Intro to Hemostasis

AMY BUENING MLS (ASCP)^{CM}

Today's Discussion

Hemostasis

Vascular Intima in Hemostasis

Coagulation Cascade

Cell-based model of Coagulation

Coagulation Regulatory Mechanisms

Classification of Factors

Fibrinolysis

Hemostasis

Hemostasis

- How your body stops bleeding
- System of checks and balances in which the blood vascular system, platelets, and coagulation proteins work together to ensure the fluidity of blood
- When out of balance can lead to hemorrhage (bleeding) or thrombosis (clotting)
- Involves the interaction of vasoconstriction, platelet adhesion and aggregation, and coagulation enzyme activation to stop bleeding
- Consists of
  - Primary hemostasis
  - Secondary hemostasis
  - Fibrinolysis

Hemostasis

Primary hemostasis

- Role of blood vessels and platelets in the initial response to a vascular injury or to dying or damaged endothelial cells
- Blood vessels contract to seal the wound or reduce the blood flow (vasoconstriction)
- Platelets become activated
  - Will adhere to the site of injury, secrete contents of their granules
- Platelets aggregate with other platelets to form a platelet plug

- Initial, rapid, short lived response to vessel damage

Hemostasis

Secondary Hemostasis

- Activation of series of coagulation proteins in the plasma
  - Mostly serine proteases
  - Inactive- zymogens (proenzymes) that become activated during the process of coagulation
- Generate thrombin
  - Enzyme that converts fibrinogen to a localized fibrin clot
- Delayed, long-term response

Hemostasis

Fibrinolysis

- Gradual digestion and removal of the fibrin clot as healing occurs

Vascular Intima in Hemostasis

Blood Vessel

- Carry blood throughout the body
- Structured into 3 layers:
  - Inner layer (vascular intima)
    - Endothelial cells
      - Essential in immune response, vascular permeability, proliferation, and hemostasis
  - Middle layer (vascular media)
  - Outer layer (vascular adventitia)

Anticoagulant Properties of Vascular Intima

- Endothelial cells form a smooth inner surface of the blood vessel

- Prevents thrombosis by inhibiting platelet aggregation, preventing coagulation activation and propagation, and enhancing fibrinolysis
  - Prevents turbulence that can activate platelets and coagulation enzymes

- Endothelial cells form a physical barrier separating procoagulant proteins and platelets in the blood from collagen, tissue factor, and smooth muscle cells

Anticoagulant Properties of Vascular Intima

- Endothelial cells synthesize and secrete substances that maintain normal blood flow
  - Prostacyclin- platelet inhibitor and vasodilator
  - Nitric oxide- induces smooth muscle relaxation- inhibits platelet activation
  - Tissue factor pathway inhibitor (TFPI)- controls activation of tissue factor pathway (extrinsic pathway)

- Synthesize and express inhibitors of thrombin formation
  - Thrombomodulin
  - Heparan sulfate

Procoagulant Properties of Vascular Intima

- Tear in the vascular intima (ECs and subendothelial matrix)
  - Promote coagulation
  - Induce vasoconstriction in arteries and arterioles
  - Smooth muscle cells contract
- Exposed collagen binds and activates platelets
- Endothelial cells secrete von Willebrand factor (VWF)
  - Released from storage sited called weibel-palade bodies
  - Necessary bridge that binds platelets to exposed subendothelial collagen
  - “carpet” on which platelets assemble
- ECs secrete and coat themselves with P-selectin
  - Adhesion molecule that promotes platelet and leukocyte binding
- ECs secrete adhesion molecules ICAMs and PECAMs
- Exposes Tissue Factor (TF)

Fibrinolytic Properties of Vascular Intima

- Endothelial cells support fibrinolysis

- Secretion of:
  - Tissue plasminogen activator (TPA)
  - Plasminogen activator inhibitor-1 (PAI-1)
  - Thrombin activatable fibrinolysis inhibitor (TAFI)

