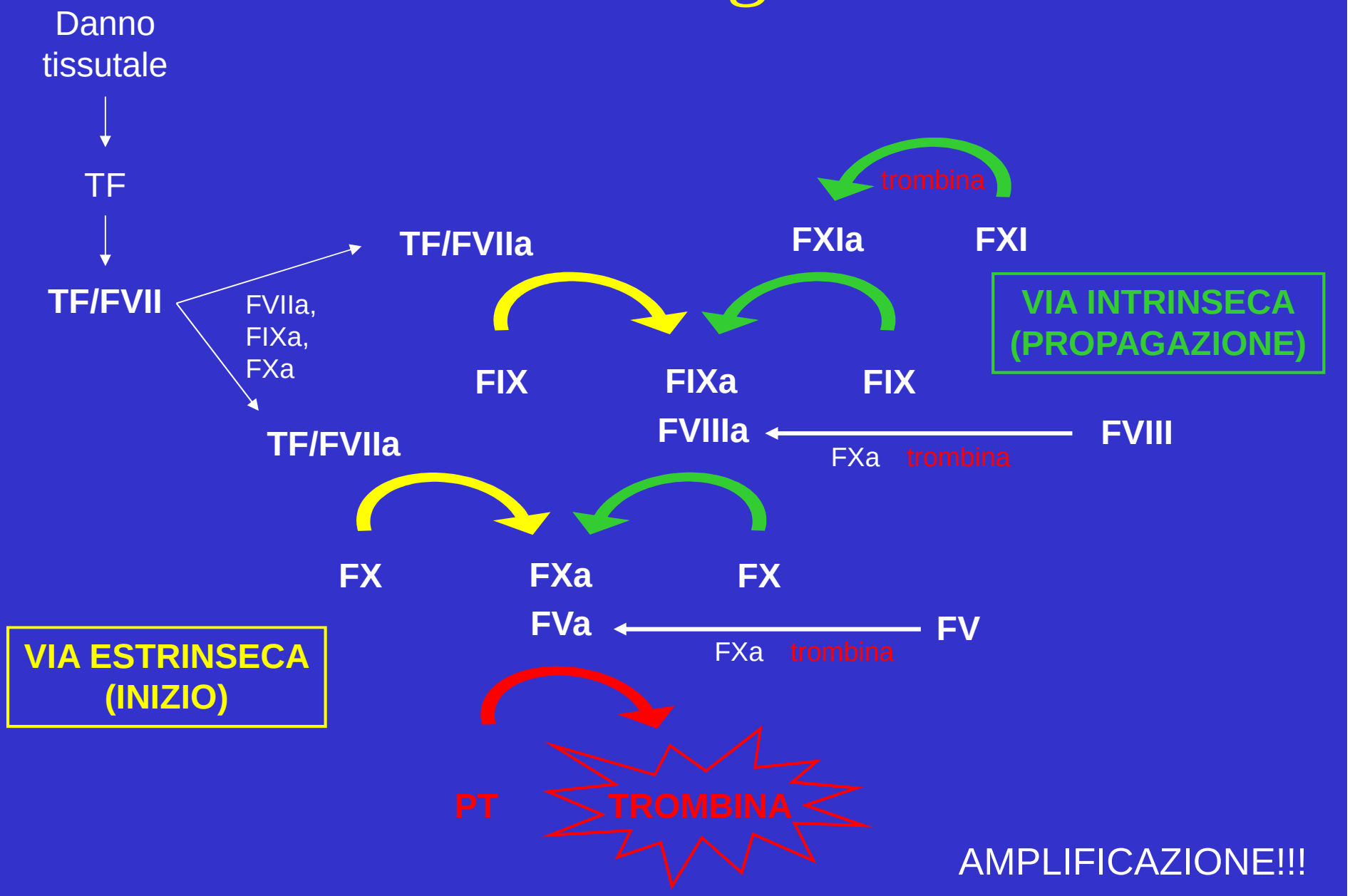
Coagulazione del sangue

- Attivazione sequenziale di proteine (fattori della coagulazione) circolanti in forma inattiva
- Formazione di un reticolo di fibrina che intrappola gli elementi corpuscolati del sangue

Fattori della coagulazione

- Zimogeni/serin-proteasi: FVII, FIX, FX, FXI, PT
- Cofattori non enzimatici: FV e FVIII
- Proteina strutturale: fibrinogeno

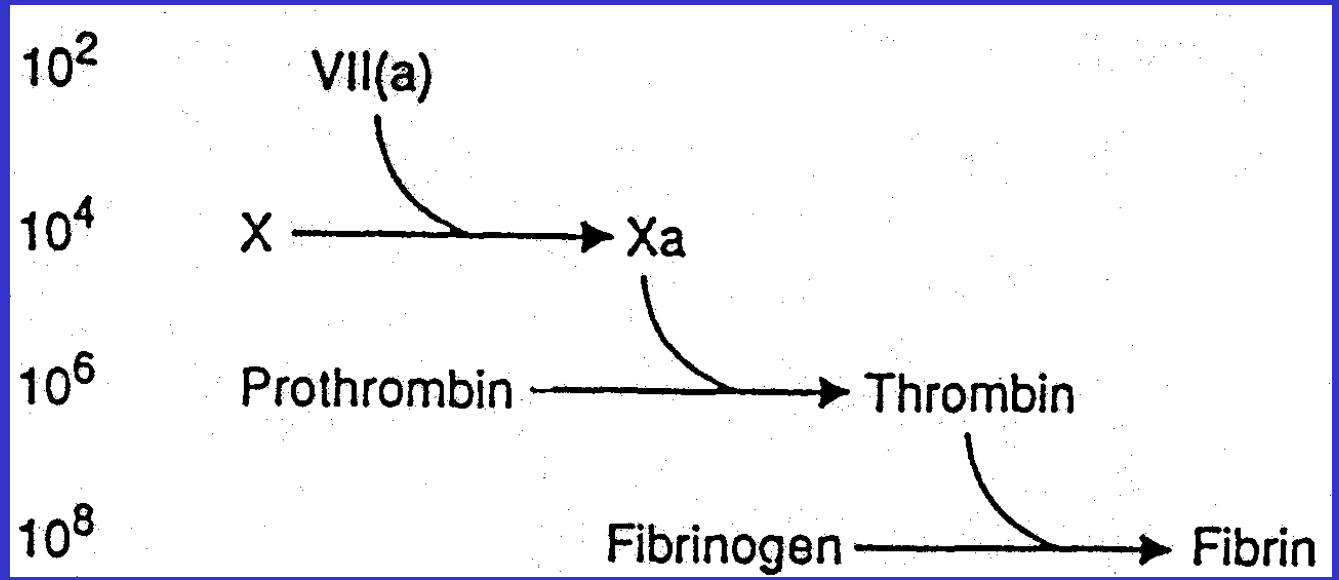
Cascata coagulativa



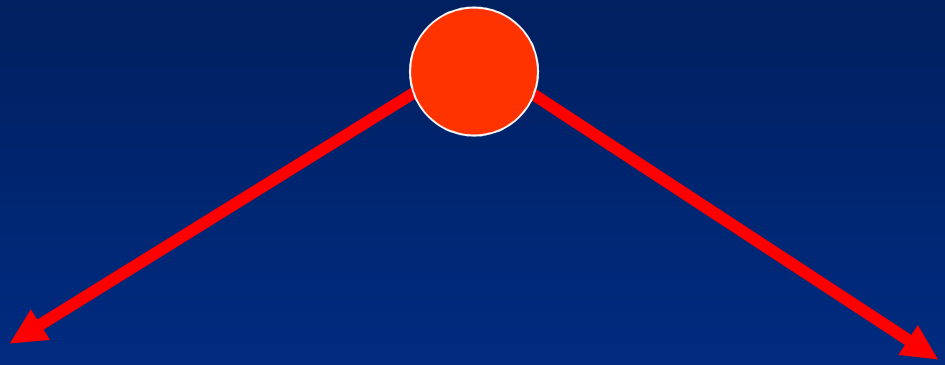
Fibrin clot

General Characteristics of the Coagulation Cascade

- The predominant mechanism illustrated in the activation pathway is that of an **enzyme amplification cascade**. One enzyme at the start of the activation pathway can activate many molecules of a given substrate



