



U.S. Food and Drug Administration

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FDA Workshop: Risk Mitigation
Strategies to Address Procoagulant
Activity in Immune Globulin Products
May 17, 2011 Bethesda, MD



Laboratory investigations of products associated with thrombotic events

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Regulation of thrombin generation by Factor XI

Sources of activation:

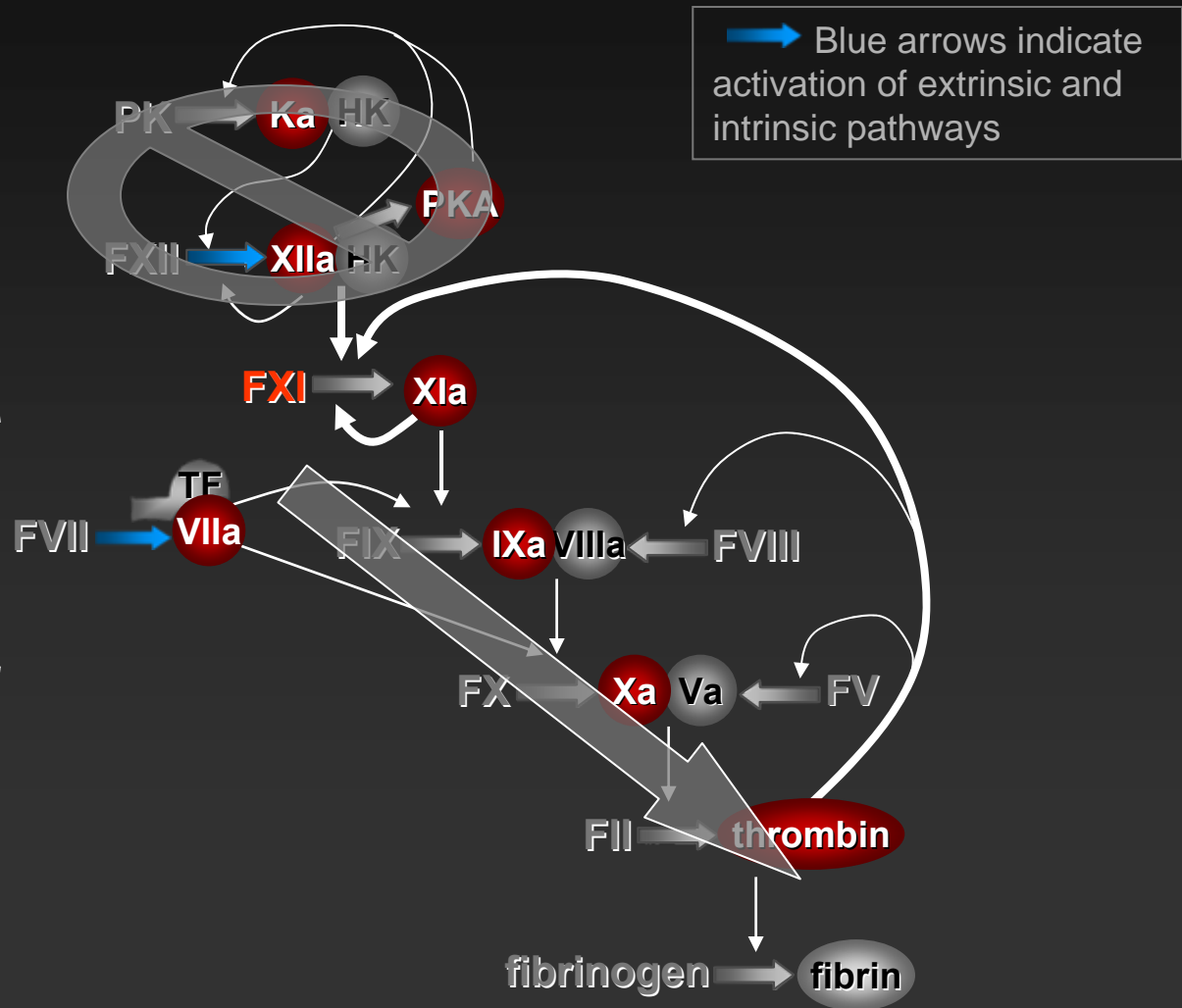
- Artificial surfaces
- Protein aggregates
- Denatured proteins
- Plasma storage
- Certain plasma fractionation steps
- PolyP (platelets)

contact pathway

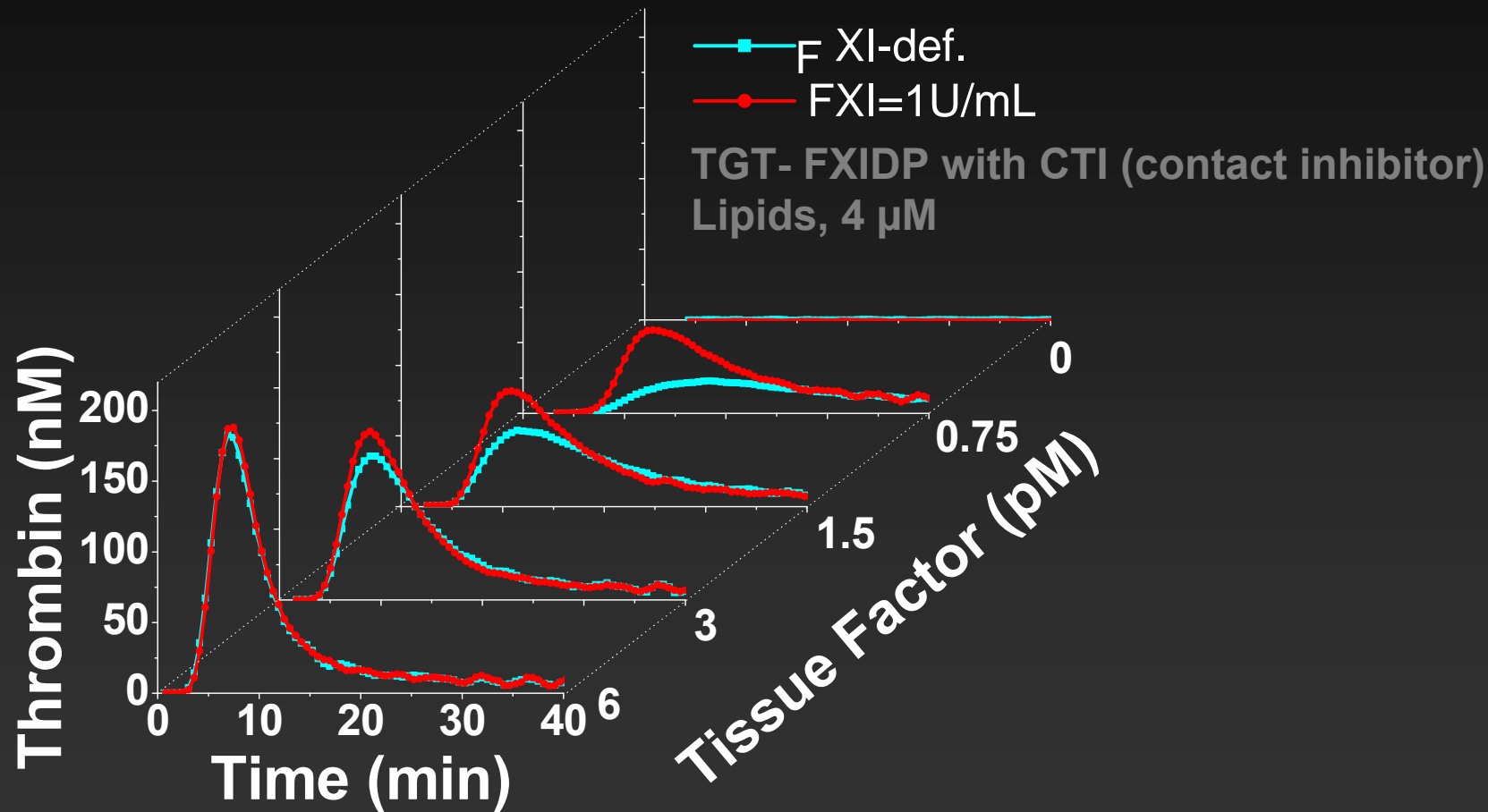
Sources of activation:

