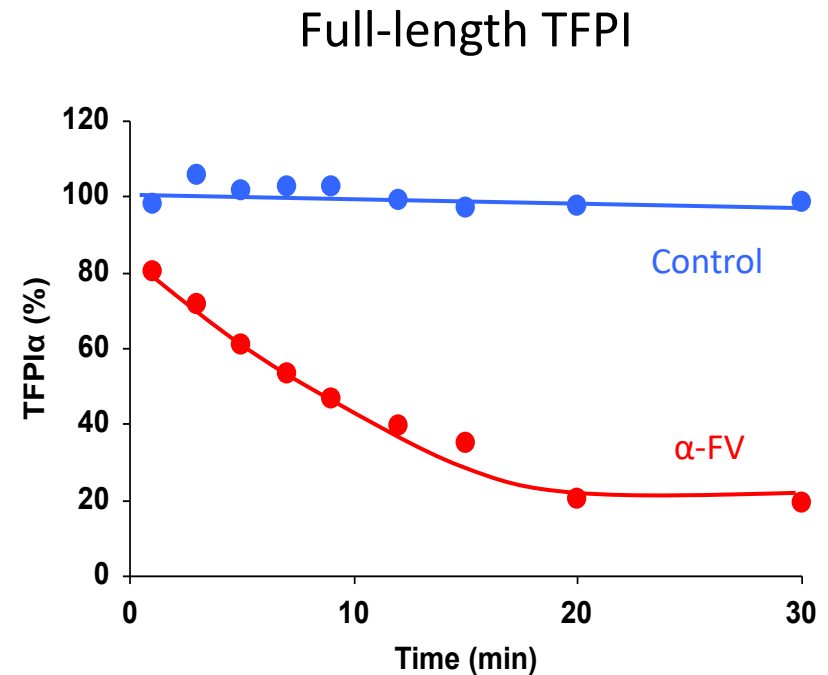
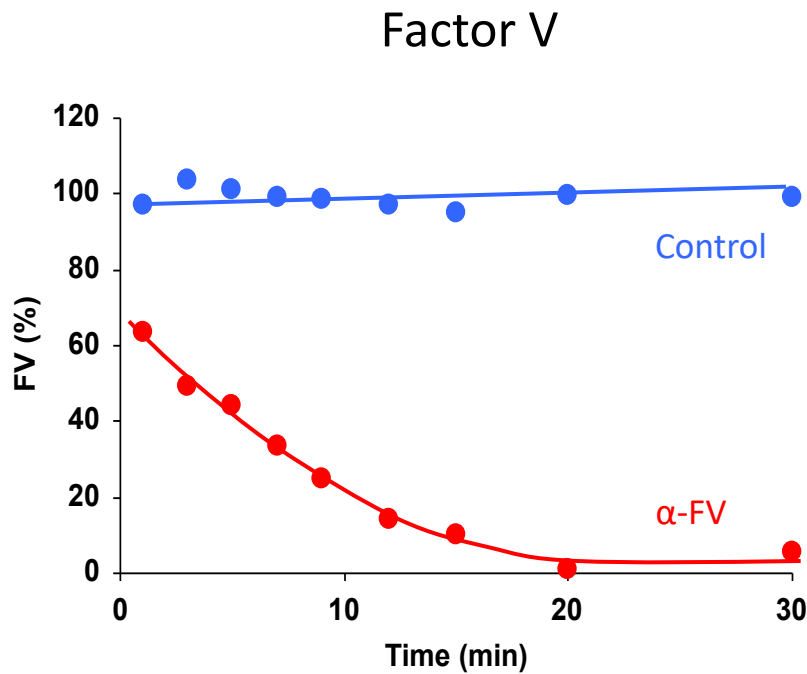
New insights

- Plasma TFPI: interactions with factor V and protein S
- Anticoagulant functions of TFPI
- TFPI and bleeding

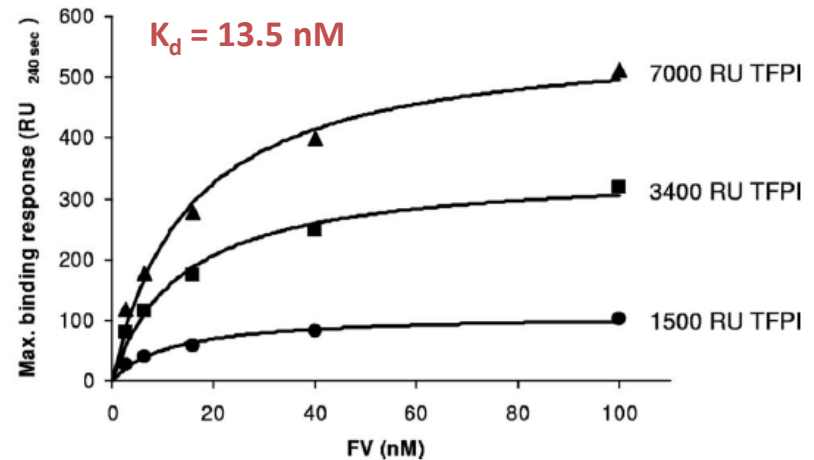
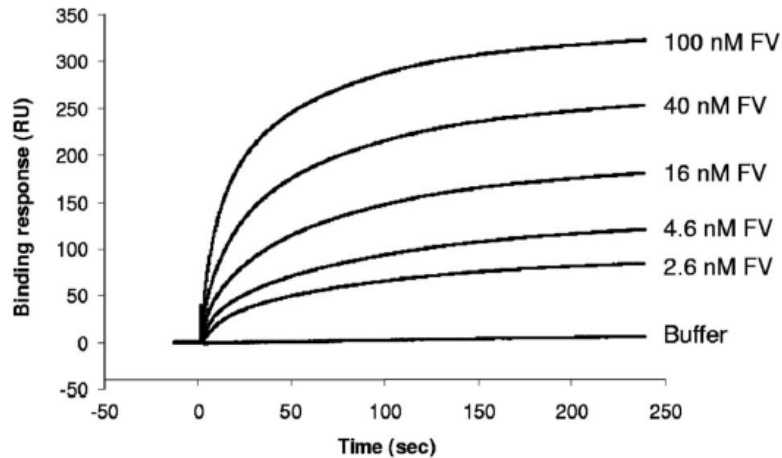
Low TPI levels in factor V deficiency