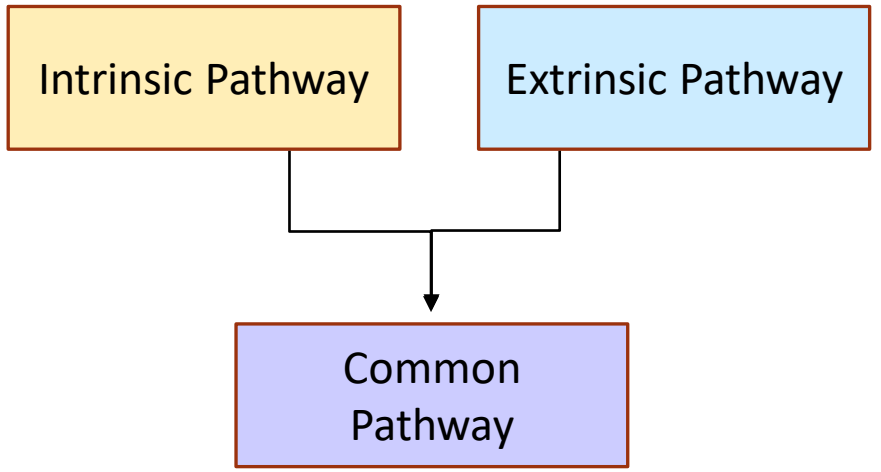
Coagulation Cascade

Coagulation Cascade

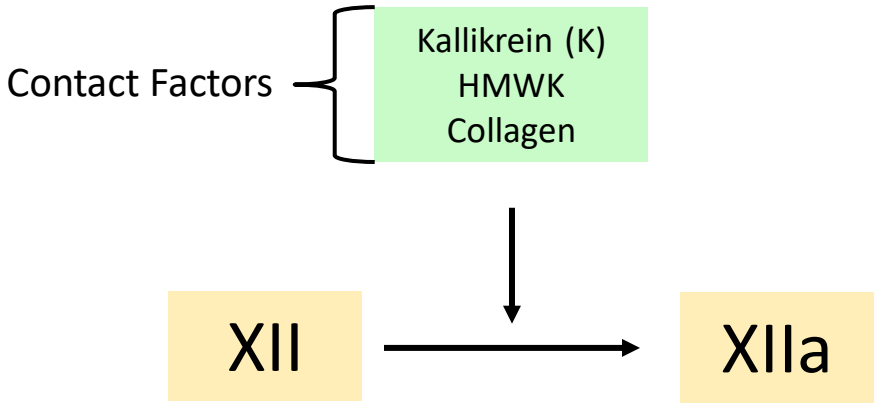
- Consist of plasma proteins that are named using roman numerals
- Zymogens
  - Inactive factors in the plasma
- Produced in the liver
  - Except for FVII that is partially made in the liver
  - Can also be made and released from EC, monos, and platelets
- After activation, they are kept in check by specific factor inhibitors
- All factors demonstrate different half-lives
  - FVII has the shortest half life
- Sequential activation of coagulation factors with the final step being the conversion of fibrinogen to fibrin

Coagulation Cascade

- Cascade pathway where one factor, when activated, activates the next factor in the sequence
- 3 pathways present in the cascade
  - Intrinsic pathway
  - Extrinsic pathway
  - Common pathway
- Procoagulant factors participate in complex formation
- Some of the procoagulants are serine protease enzymes
  - Thrombin (IIa), VIIa, IXa, Xa, XIa, XIIa, pre-K
  - Exception: Factor XIII is a transglutaminase
- Procoagulant cofactors
  - Tissue factor (TF), V, VIII, and HMWK