- Vascular wall injury
- Cancer cells
- Atherosclerotic plaque

extrinsic pathway

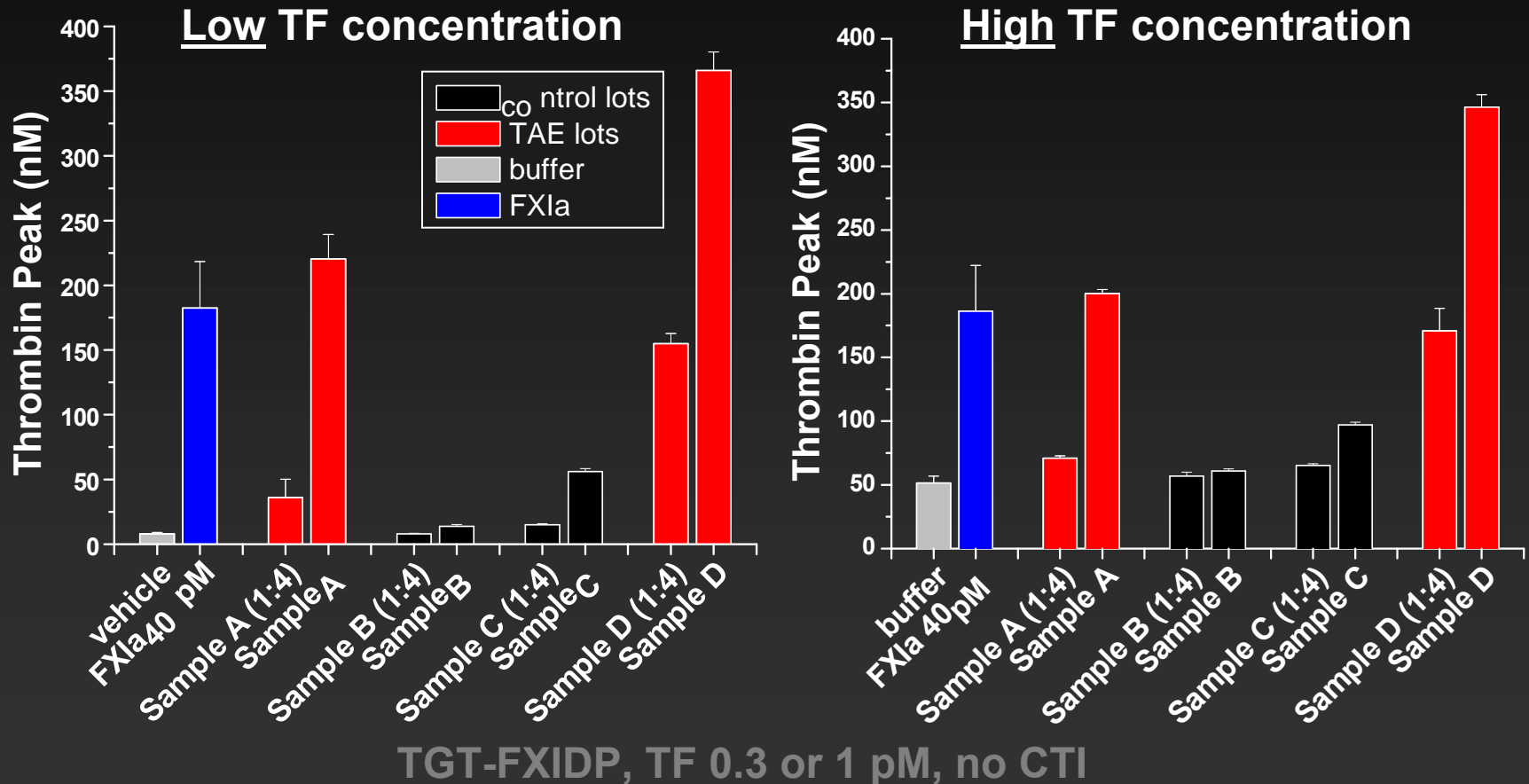


Concentration of TF stimulus regulates contribution of FXI



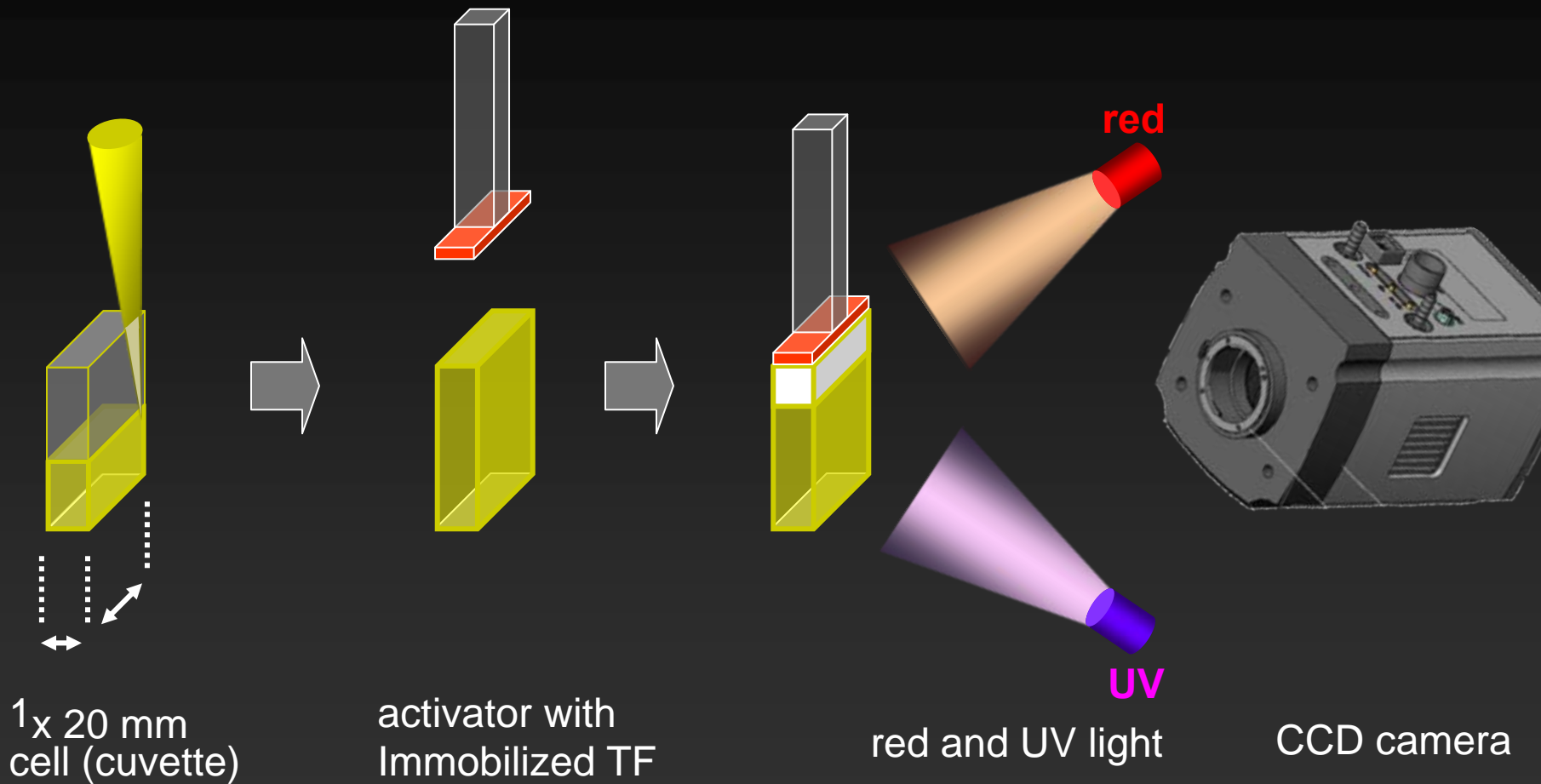
(Cawthern et al, 1998; Oliver et al, 1999;
Keularts et al, 2001; Kravtsov et al, 2009)

Procoagulant activity of lots implicated in Thrombotic Adverse Events (TAEs)



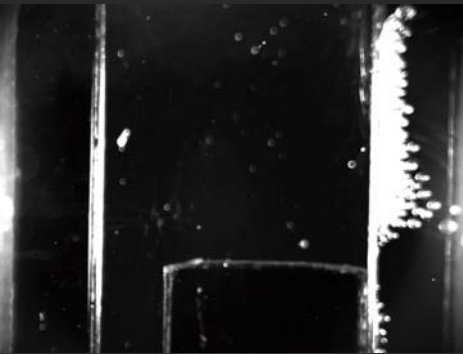
- TAE lots promote (or induce?) coagulation in FXI-def. plasma
- Better range at low TF, suggesting independence from TF