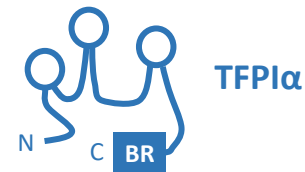
TFPI circulates in complex with factor V



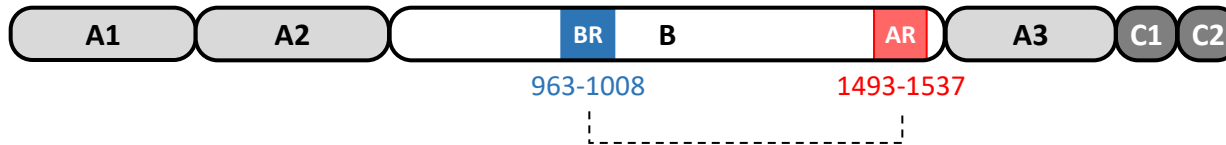
Binding of factor V to immobilised TFPI α



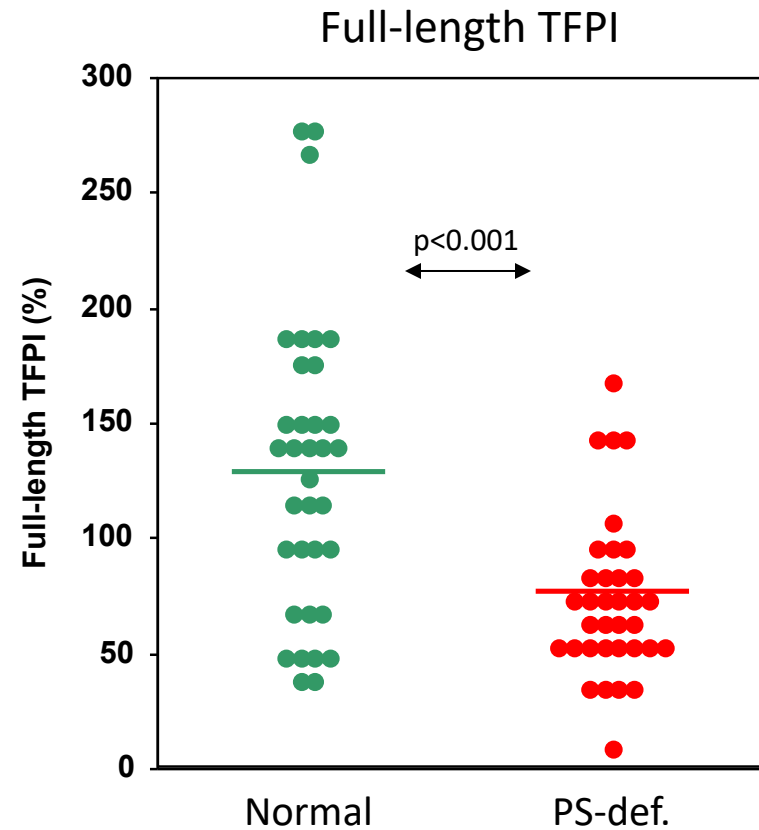
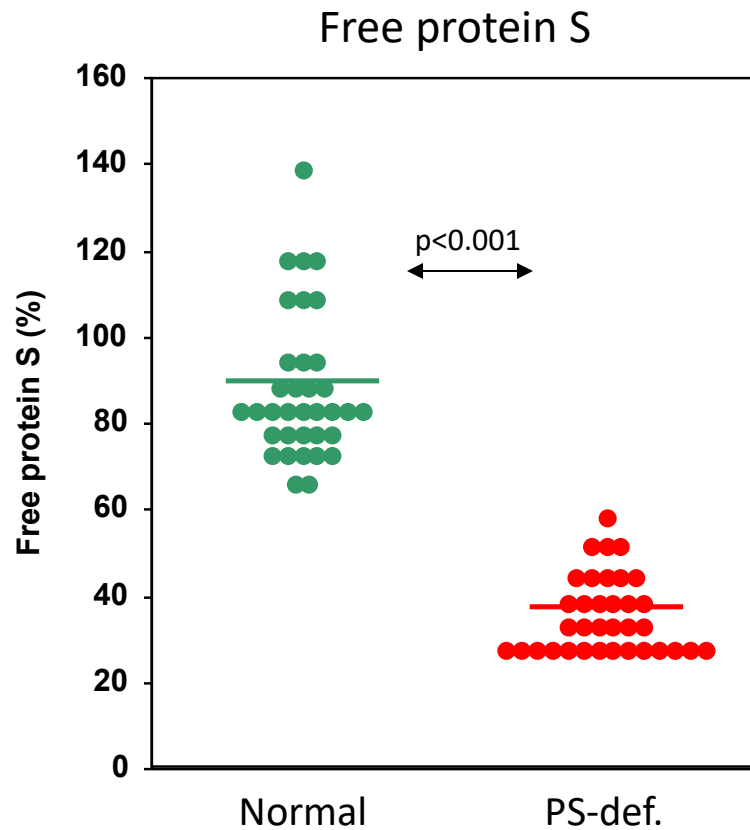
FV VRQDGGKSRLLKKSQFLIKTRKKKK EK
 TFPI α GFIQRISKGGLIKTKRKRKKQRVKIAAYEEIFVKNM