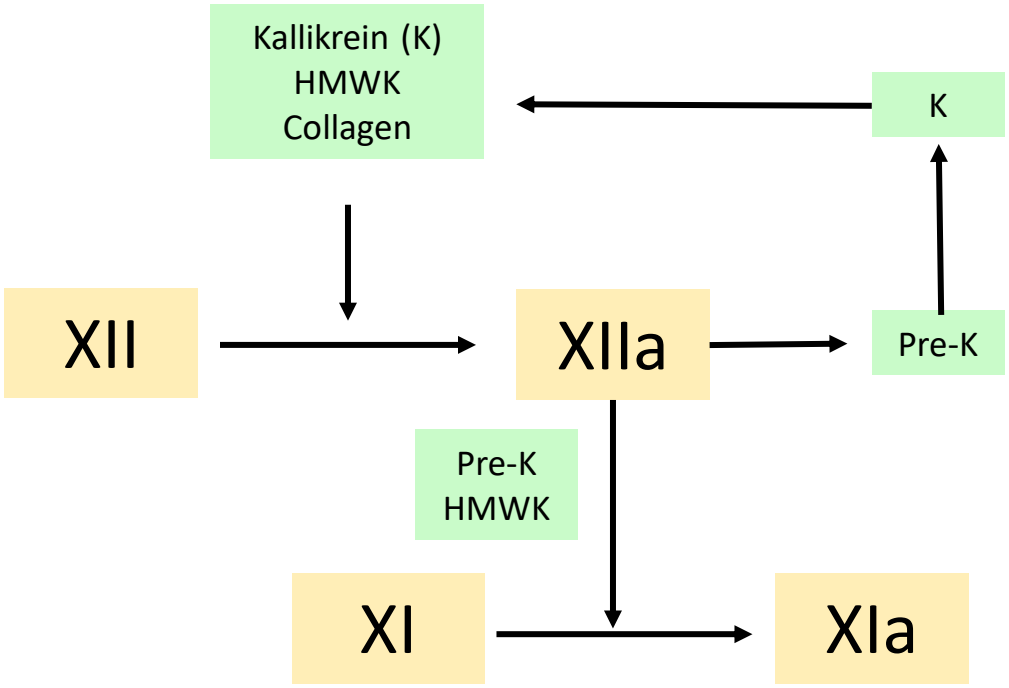
Intrinsic Pathway



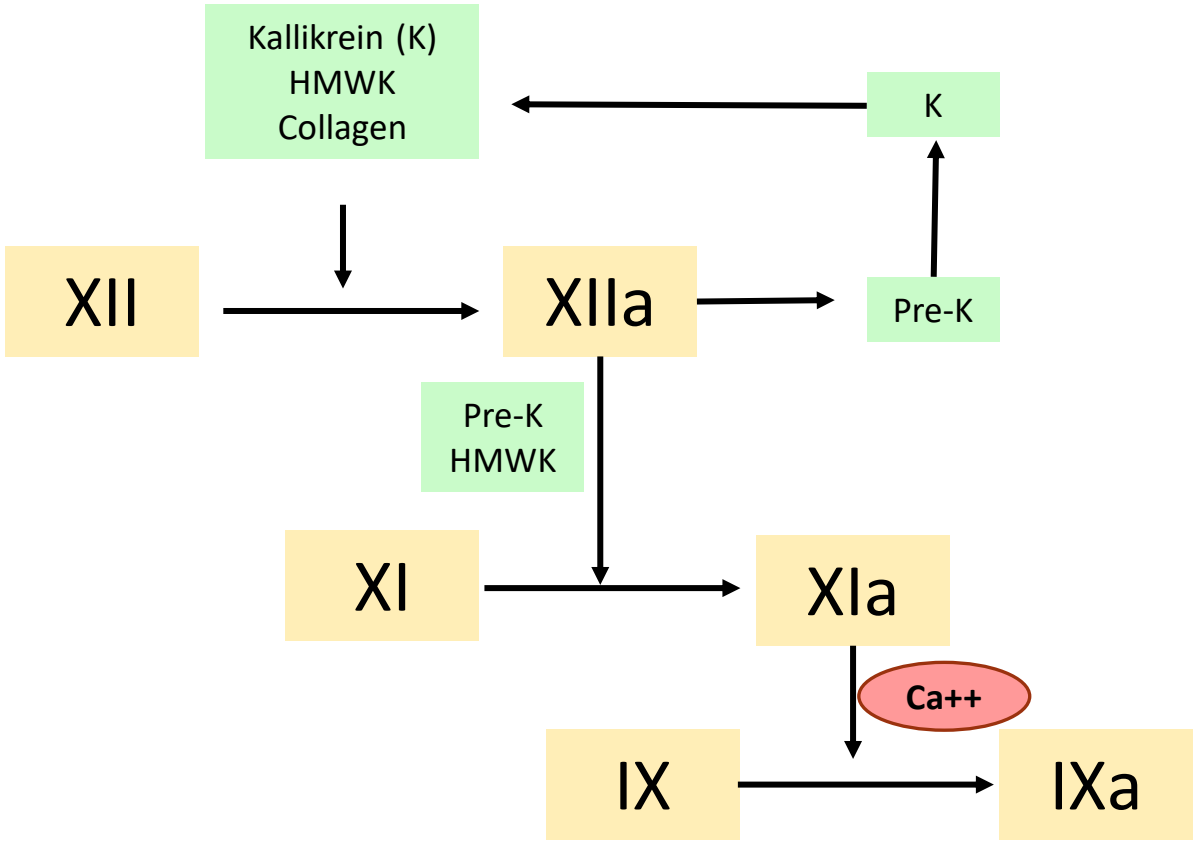
- Intrinsic Pathway: Initiated by negatively charged phospholipids (on the surface of the platelet)
  - Called intrinsic because factor XII was able to be found in the blood