Videomicroscopy of Clot Formation



- clotting is activated by “exposed TF-bearing surface”
- clot expands in size

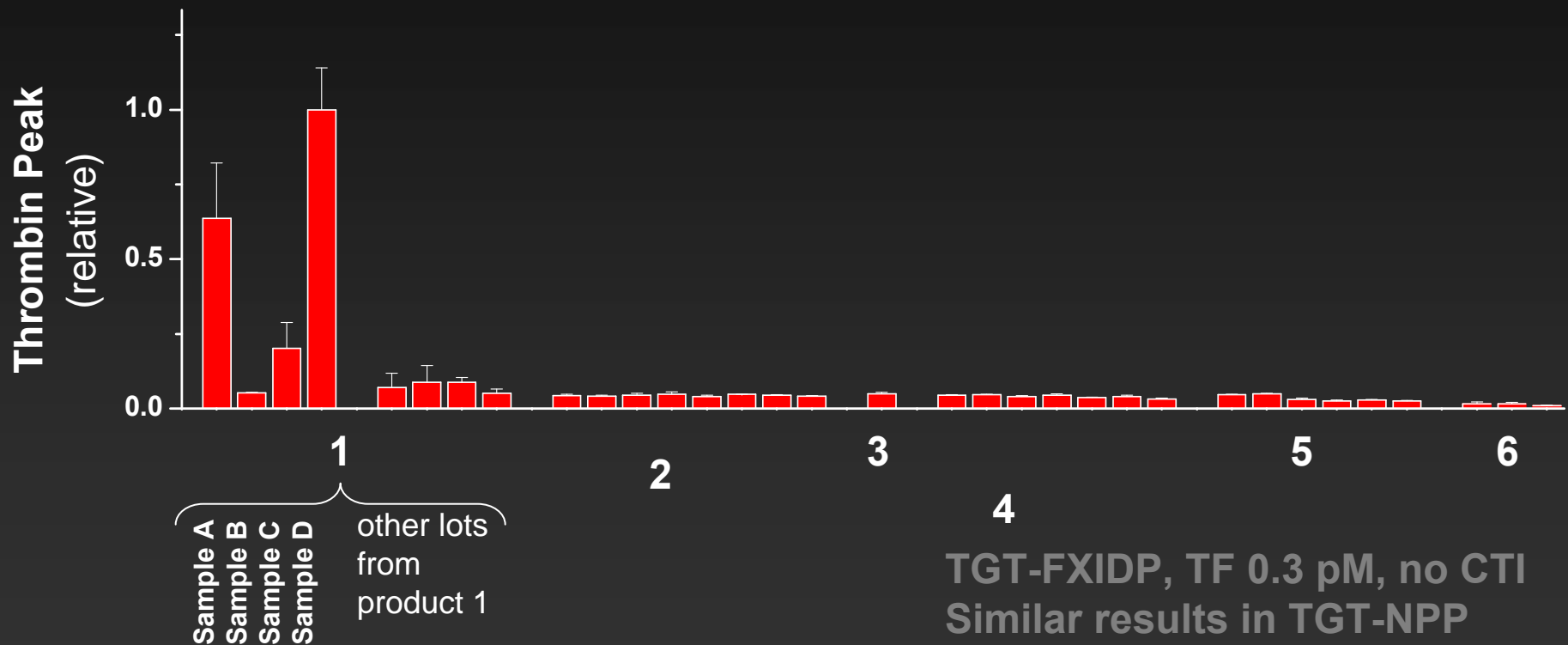
TF-dependent and independent clot formation



Procoagulant substance can induce clotting directly and can promote TF-induced clot growth

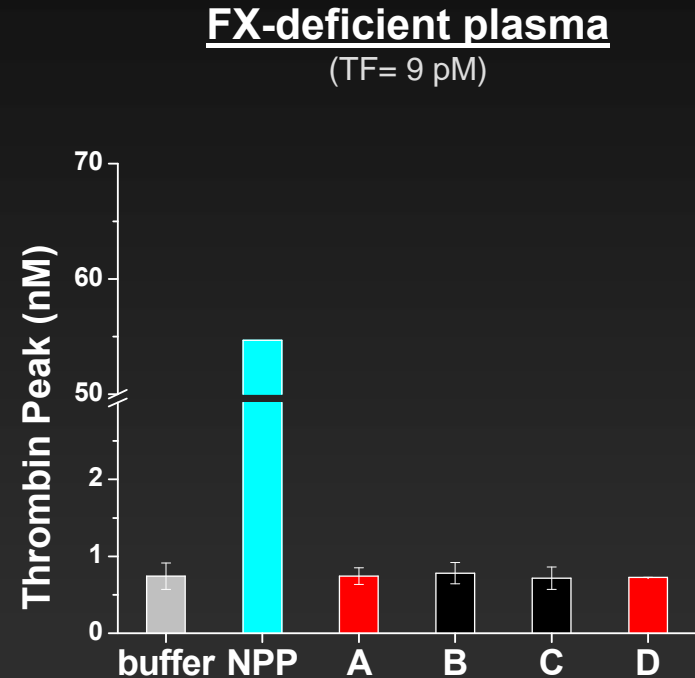
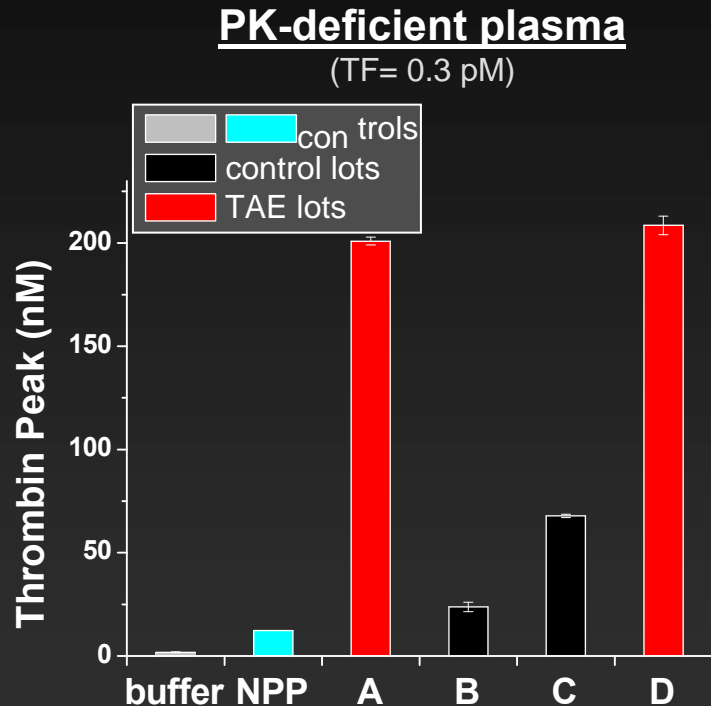
Relative Procoagulant Activity of 6 products

(Note: more products with different activities have been identified since the time of this experiment)



TAE-Implicated lots have very high procoagulant activity compared to other products and other lots of the same product

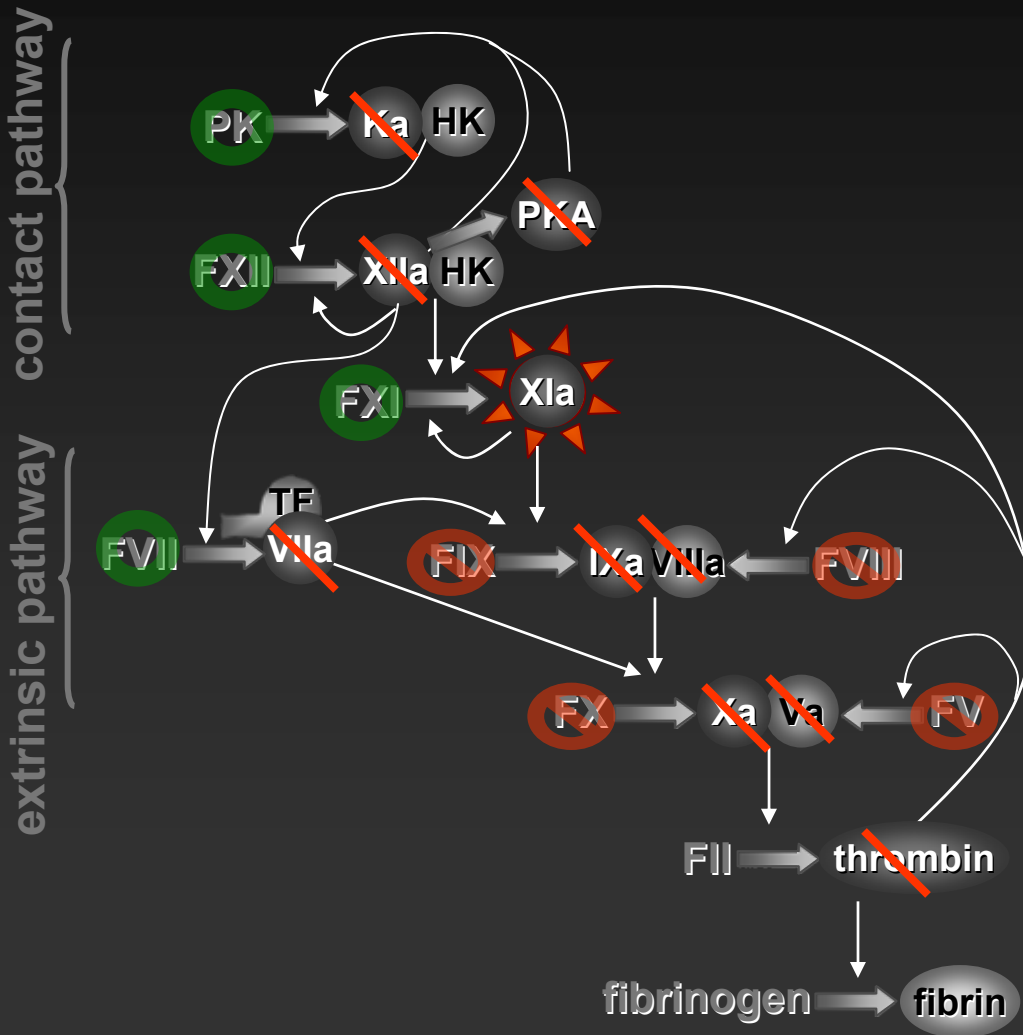
Effect of single factor deficiency on procoagulant activity of implicated lots