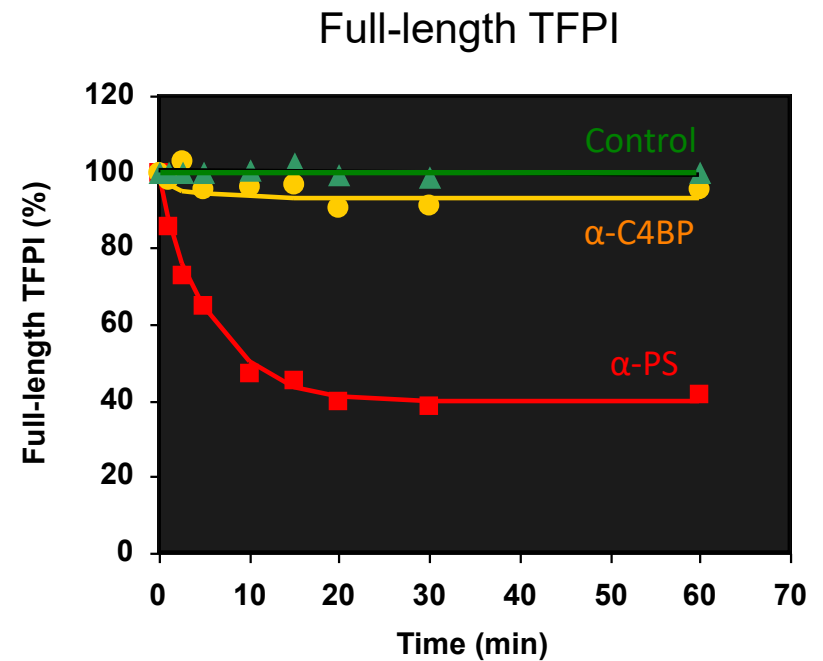
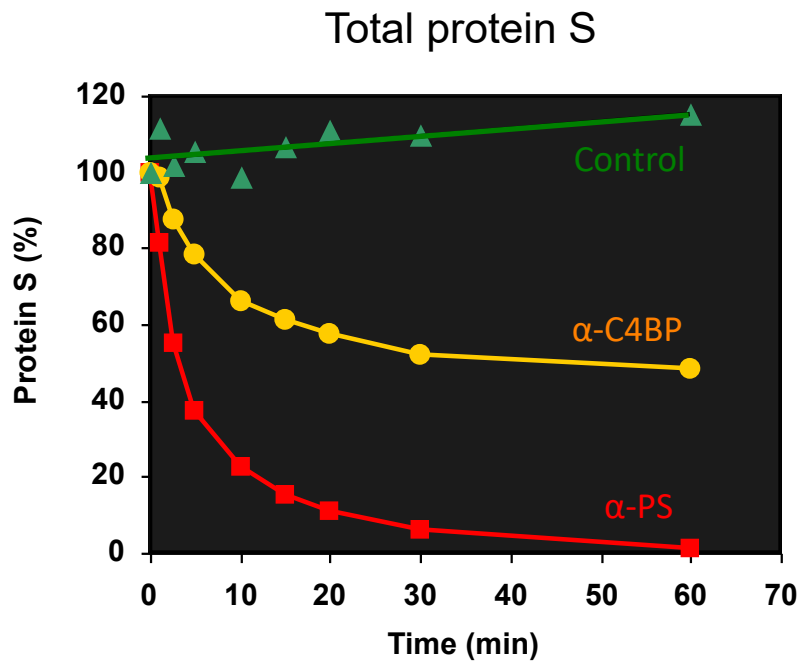
FV



Low TPI levels in protein S deficiency



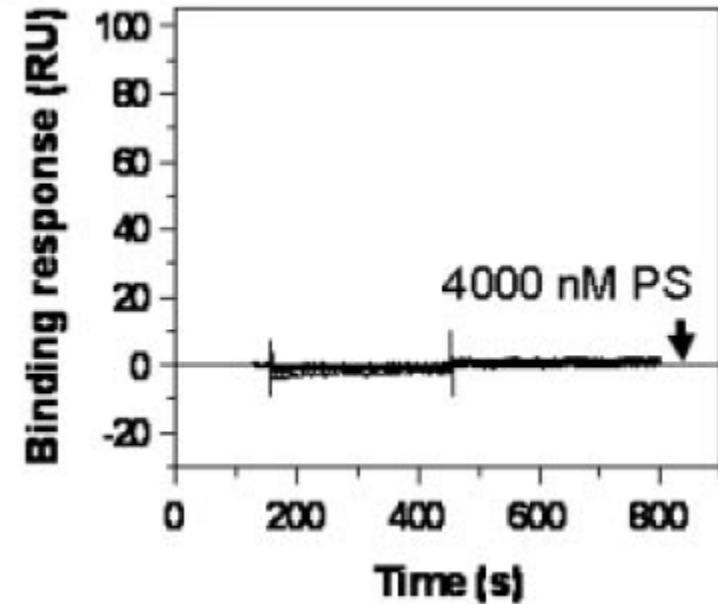
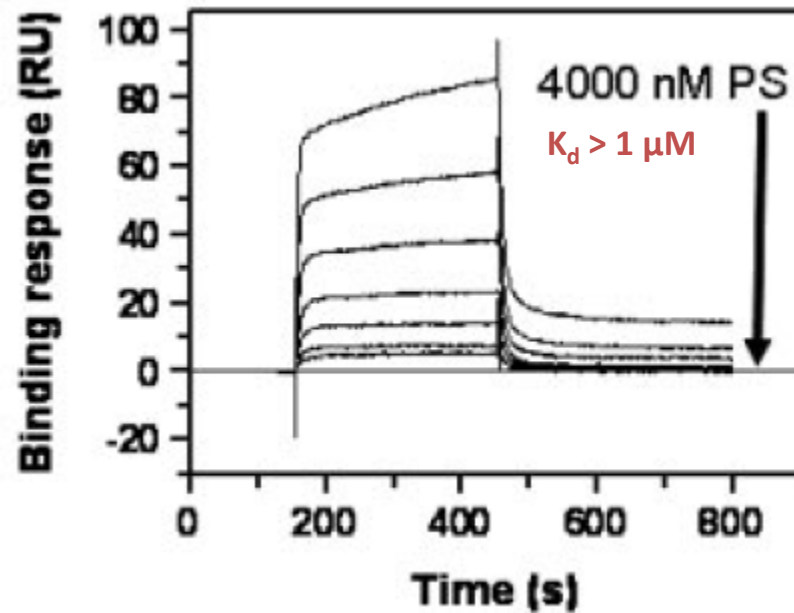
TFPI circulates in complex with (free) protein S



Binding of protein S to immobilised TFPI α

WT-TFPI α

TFPI α - Δ K3



Conclusions (I)