COAGULAZIONE



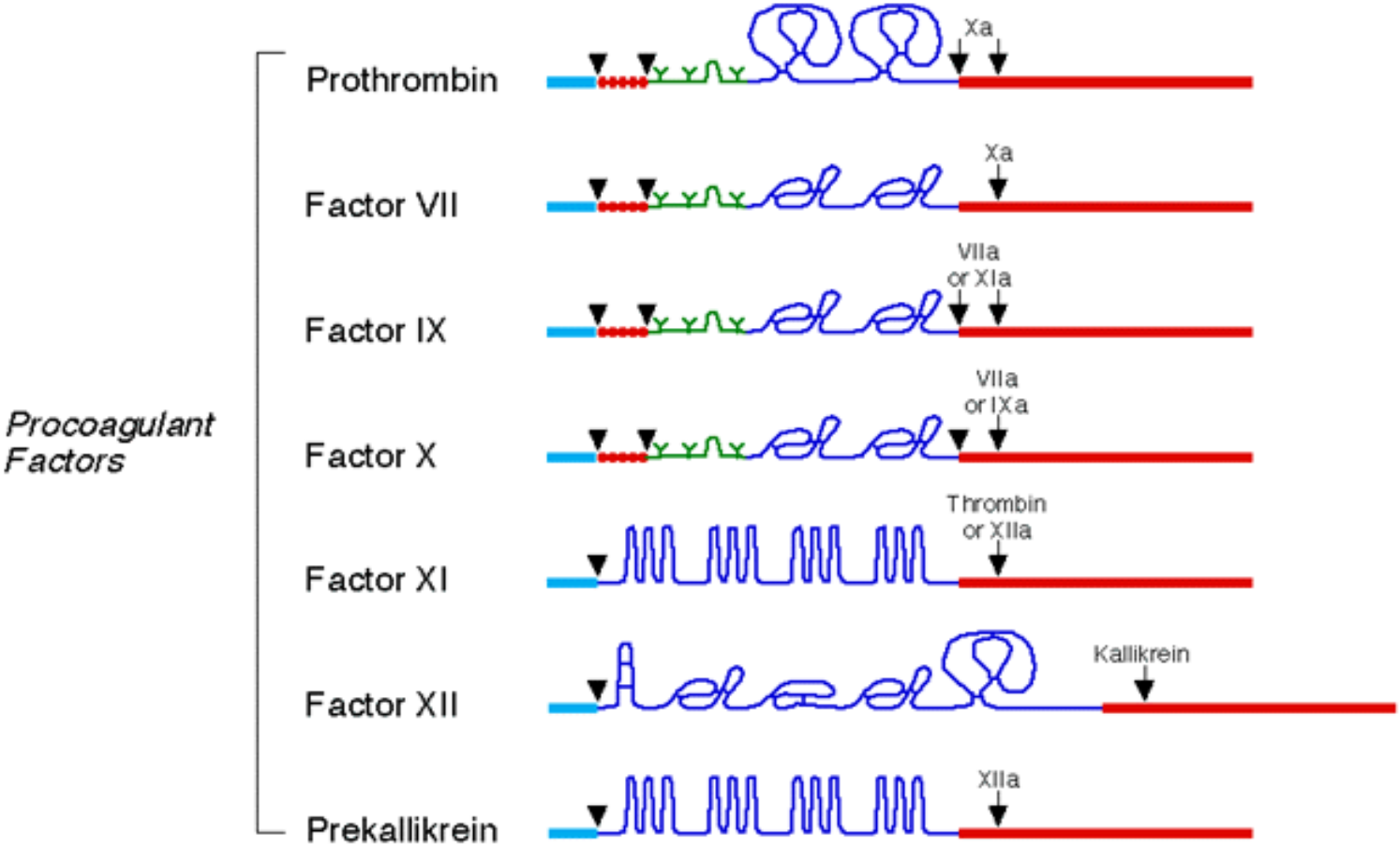
Fattori coagulativi

Inibitori fisiologici

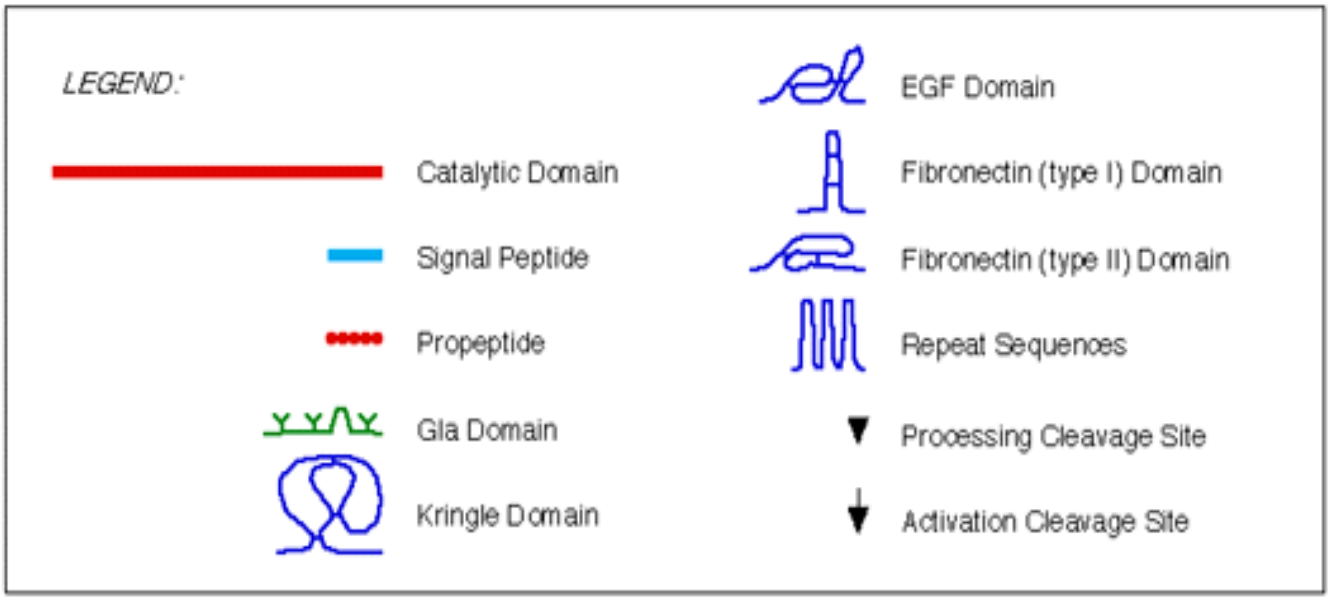
FATTORI COAGULATIVI

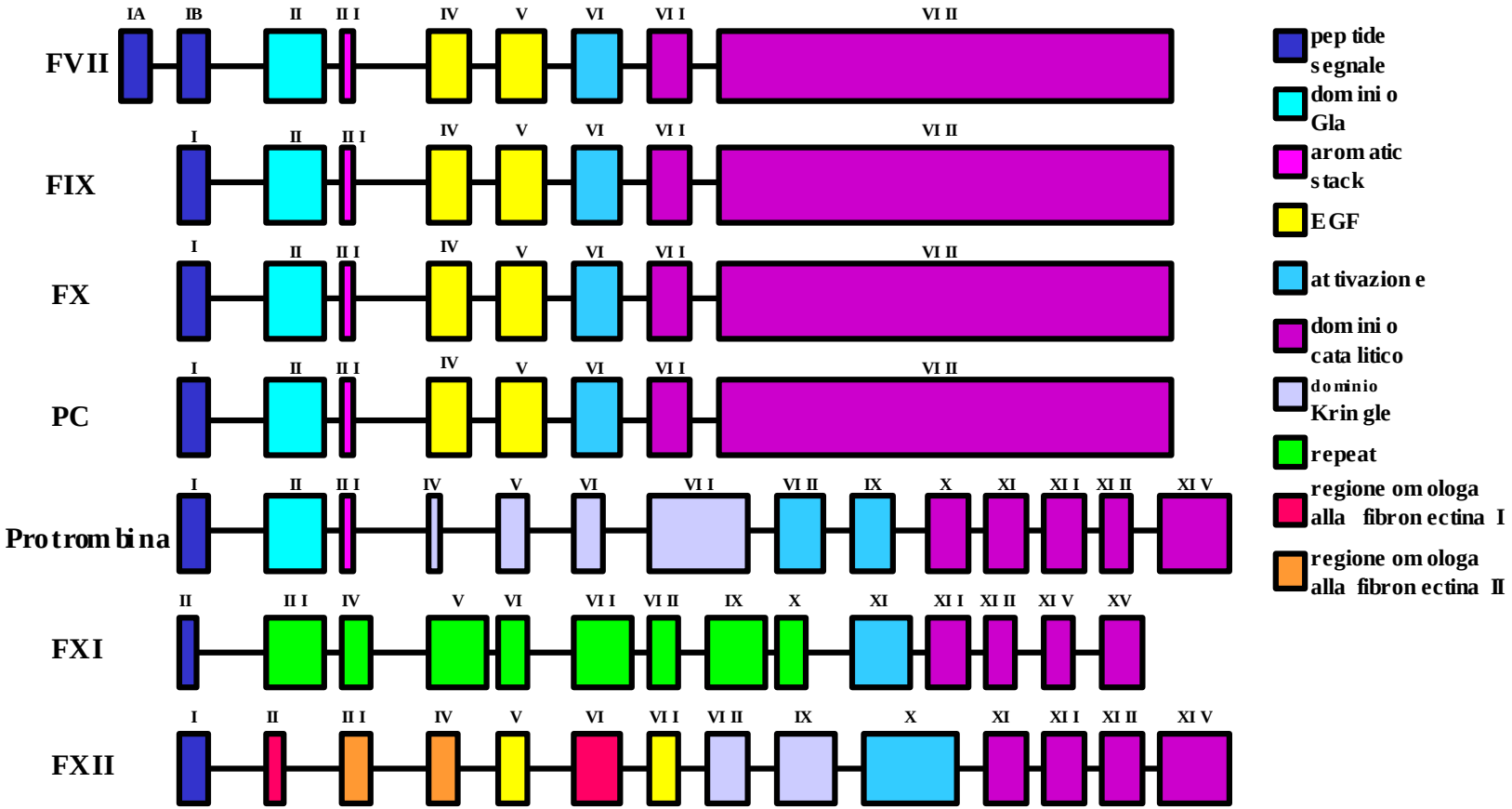
fattore	PM	Gene(Kb)	Cromos.	funzione
XIII	320,000			Stab.fib
XII	80,000	12	5	zimogeno
XI	160,000	23		zimogeno
IX	56,000	34	X	zimogeno
VII	50,000	13	13	zimogeno
VIII	330,000	185	X	cofat.
V	330,000	7,0	1	cofat.
X	56,000	22	13	cofat.
II	72,000	21	11	zimogeno
I	340,000		4	strut.