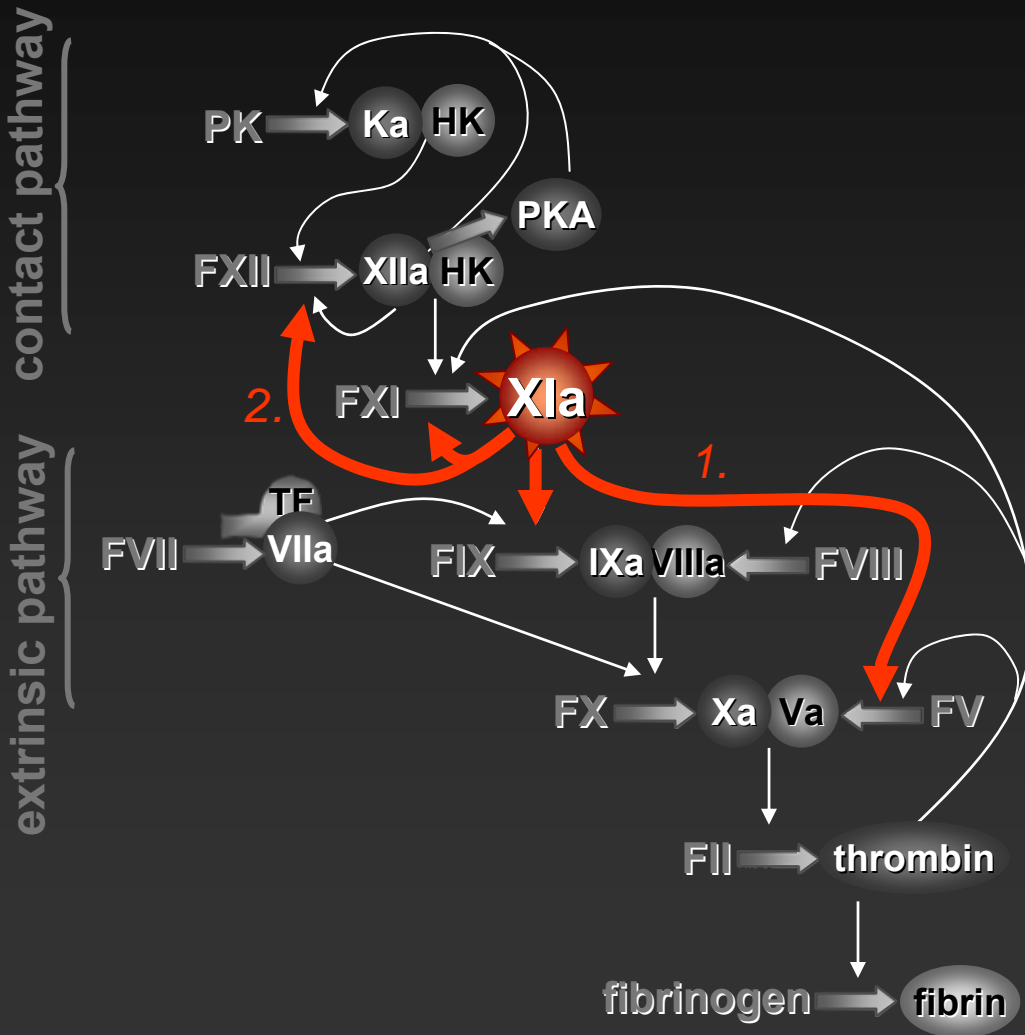
	NPP	XII (-)	PK (-)	XI (-)	VII (-)	PAI1 (-)	IX (-)	VIII (-)	X (-)	V (-)
Inhibition of TAE lots	no	no	no	no	no	no	yes	yes	yes	yes

TGT without addition of CTI

Effect of single factor deficiency on procoagulant activity of implicated lots



Procoagulant involvement of Factor XIa

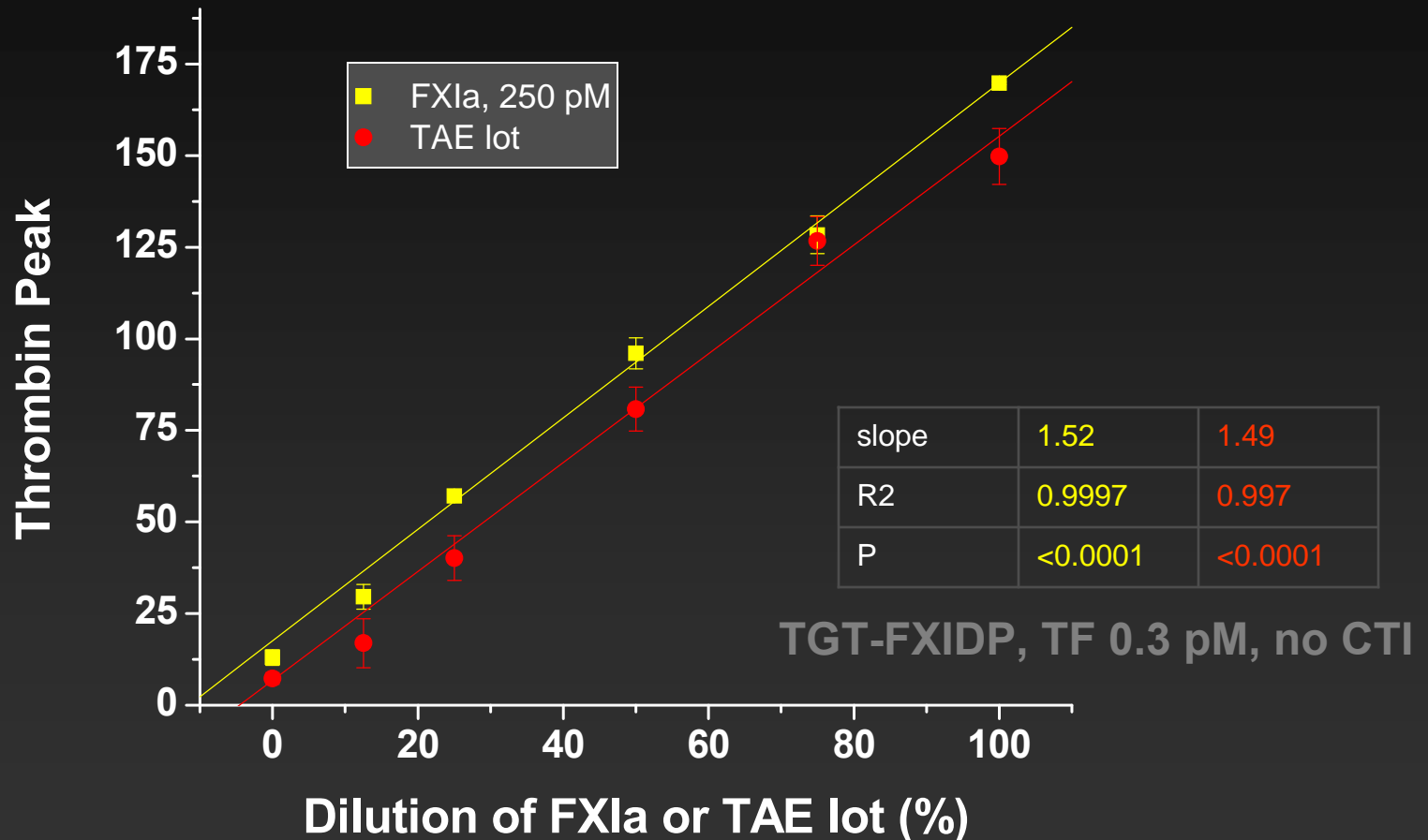


New FXIa activities:

1. Whelihan et al, 2010

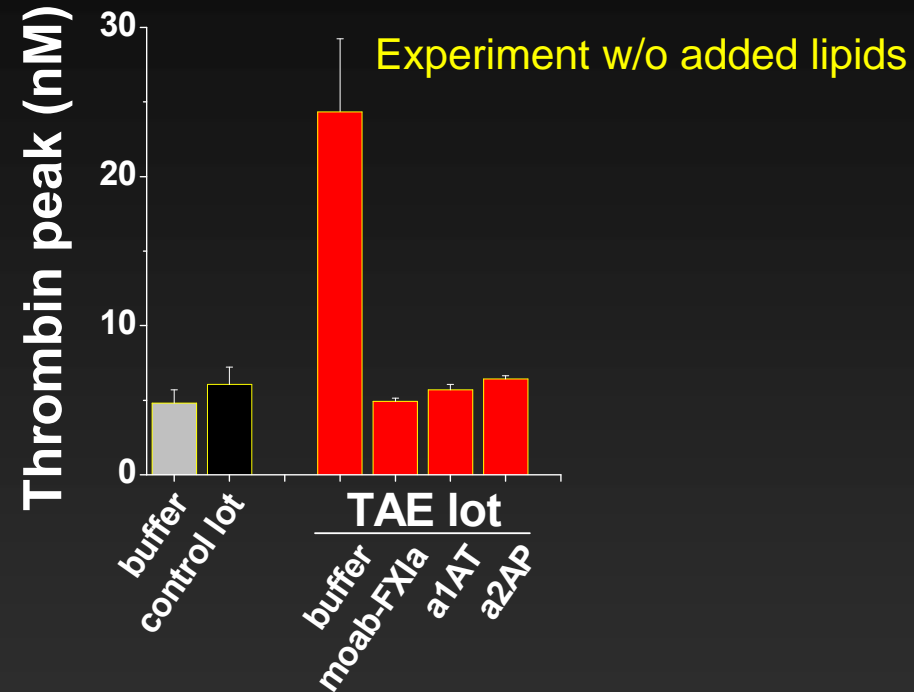
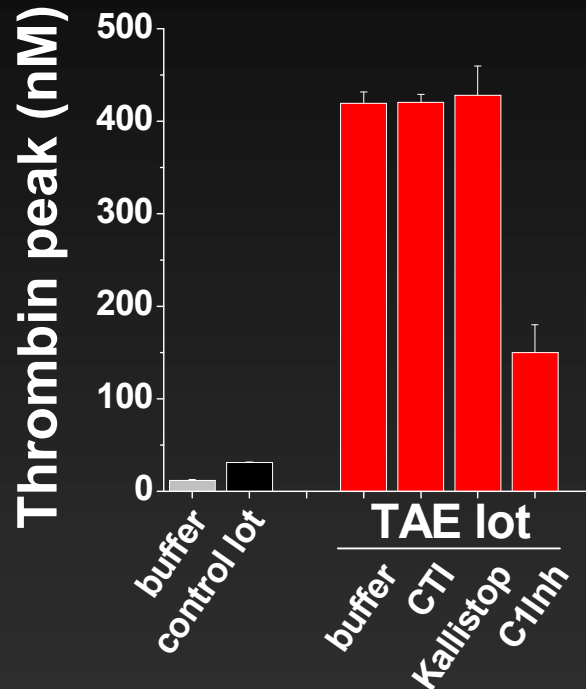
2. Matafonov et al, 2011

TGT response to Factor XIa and implicated lot



Similar results were obtained when FXIa was spiked into five different IGIV products

Pre-incubation of implicated lot with inhibitors of contact factors



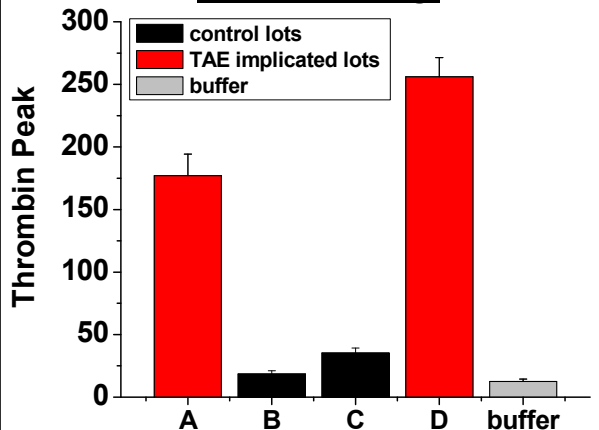
	CTI	KalliStop	C1-Inh	α 1AT	α 2AP	α -FXIa ab
FXIa			+	+	+	+
kallikrein		+	+	+		
FXIIa	+	+	+	+	-/+	
TAE lot: TGT-FXIDP	no	no	yes*	yes*	yes*	yes*
SN13a assay	no	partial	yes	n.t.	n.t.	no

*Inhibition was incomplete in the presence of lipids n.t. – not tested

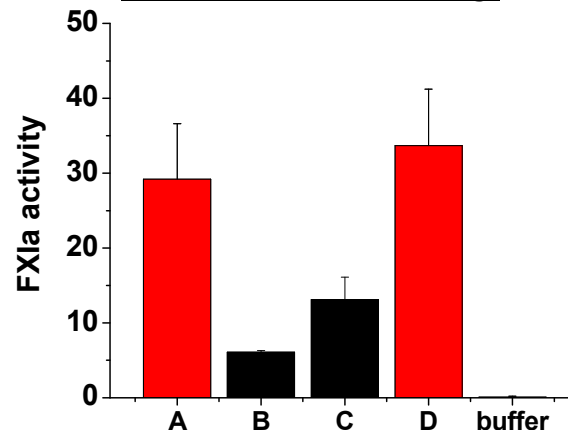
Correlation between TGT, Factor Xla activity and FXI:Ag

- TGT in FXI-deficient plasma
- Fluorogenic FXIa substrate SN13a
- FXI-ELISA (sensitive to FXI and FXIa)