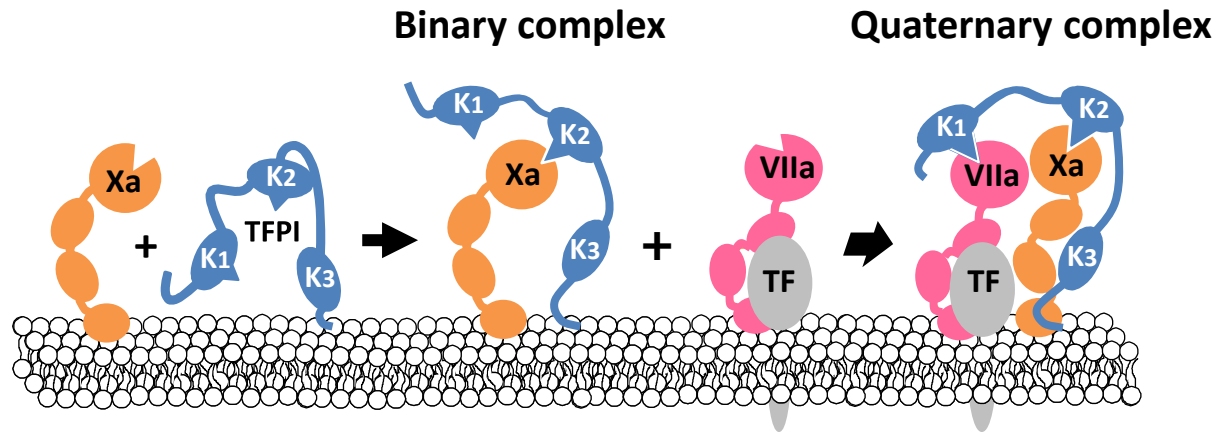
- Plasma full-length TFPI circulates in non-covalent complexes with factor V and free protein S
- These complexes protect full-length TFPI from truncation and/or clearance
- The low TFPI level of factor V-deficient patients mitigates the bleeding tendency of these patients
- The low TFPI level in protein S-deficient patients contributes to the hypercoagulable state of these patients

New insights

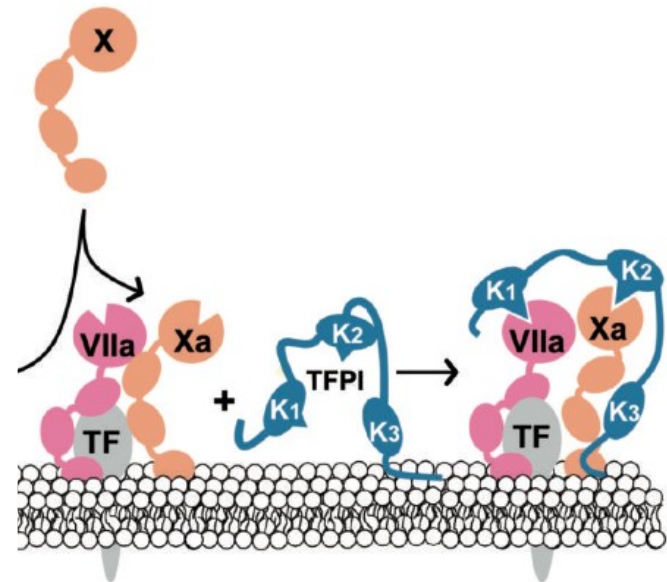
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- TFPI and bleeding

Inhibition of TF/FVIIa and FXa by TFPI α

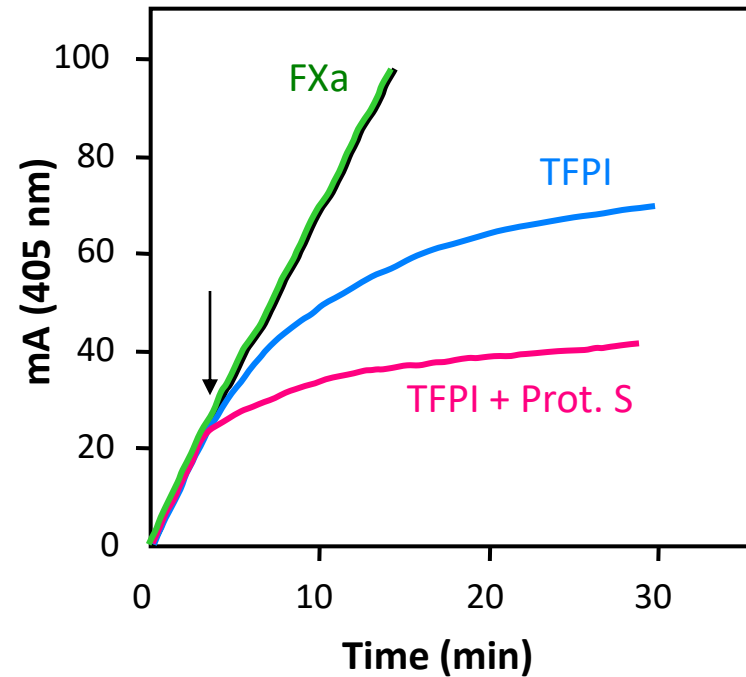
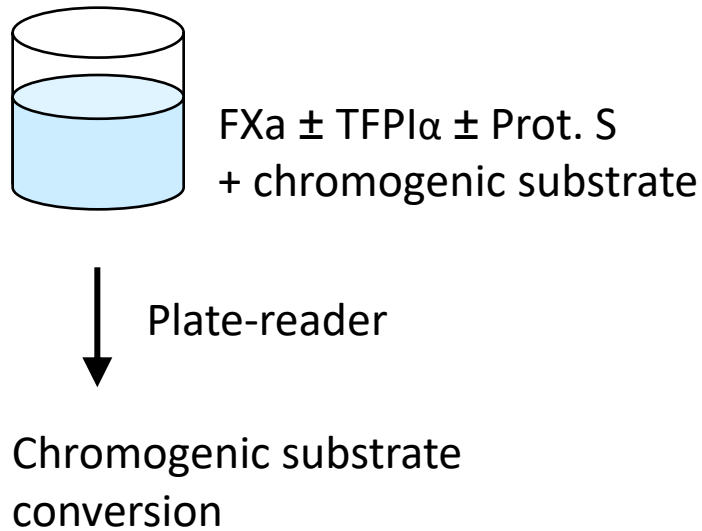
Model I