- **1- enzimi della coagulazione sono simili alle proteasi digestive tripsina e chimotripsina: proteasi seriniche**
  - **-dominio catalitico C-terminale: simile tripsina**
  - **dominio N-terminale: specifico**



Dominii proteici nei fattori della coagulazione





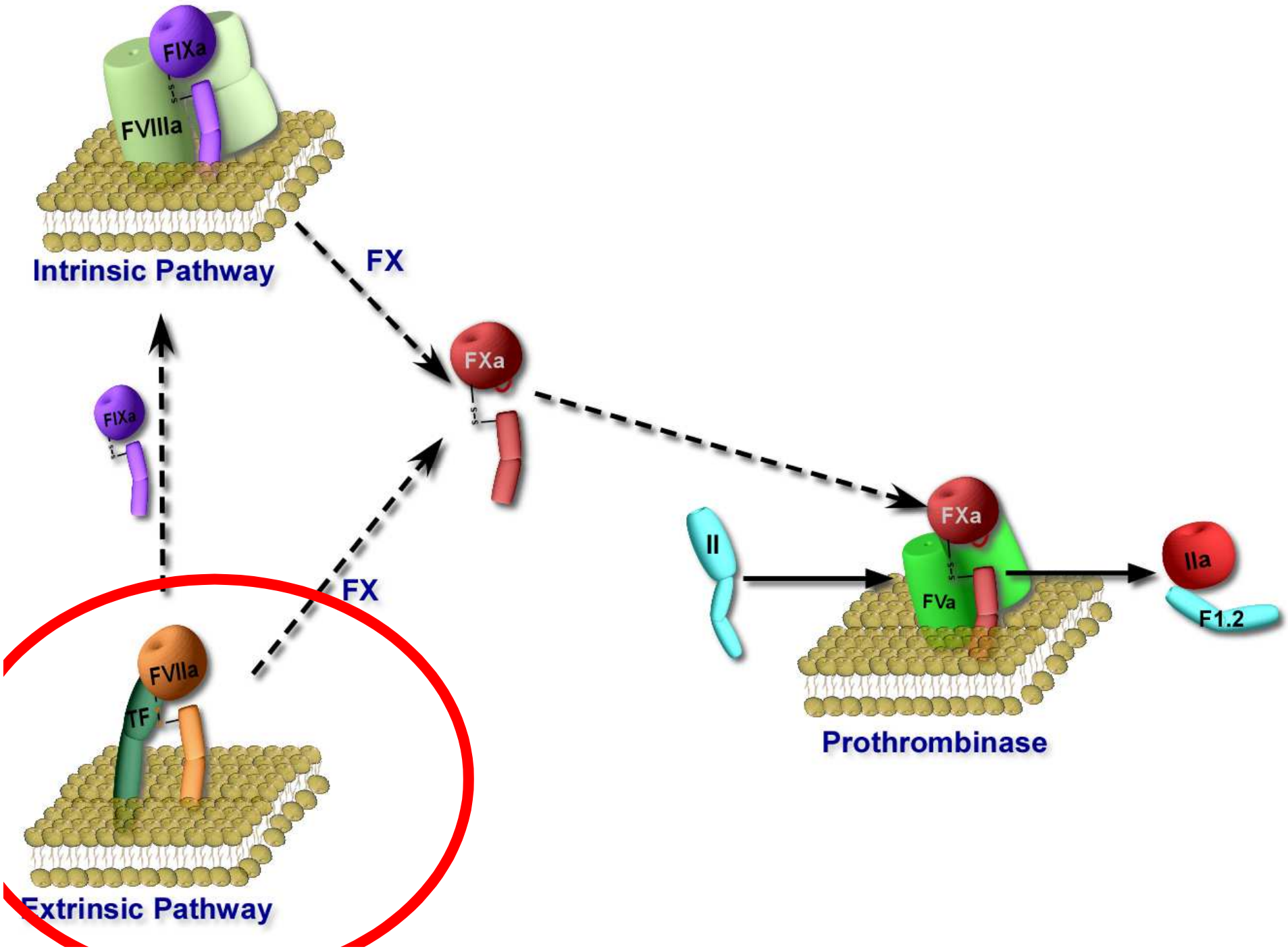
Struttura genica e proteica di serina proteasi della coagulazione

IL FATTORE TISSUTALE

- Proteina transmembrana che serve come recettore di superficie e cofattore essenziale per il FVIIa

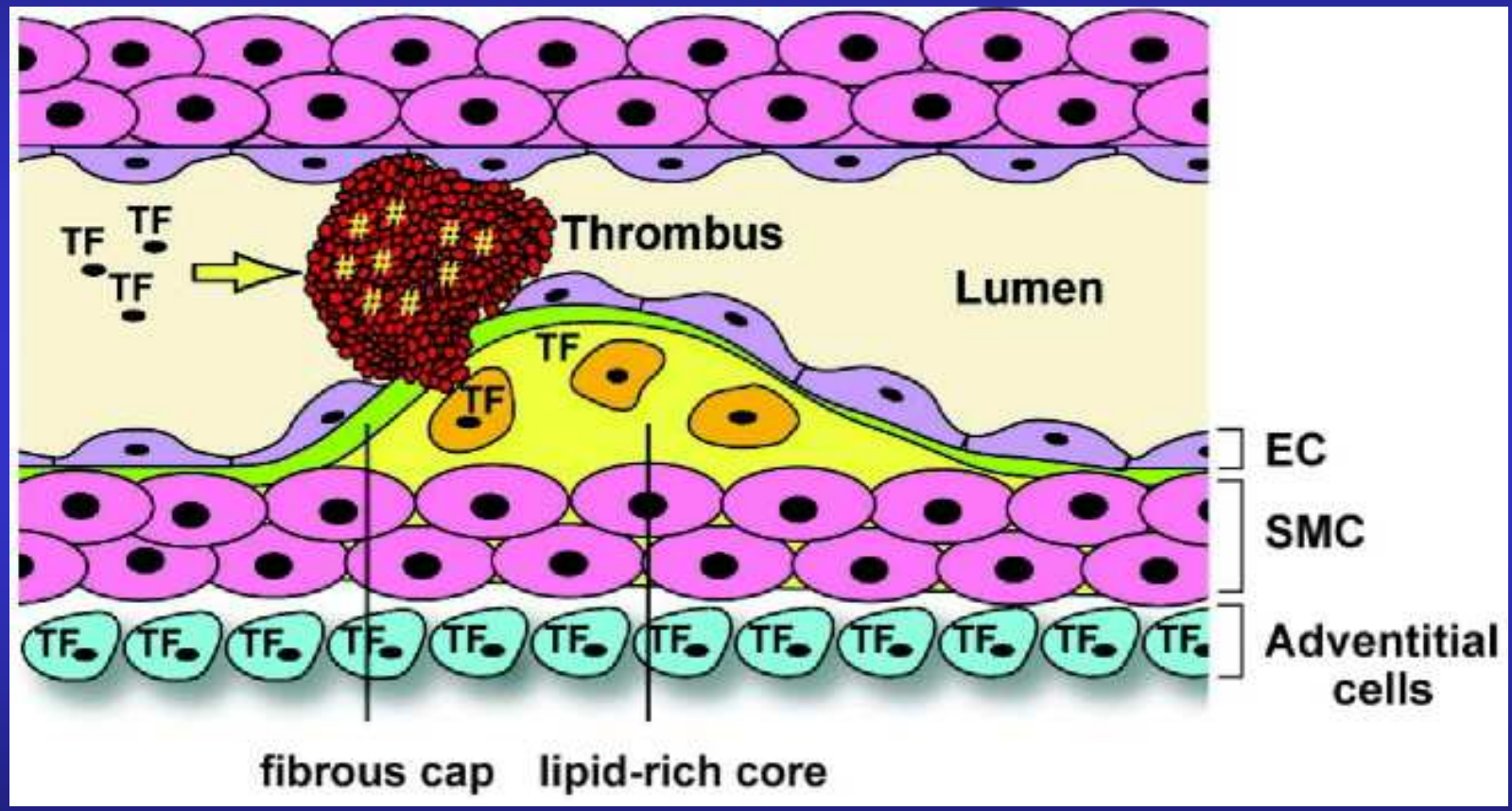
- Legandosi il TF al FVIIa il complesso TF-FVIIa acquista attività catalica e attiva i fattori X e IX