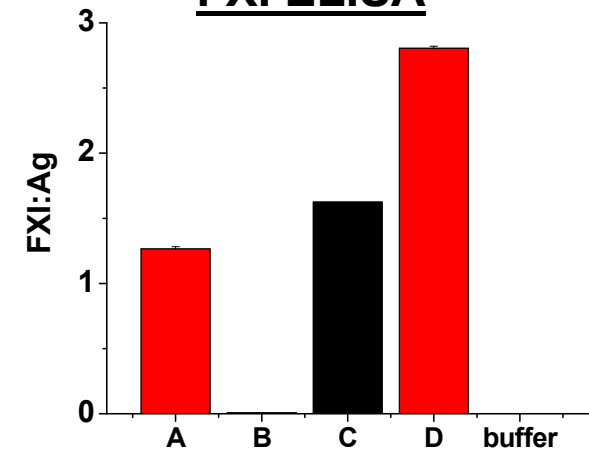
TGT assay



Factor Xla assay

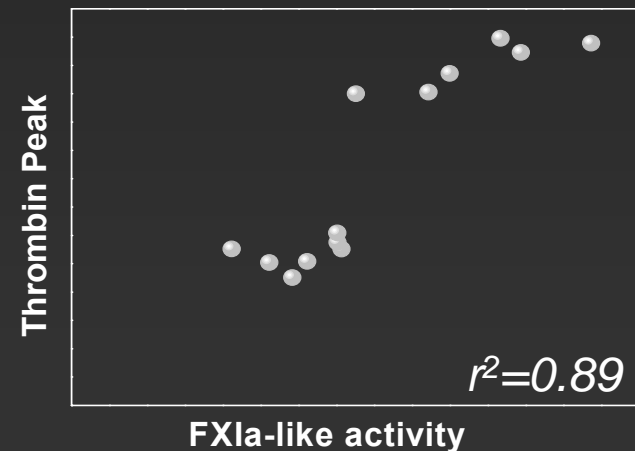
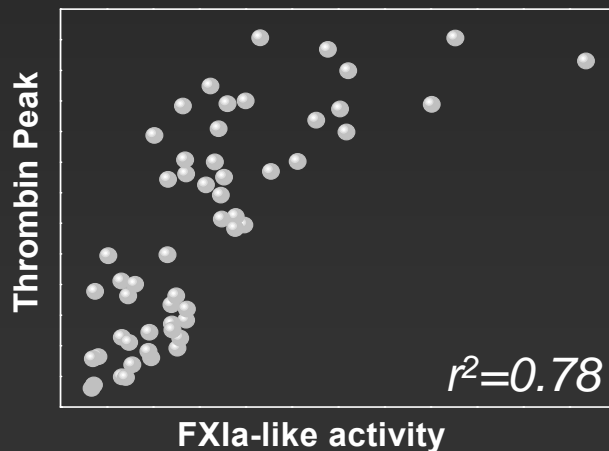
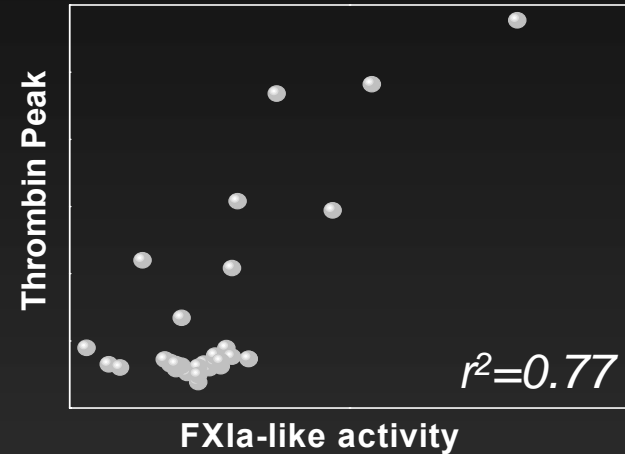
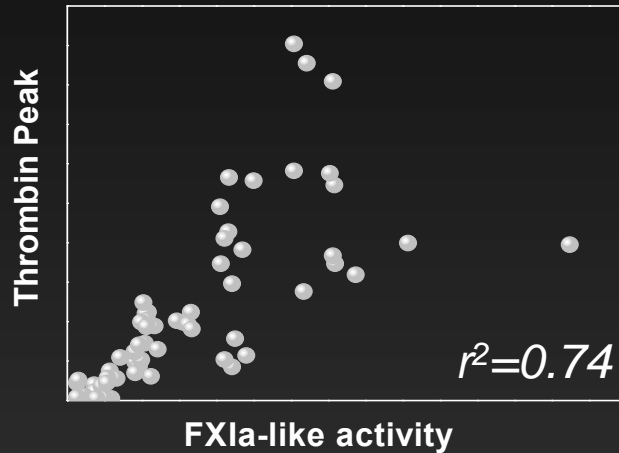


FXI ELISA



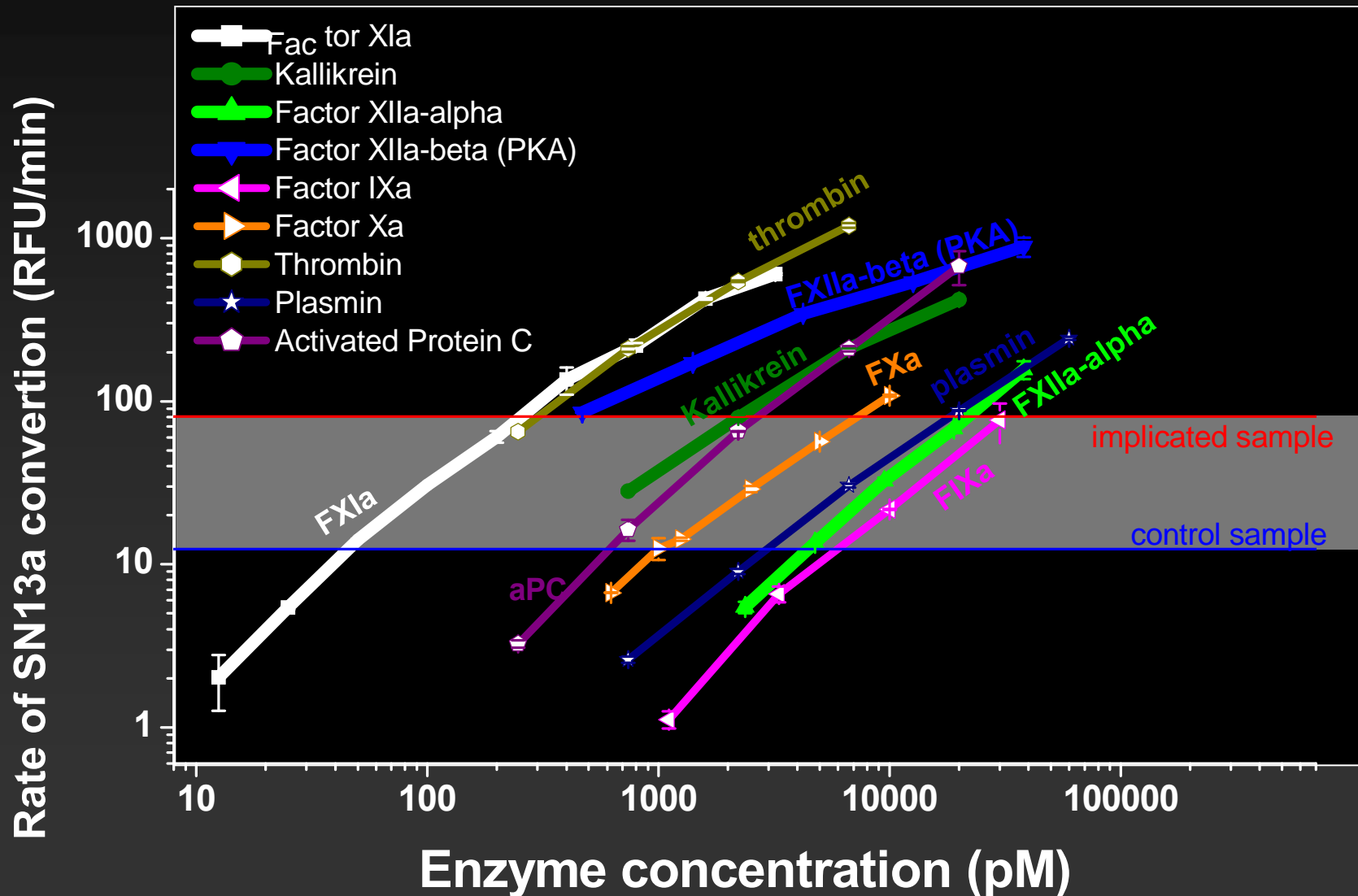
FXI antigen does not predict procoagulant activity