Model II

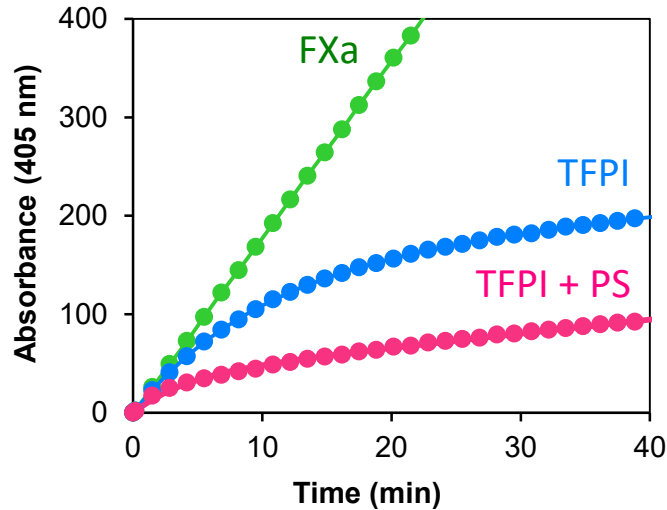


Protein S stimulates FXa inhibition by TFPI

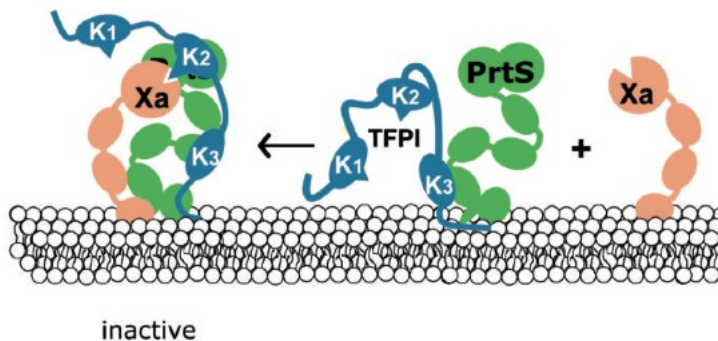
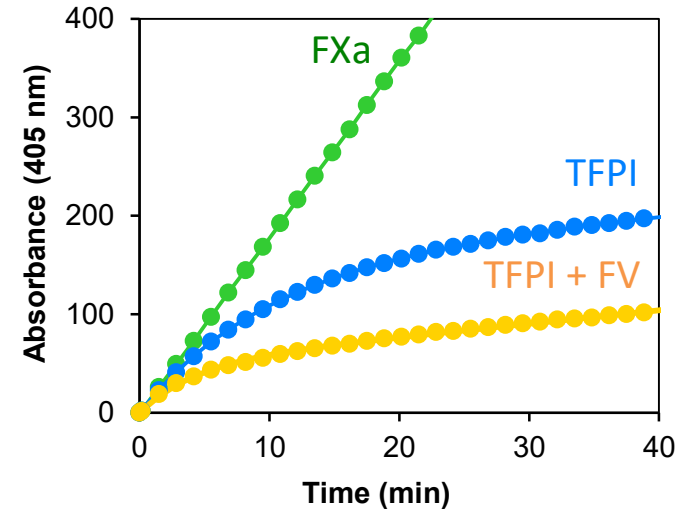