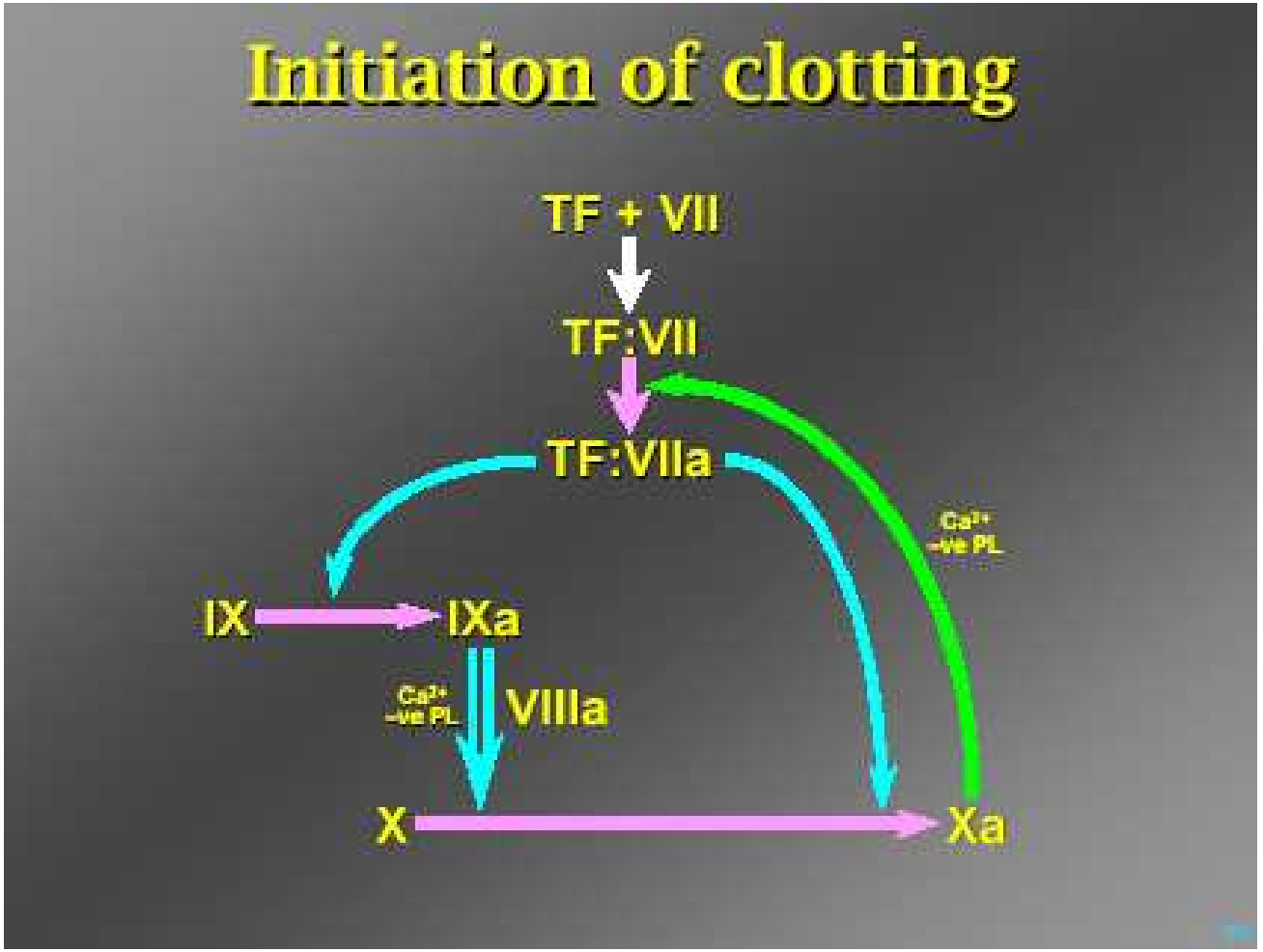
- Presente sulle membrane dei fibroblasti, cellule tumorali. *In condizioni normali non presente nella membrana di monociti e cellule endoteliali*, nelle quali però può essere espresso a seguito diversi stimoli

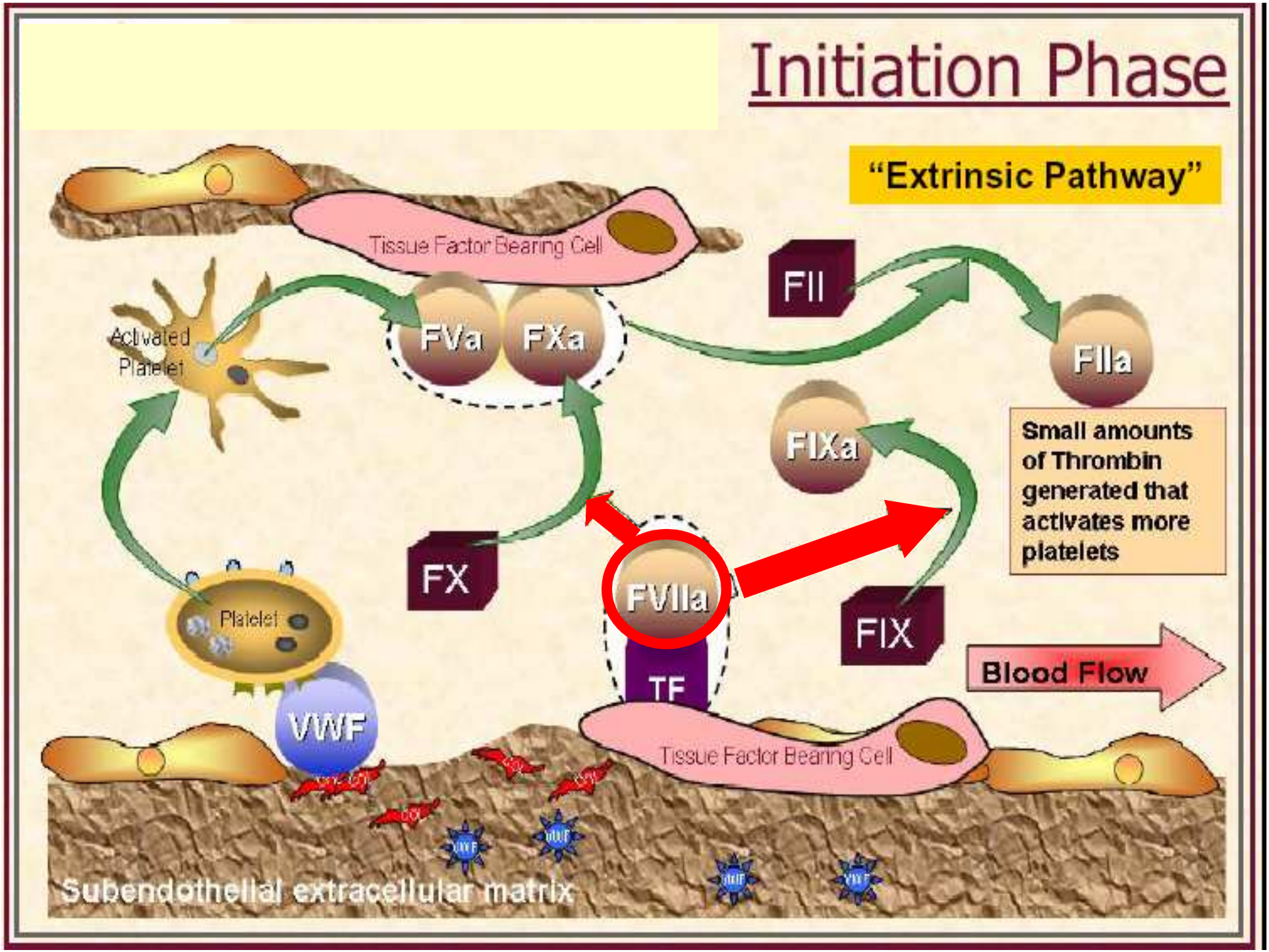

FIXa
FVIIIa
Intrinsic Pathway
FX
FXa
FIXa
FX
FVIIa
TF
Extrinsic Pathway
II
FXa
FVa
IIa
F1.2
Prothrombinase