Correlation between TGT and FXIa in four products

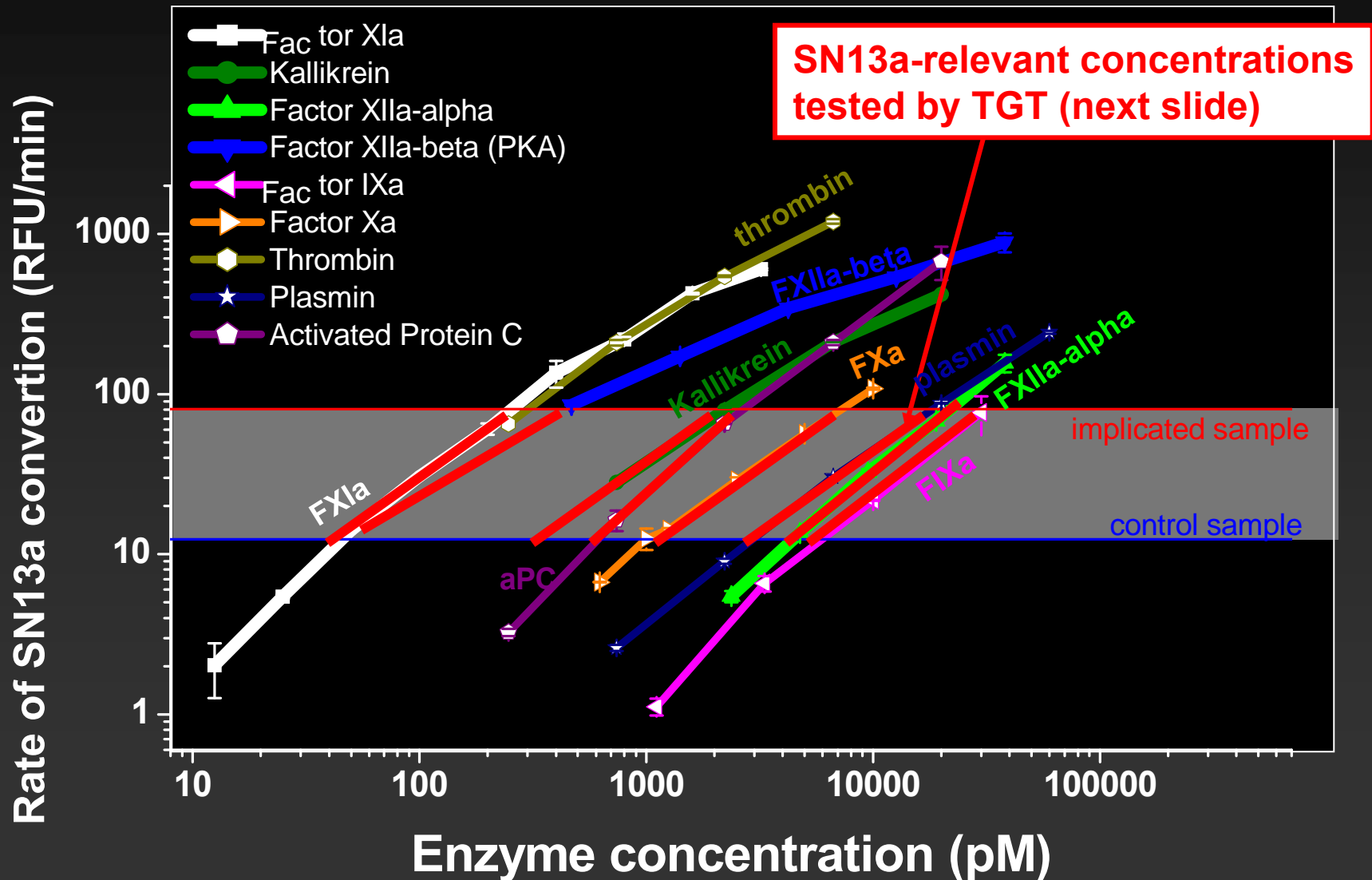


Note: Scales on each graph are different, i.e. products cannot be compared

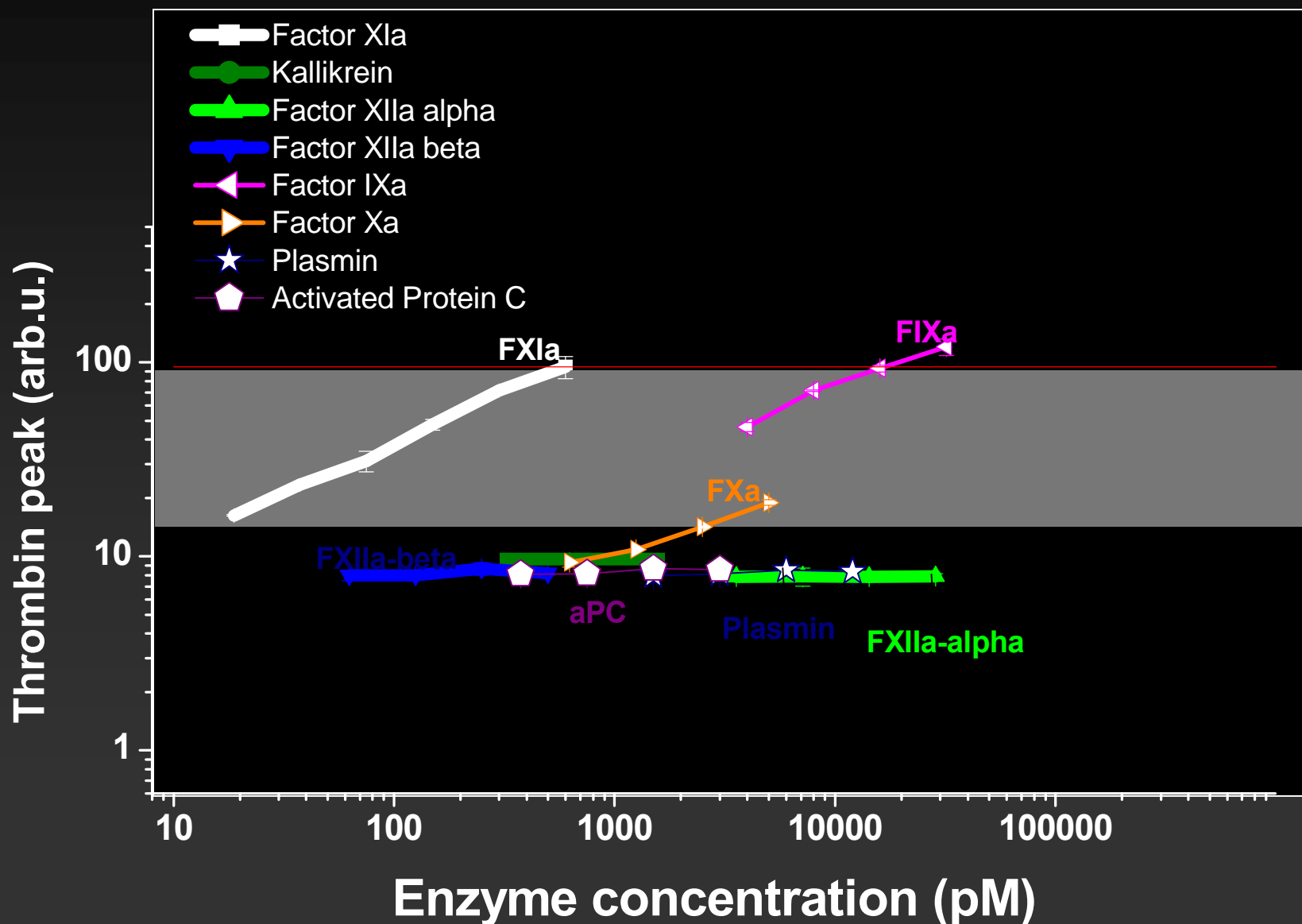
Specificity of FXIa substrate SN13a



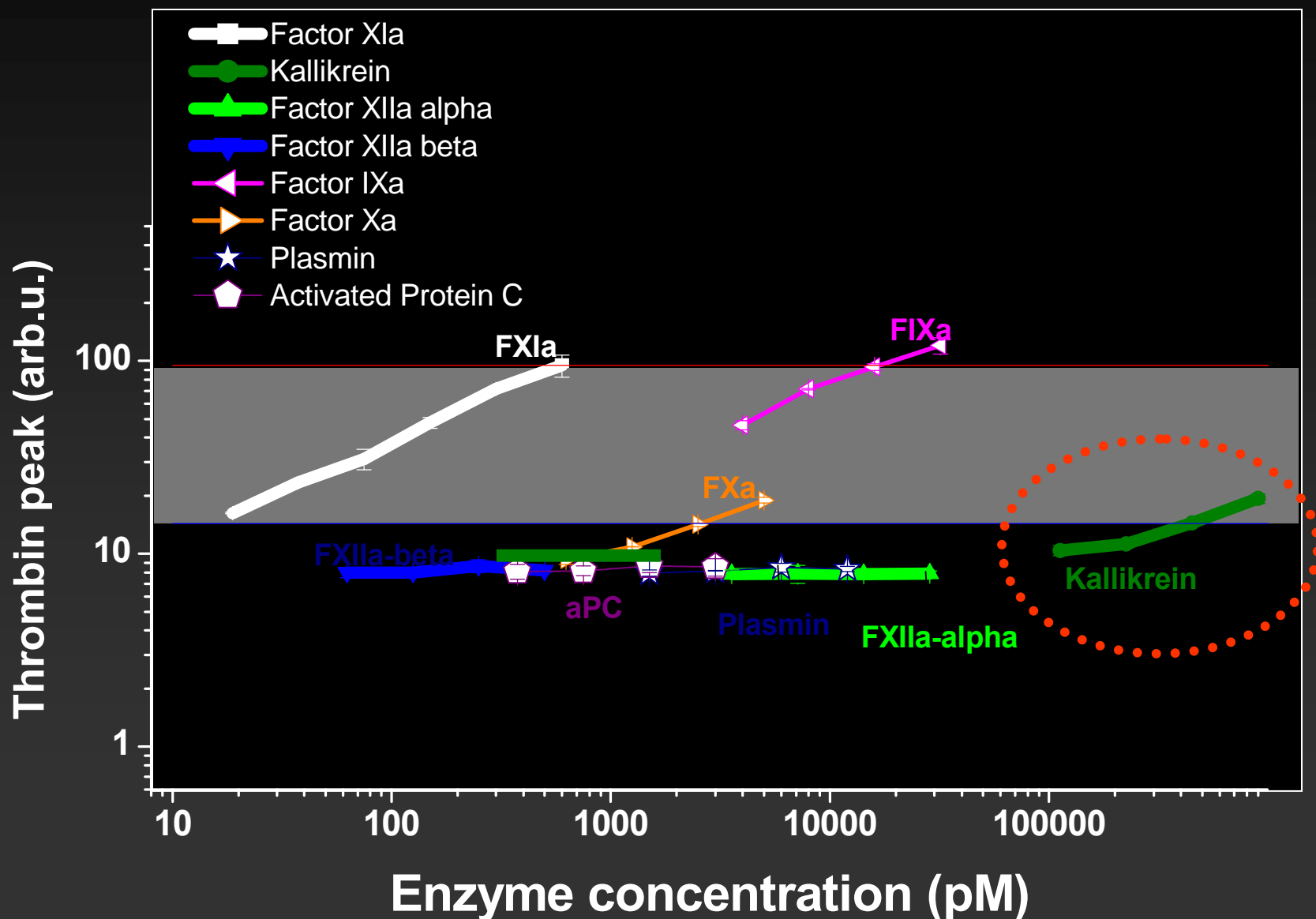
Specificity of FXIa substrate SN13a: 'relevant' enzyme concentrations