Factor V stimulates FXa inhibition by TFPI

Effect of 80 nM protein S

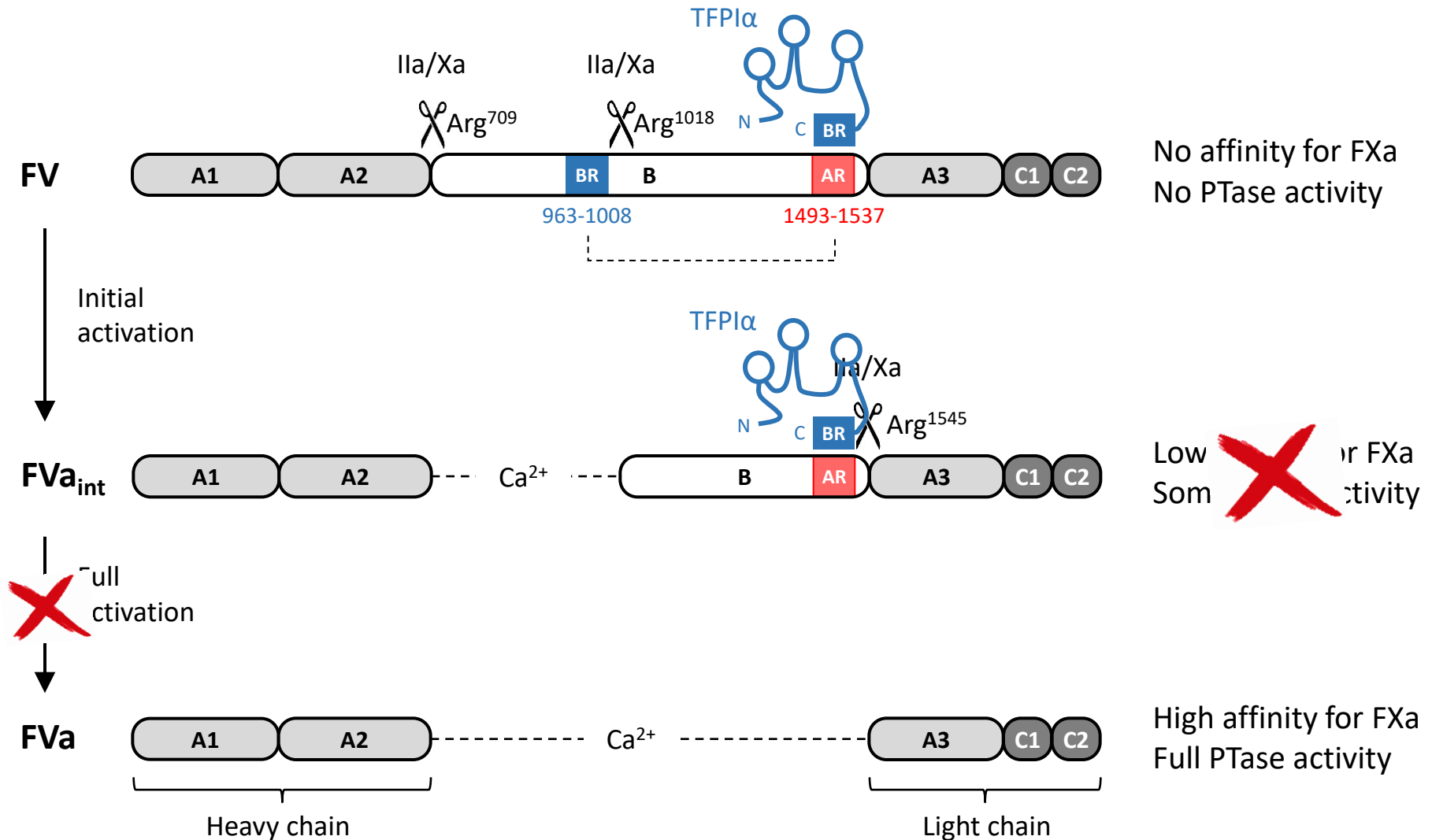


Effect of 20 nM factor V

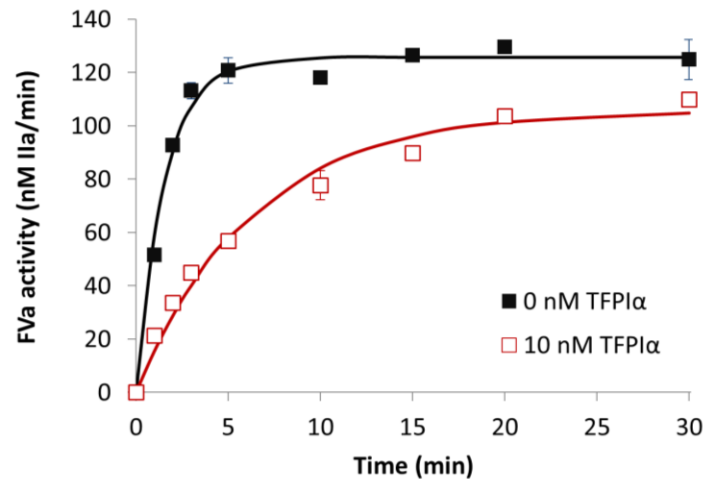
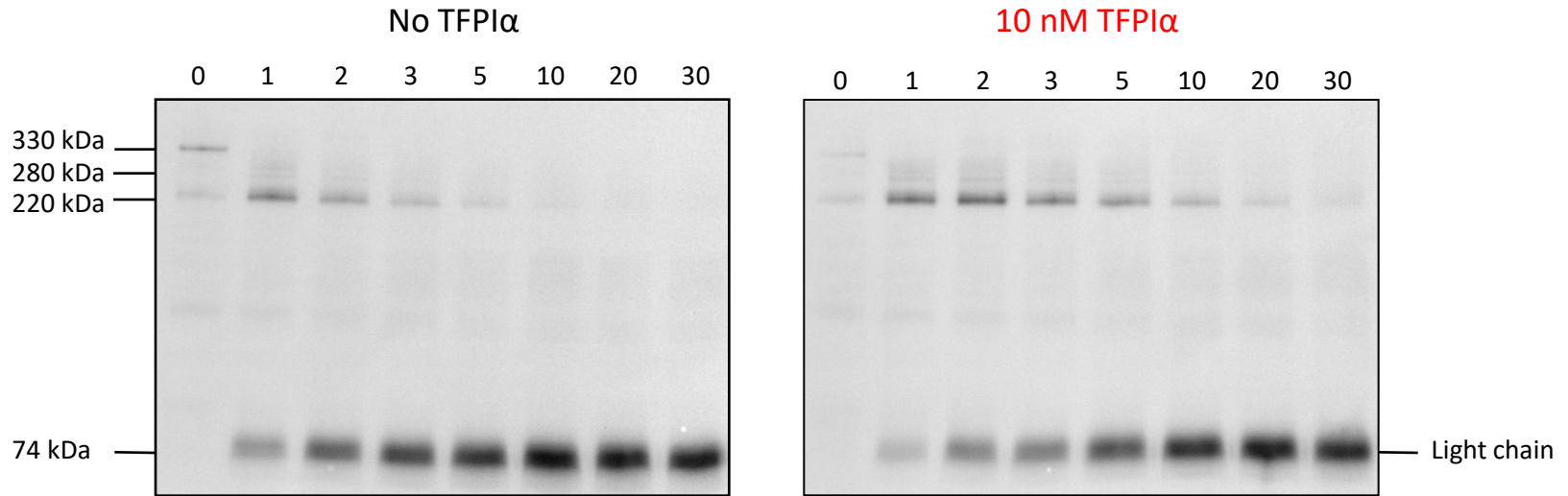


Protein S and factor V are thought to act by enhancing binding of TFPI to phospholipids

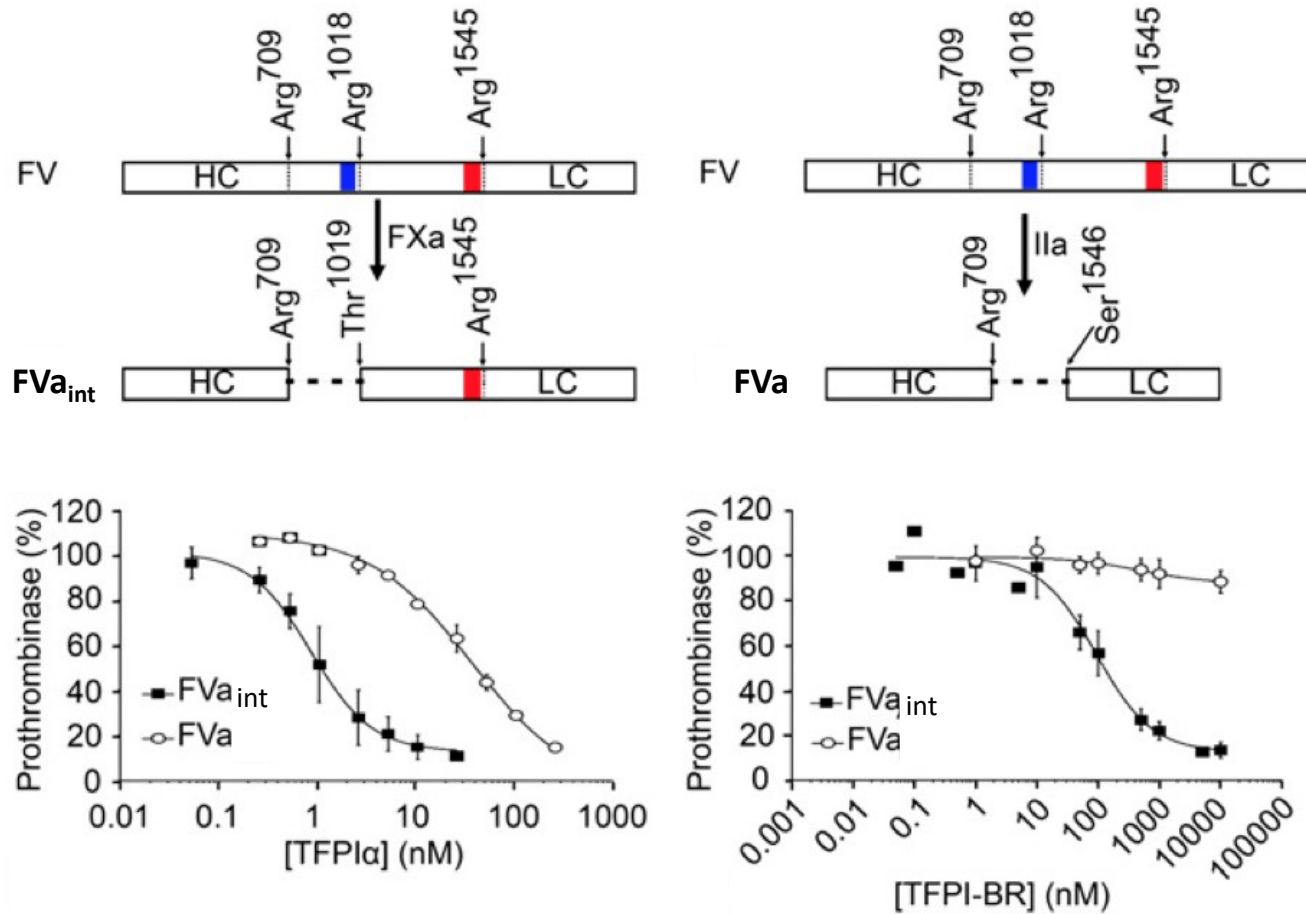
Inhibition of FV activation/activity by TFPI α