Agenti in grado di indurre l'espressione di TF sulle cellule endoteliali

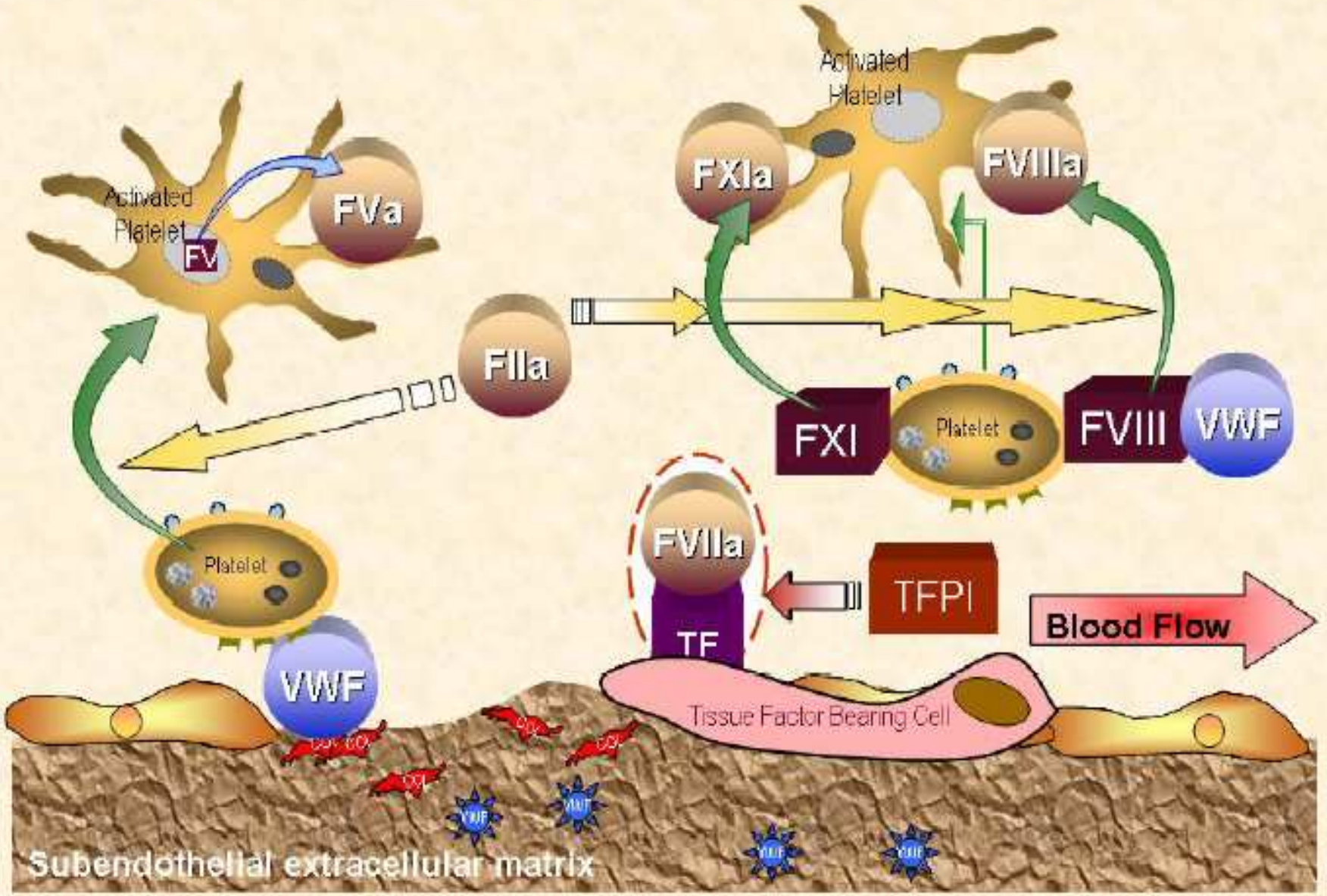
- Virus, batteri
- Endotossine
- Complessi immuni
- Proteina C reattiva
- Interleuchine (IL-1, TNF)
- Trombina


TF
TF
TF
Thrombus
Lumen
TF
TF
EC
SMC
TF
Adventitial cells
fibrous cap
lipid-rich core

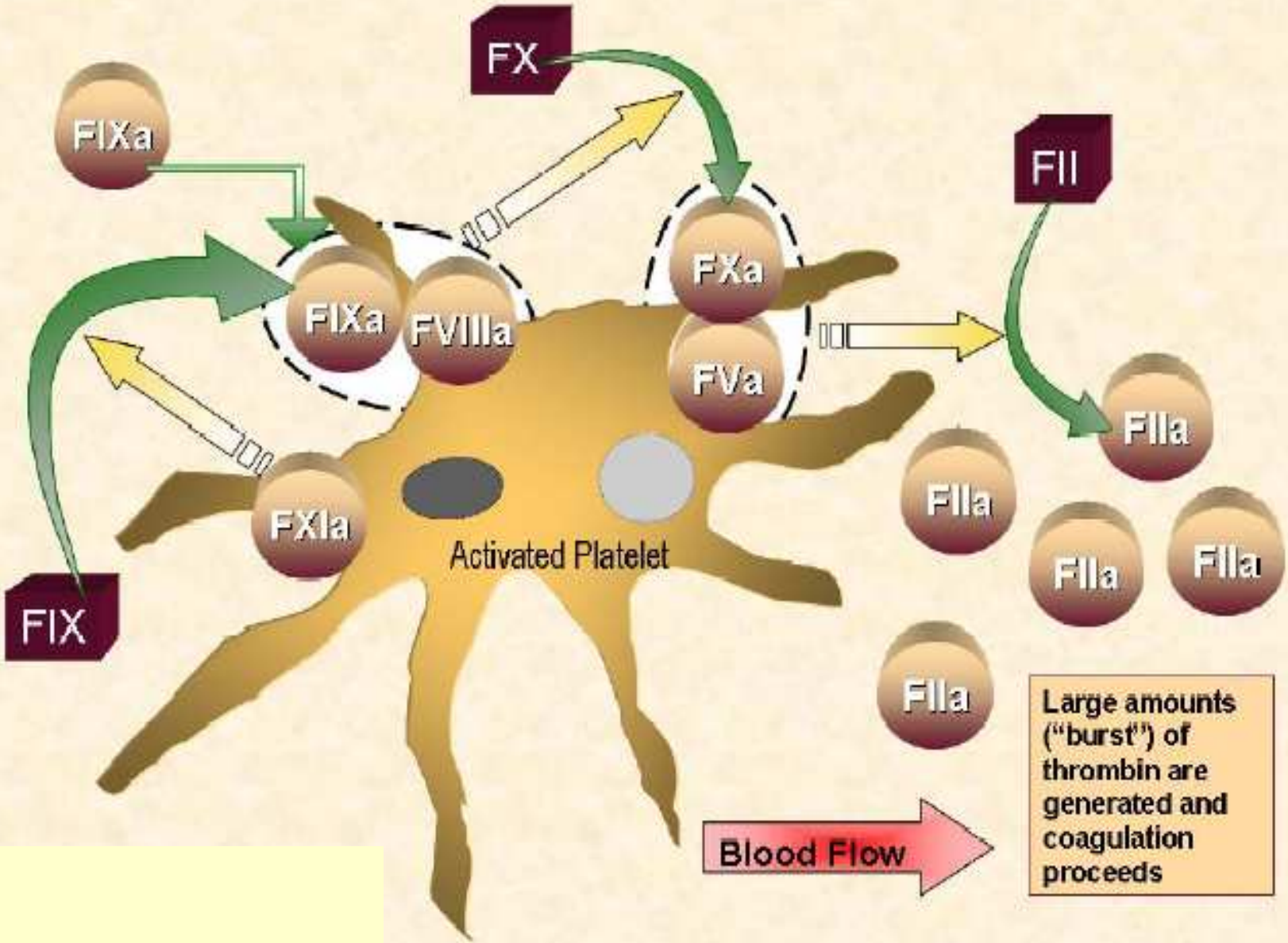

Initiation of clotting
TF + VII
TF:VII
TF:VIIa
Ca2+
-ve PL
IX
IXa
Ca2+
-ve PL
VIIIa
X
Xa