TGT on FXI def plasma is specific to FXIa (similar results in normal plasma)

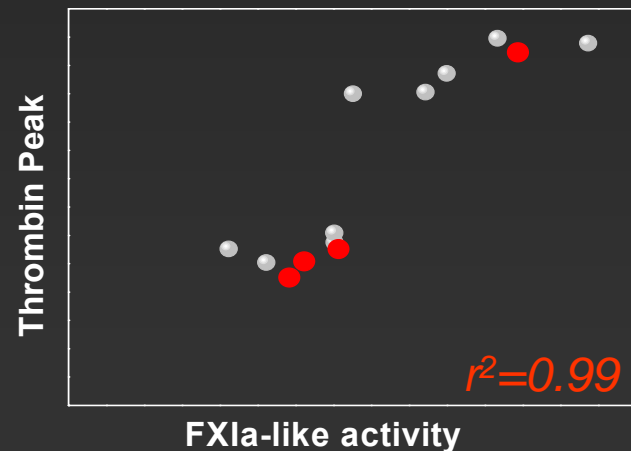
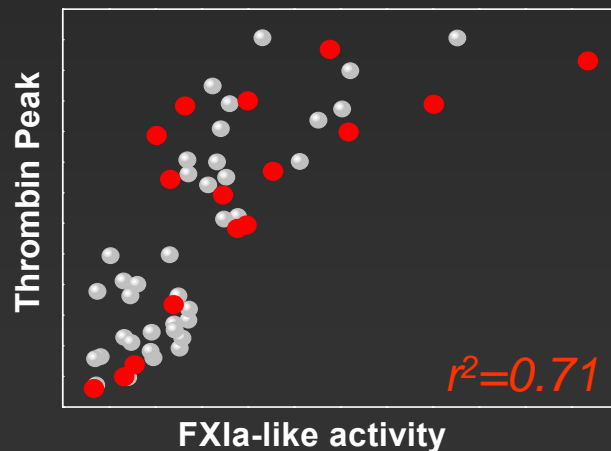
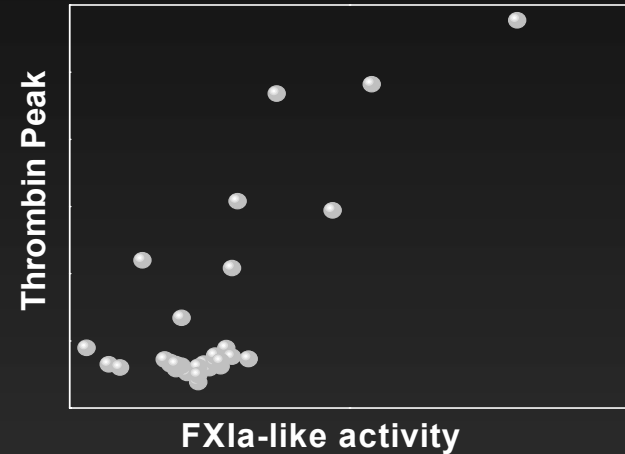
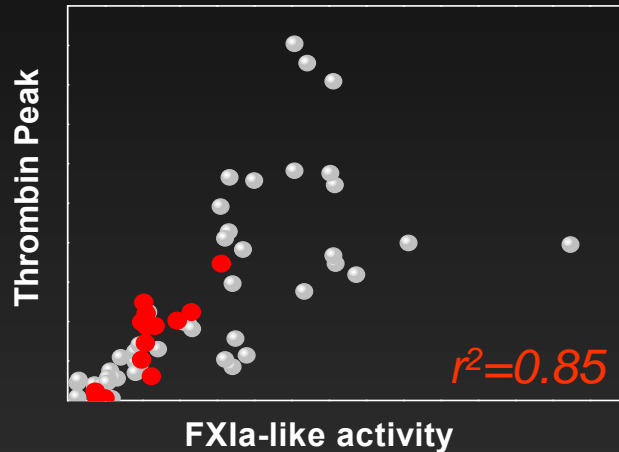


TGT on FXI def plasma is specific to FXIa (similar results in normal plasma)



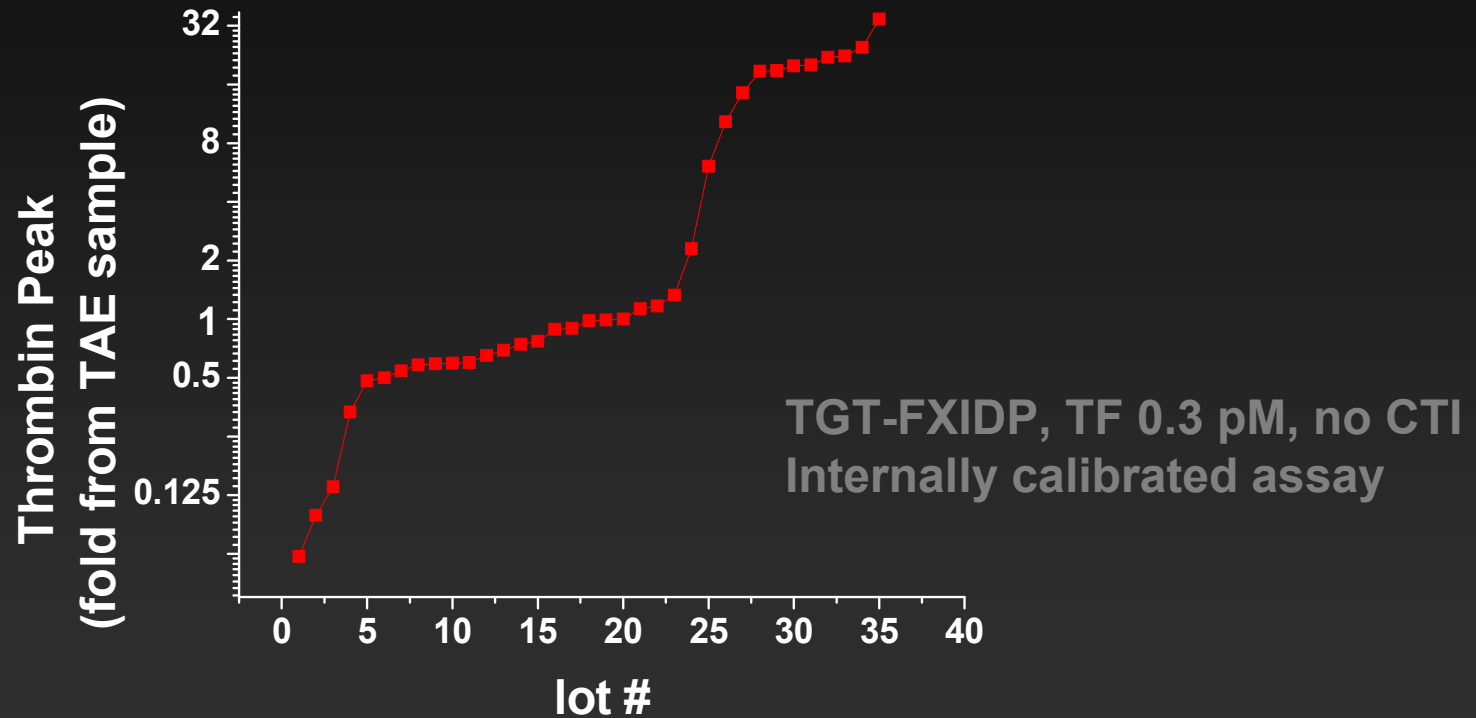
Relative activity of TAE lots: activity alone does not predict event

- Not implicated
- TAE implicated



Note: Scales on each graph are different, i.e. products cannot be compared

Common denominator among implicated lots: procoagulant activity



Limitations of our TAE lot analysis:

- Only small fraction of known TAE lots tested
- Data on lot-specific TAE case clusters not analyzed separately
- Effects of dose and other differences in patient populations

Conclusions

- FXIa is the primary procoagulant contaminant in IG products
- Other relatively non-procoagulant contaminants, e.g. kallikrein and FXI, are found in implicated lots
- Products have different FXIa activities which is likely due to variations in manufacturing
- Many TAE-implicated lots have high FXIa and TGT activities
- Procoagulant activity alone does not predict reported TAEs, i.e. host factors are likely important

Acknowledgments

Lab

Testing

Samuel Woodle (*postbac*)

Alexey Shibeko (*postdoc*)

Samples

Christine Anderson

Mei-ying Yu

OBRR

Dorothy Scott

Basil Golding

Ginette Michaud

Jay Epstein

Manufacturers

**who provided samples
for testing**