TFPI α inhibits FV activation



TFPI α inhibits prothrombinase



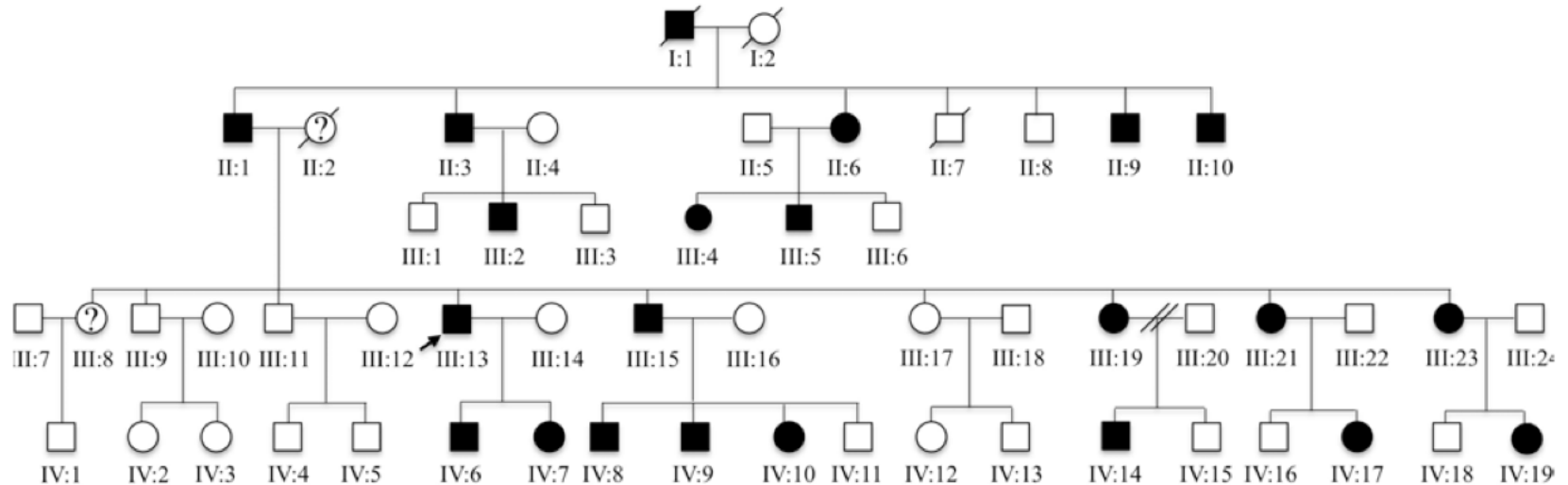
Conclusions (II)

- Protein S and factor V stimulate FXa inhibition by full-length TFPI α by enhancing TFPI binding to the phospholipid surface
- Full-length TFPI α binds to the acidic region of FV, thereby protecting the Arg¹⁵⁴⁵ cleavage site and delaying full FV activation
- Full-length TFPI α bound to partially activated FVa species prevents their incorporation in the prothrombinase complex, thereby inhibiting prothrombinase activity

New insights

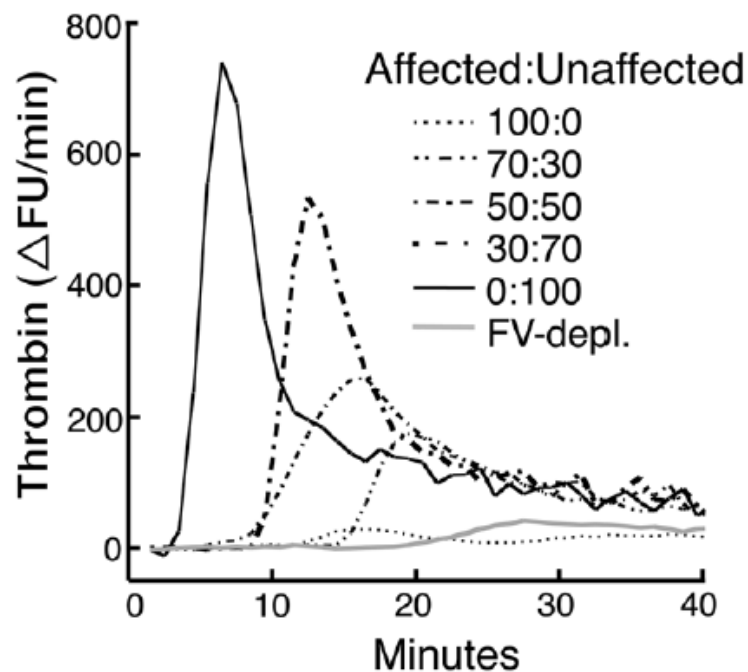
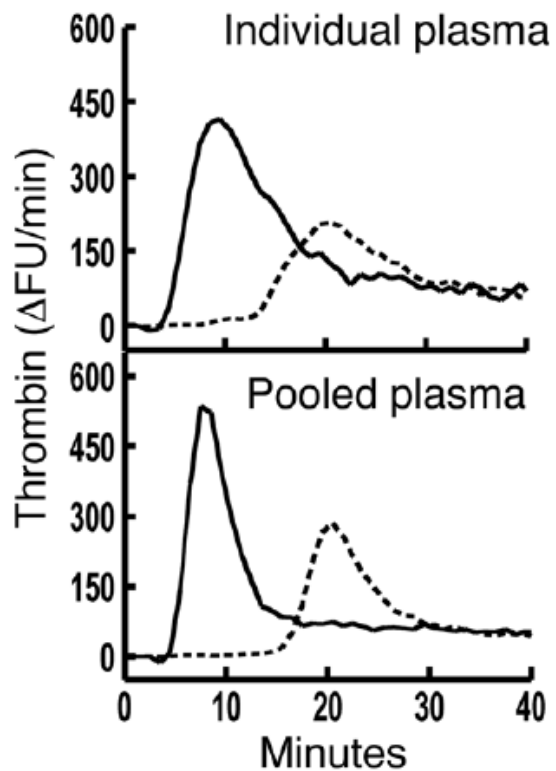
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East Texas bleeding disorder