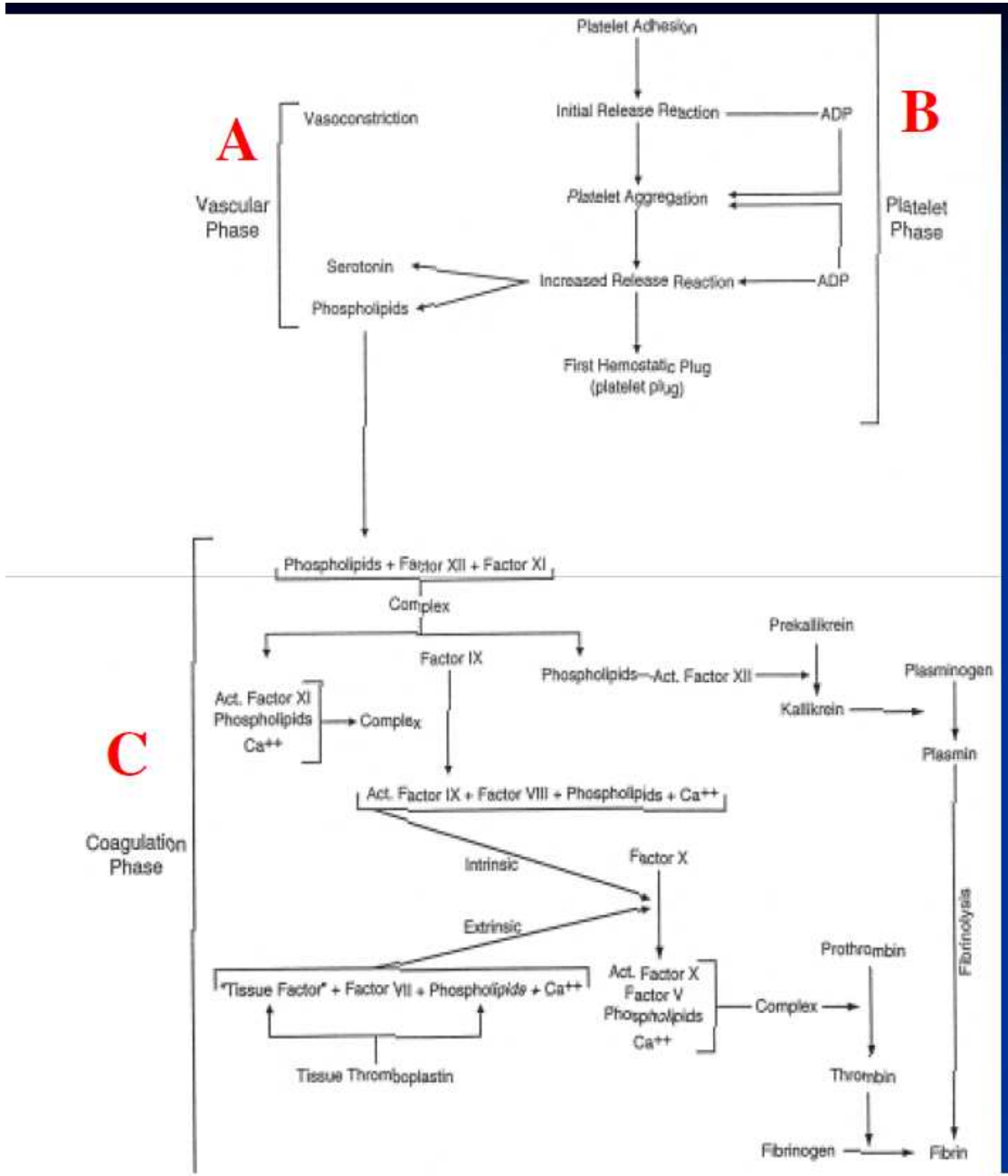

Initiation Phase
"Extrinsic Pathway"
Tissue Factor Bearing Cell
Activated Platelet
FVa
FXa
FII
FIIa
FIXa
Small amounts of Thrombin generated that activates more platelets
FX
FVIIa
FIX
Platelet
TF
Blood Flow
VWF
Tissue Factor Bearing Cell
Subendothelial extracellular matrix

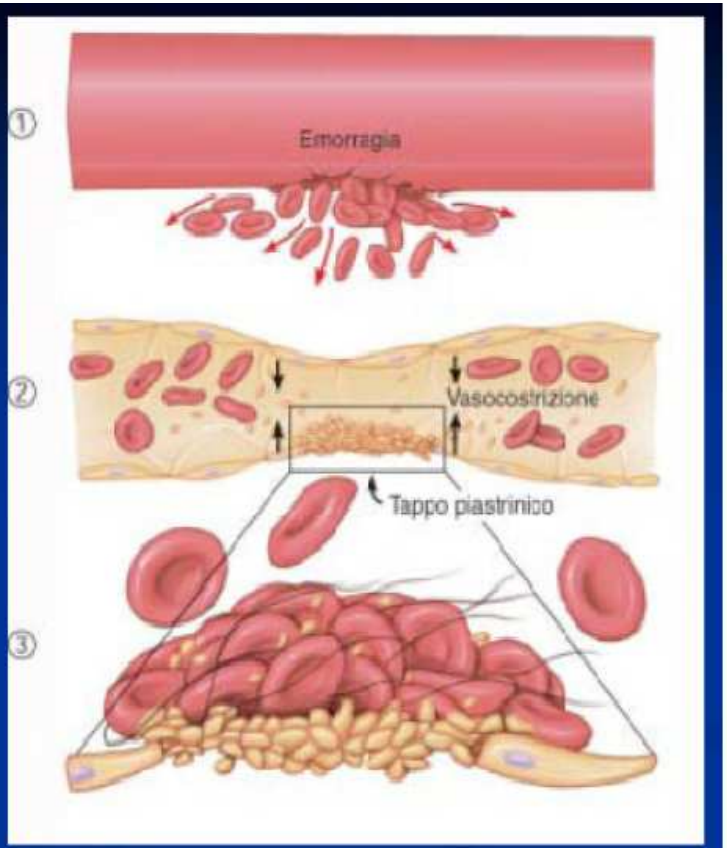
Amplification Phase



Propagation Phase

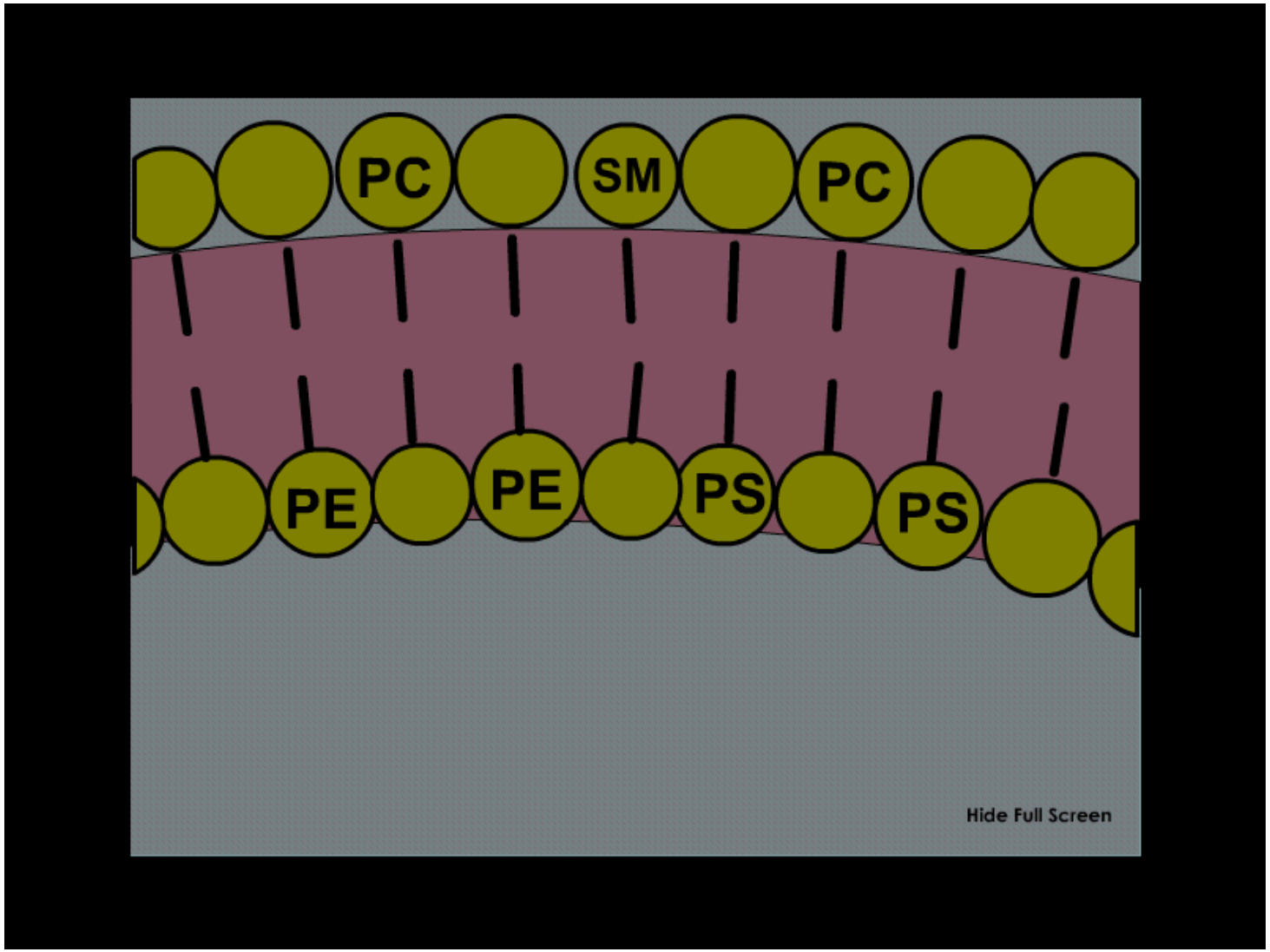



A
Vascular Phase
Vasoconstriction
Serotonin
Phospholipids
B
Platelet Phase
Platelet Adhesion
Initial Release Reaction
ADP
Platelet Aggregation
Increased Release Reaction
ADP
First Hemostatic Plug
(platelet plug)
C
Coagulation Phase
Phospholipids + Factor XII + Factor XI
Complex
Factor IX
Act. Factor XI
Phospholipids
Ca++
Complex
Act. Factor IX + Factor VIII + Phospholipids + Ca++
Intrinsic
Extrinsic
"Tissue Factor" + Factor VII + Phospholipids + Ca++
Tissue Thromboplastin
Factor X
Act. Factor X
Factor V
Phospholipids
Ca++
Complex
Prothrombin
Thrombin
Fibrinogen
Fibrin
Prekallikrein
Phospholipids
Act. Factor XII
Kallikrein
Plasminogen
Plasmin
Fibrinolysis