HMWK= HMW Kininogen (Fitzgerald)
PreK= Prekallikein (Fletcher)

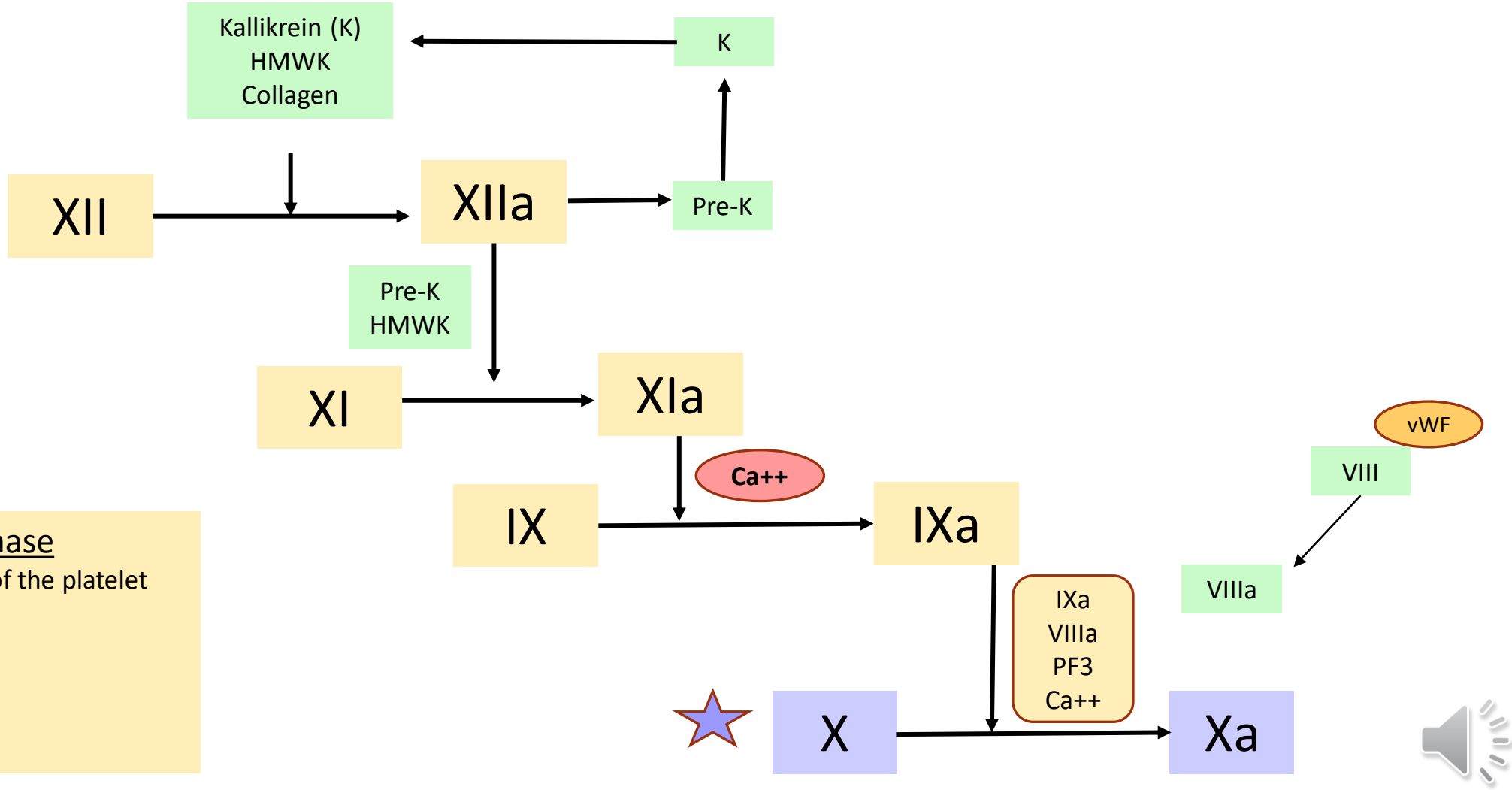
Intrinsic Pathway



Intrinsic Pathway



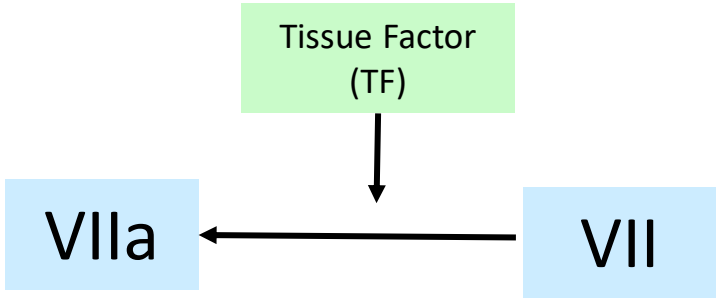
Intrinsic Pathway



Intrinsic Tenase

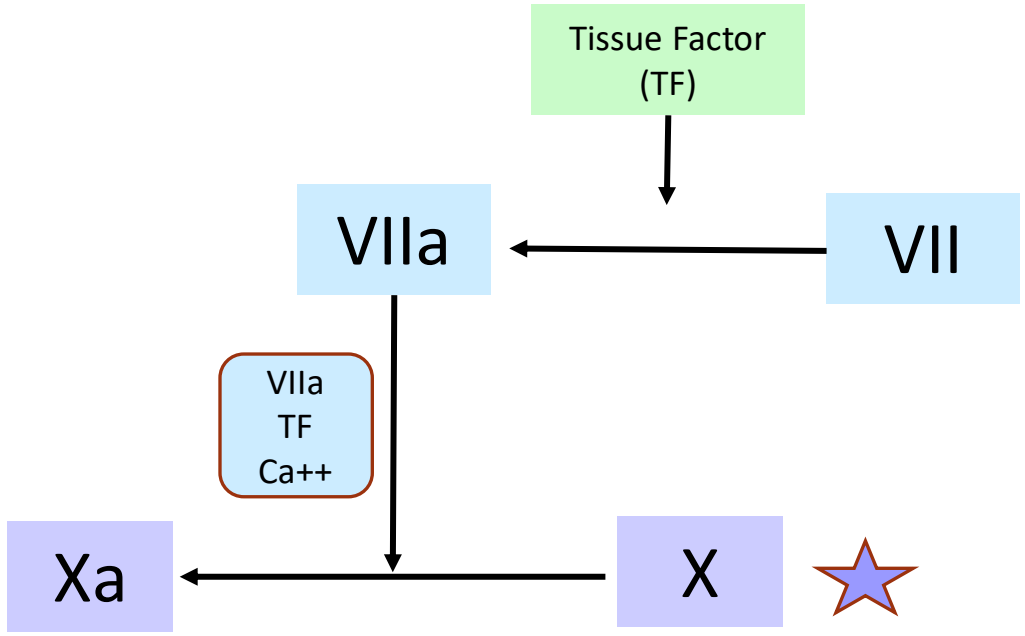
- Forms on the surface of the platelet
- Consists of:
  - IXa
  - VIIIa
  - PF_3
  - Ca^{2+}

Extrinsic Pathway