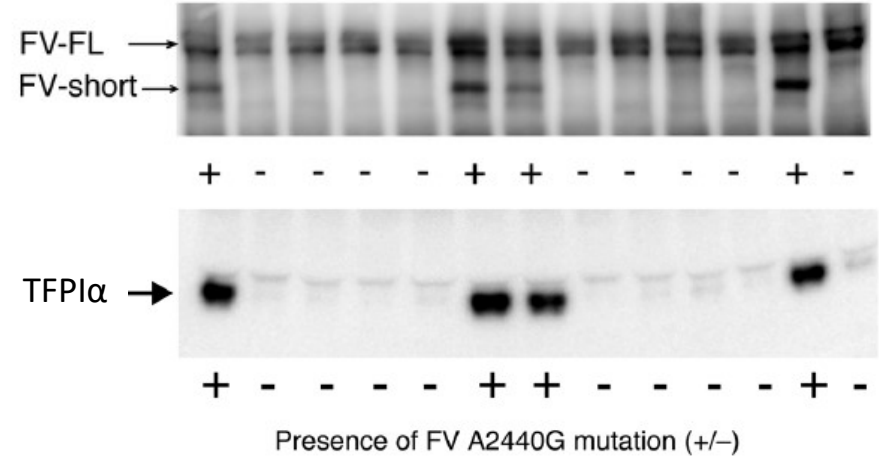
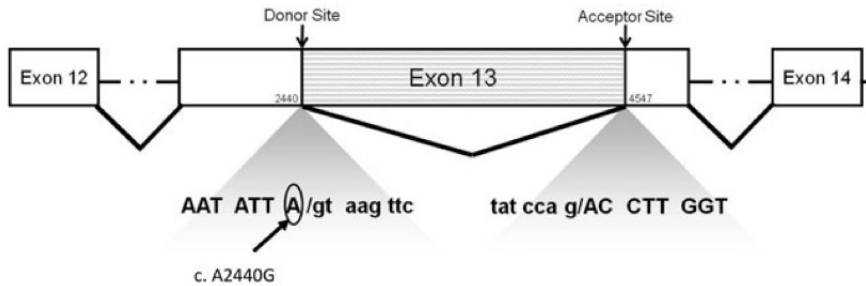
- Autosomal dominant bleeding disorder
- Prolonged PT and APTT, normal levels of all coagulation factors
- Linkage analysis points at the *F5* gene
- Sequencing identifies the *F5* A2440G (Ser756Gly?) mutation, which co-segregates with bleeding phenotype

East Texas bleeding disorder

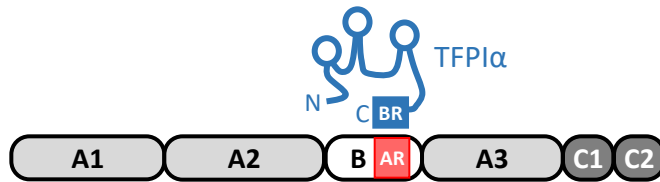


Factor V-short

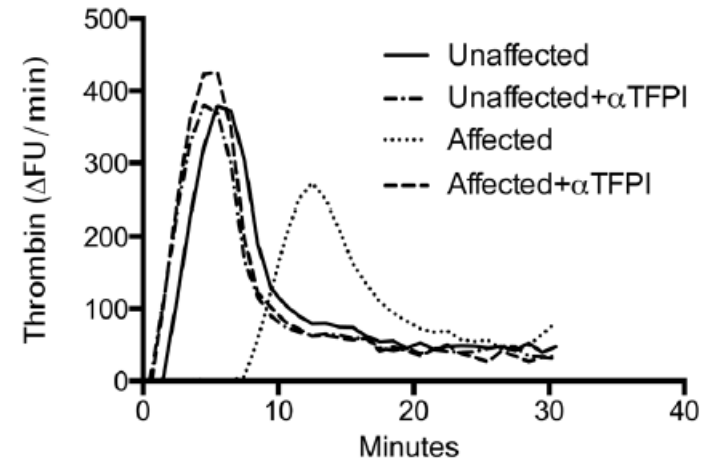
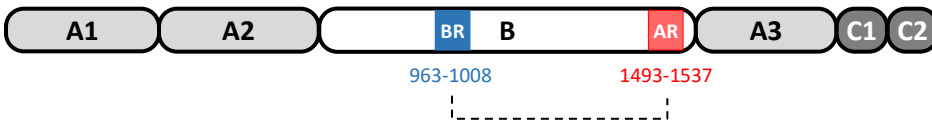
FV^{A2440G} activates alternative splicing



FV-short



FV



Conclusions (III)

- FV-short is a splicing variant of FV expressed at low levels in all individuals
- Due to the lack of the basic region, FV-short binds TFPI α with high affinity and prolong its half-life in the circulation
- Up-regulation of the expression of FV-short is associated with elevated levels of full-length TFPI α
- Elevated levels of full-length TFPI α , such as observed in the East Texas bleeding disorder, are associated with a moderate bleeding tendency