1
Emorragia
2
Vasocostrizione
Tappo piastrinico
3


4

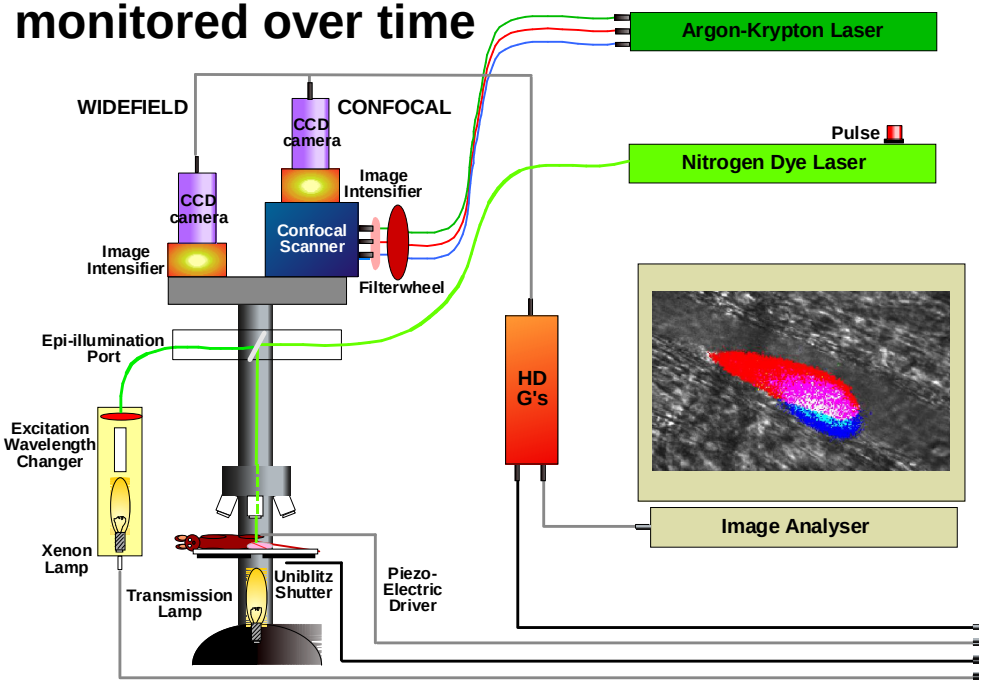
- Il tappo piastrinico contiene una striscia fosfolipidica ideale per l'attivazione della coagulazione

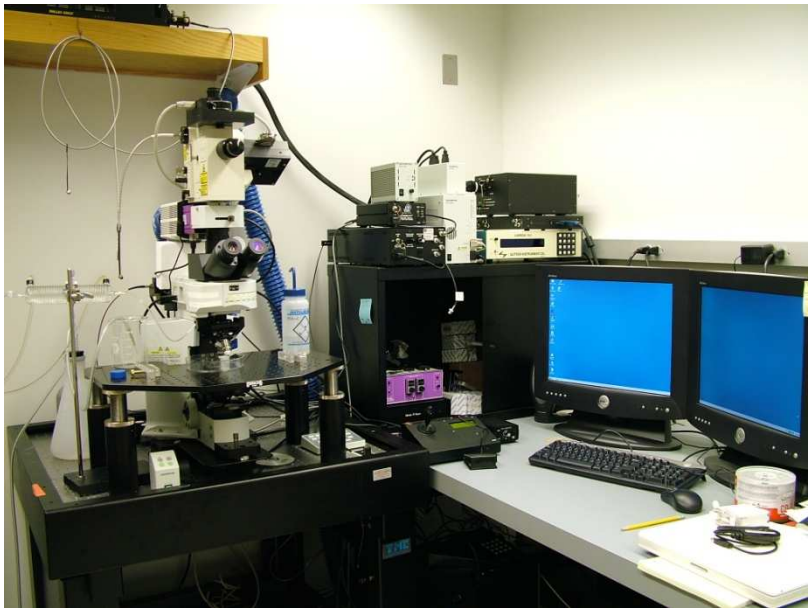


Platelet adhesion/activation
and coagulation
are strongly interconnected

Real-time Observation of Thrombus Formation: Cremaster laser injury model

- **Preparation of mouse-isolate cremaster muscle**
- **Injection of antibodies** (Alexa-555 anti-CD41;GBIIb/IIIa-Platelet recognition and **Alexa-488 anti-Fibrin**)
- **Laser injury to cremaster artery—heat injury to vessel wall**
- **Accumulation of labeled platelets and fibrin deposition (clot formation) is monitored over time**





Real-time *in vivo* imaging of thrombus formation

Platelets
Fibrin

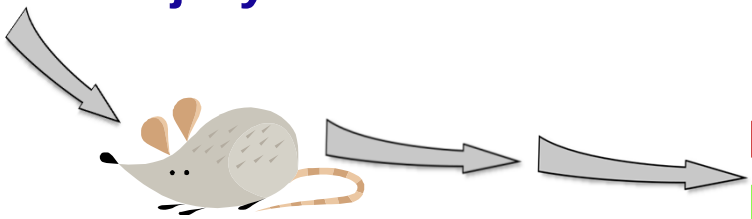
4x speed

WT littermate: 6-8 weeks

4x speed

HB, Balb/C: 6-8 weeks
Dose: 90 µg/kg FXa^{I16L}

Vessel Injury

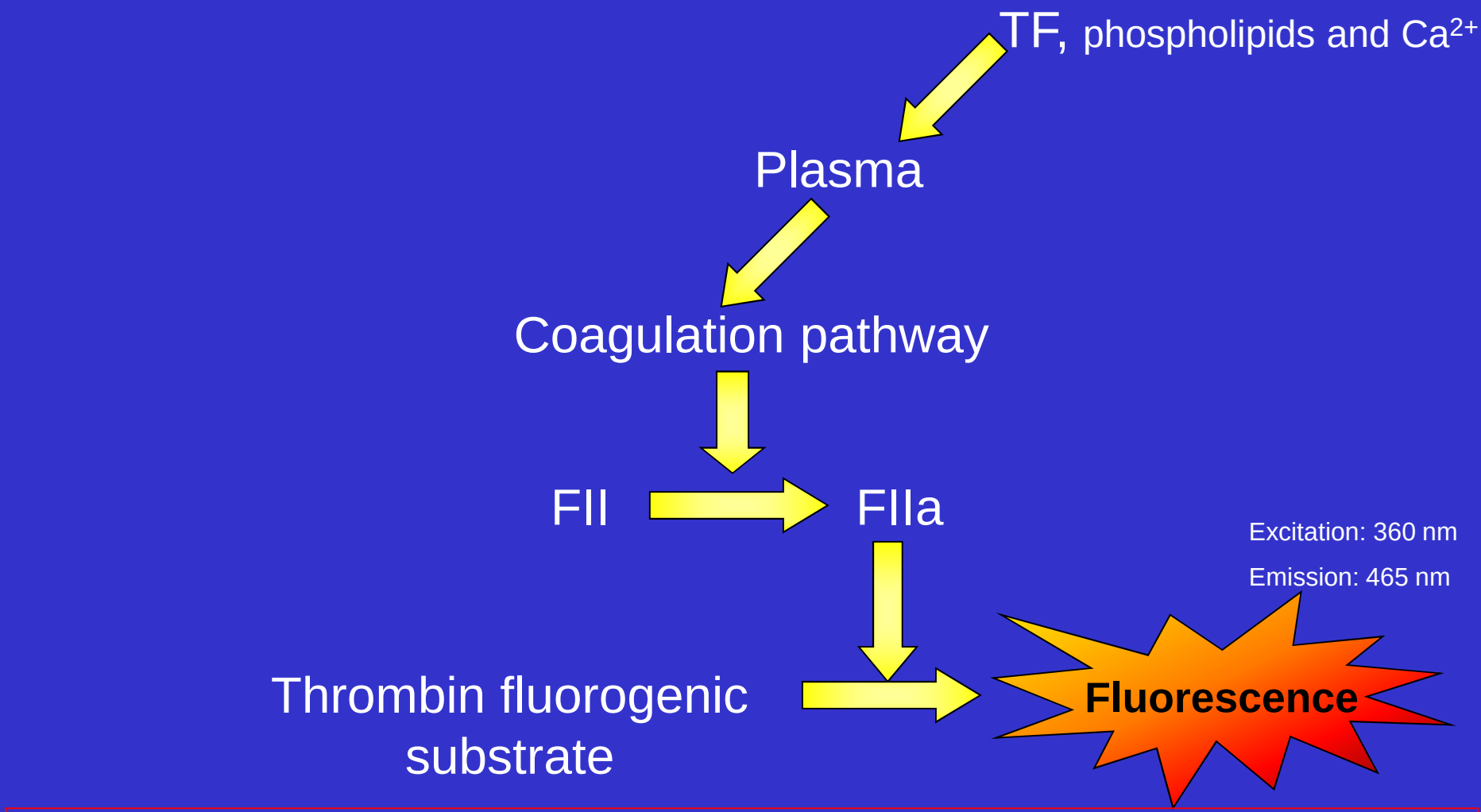


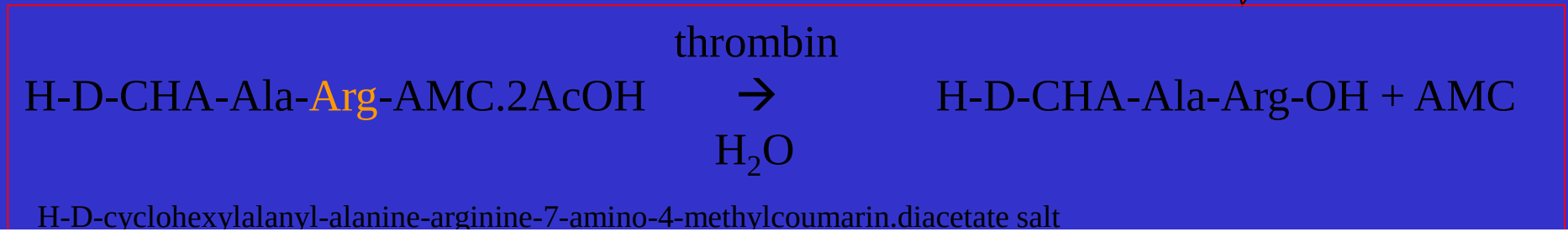
Platelet accumulation
Fibrin deposition
(at old injury site)

Possiamo monitorare l’andamento di una reazione a cascata così complessa??

USO DI SUBSTRATI CROMO e FLUOROGENICI

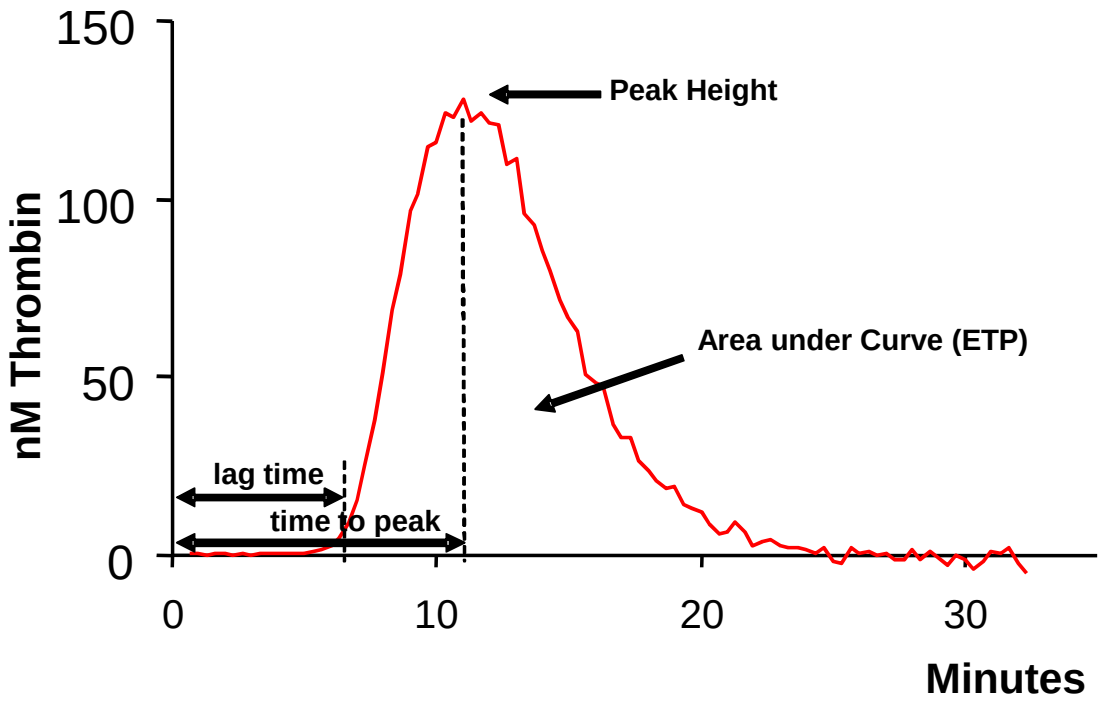
Thrombin Generation Assay



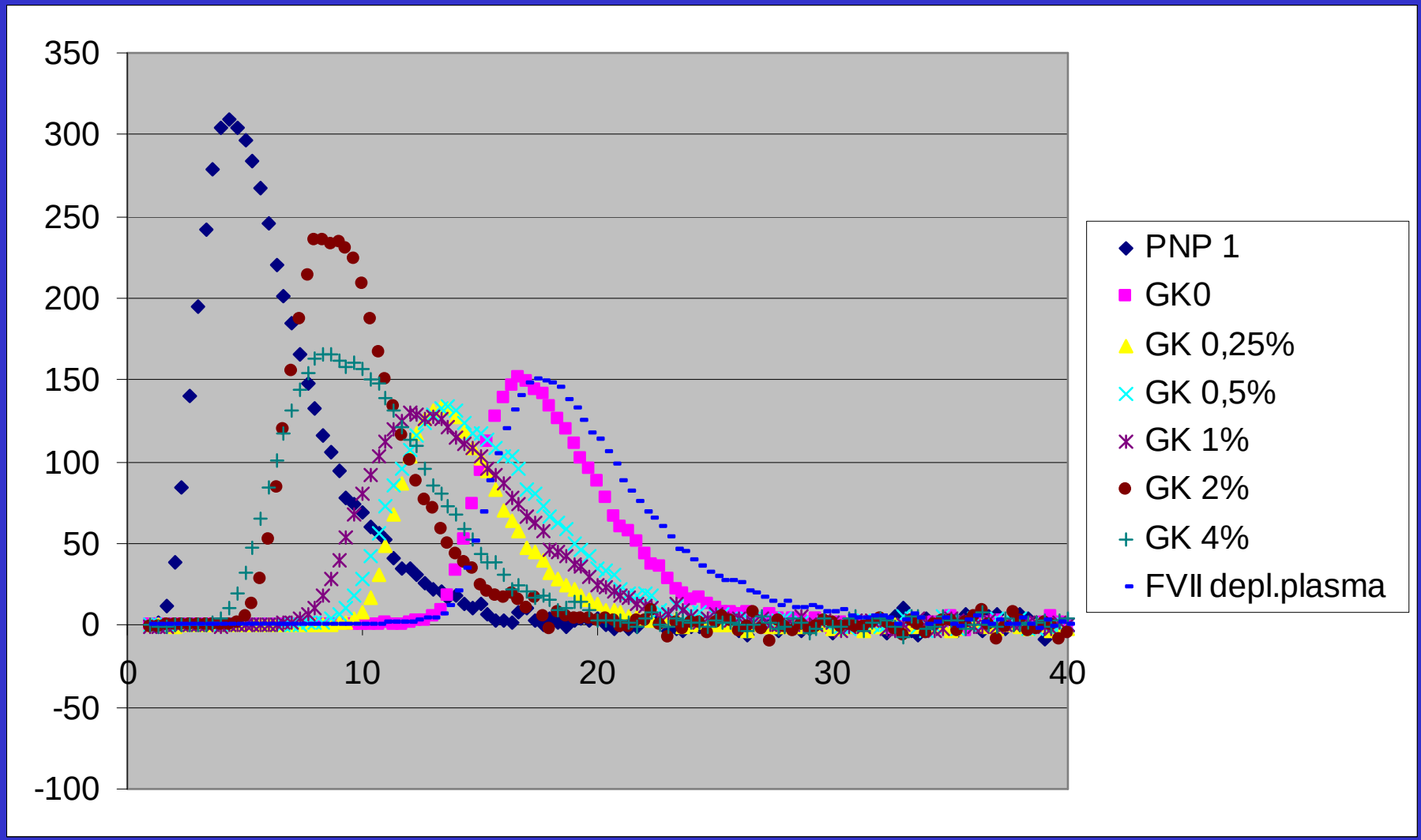


H-D-cyclohexylalanyl-alanine-arginine-7-amino-4-methylcoumarin.diacetate salt

Thrombin generation



Standard Curve in FVII deficient Plasma (G.K)



1) Cosa previene l'attività incontrollata delle serin proteasi?

2) quali sono i determinanti di specificità di queste interazioni precise e coordinate?