- Extrinsic pathway: Initiated by tissue factor
  - Called this because tissue factor (TF) is not present in your blood
    - TF is exposed through injury
- Operates on the TF-bearing cell to initiate and amplify coagulation
- Shorter and more efficient
- Primary in vivo initiation mechanism form coagulation

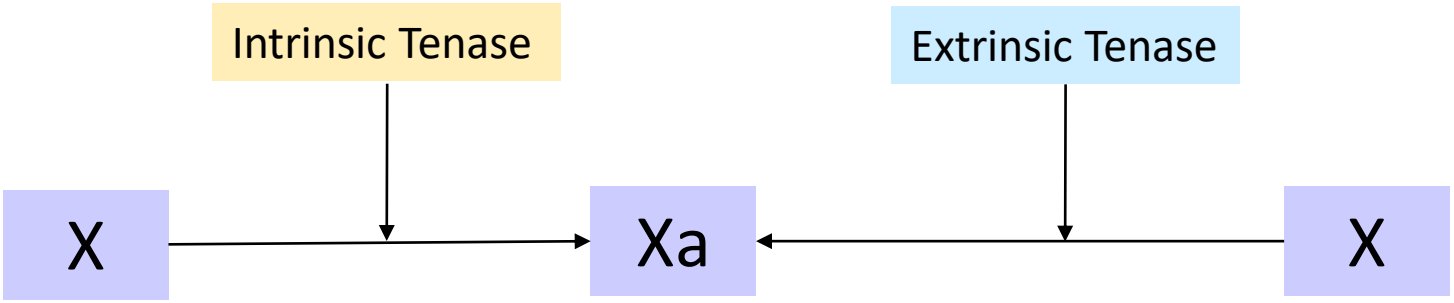
Extrinsic Pathway



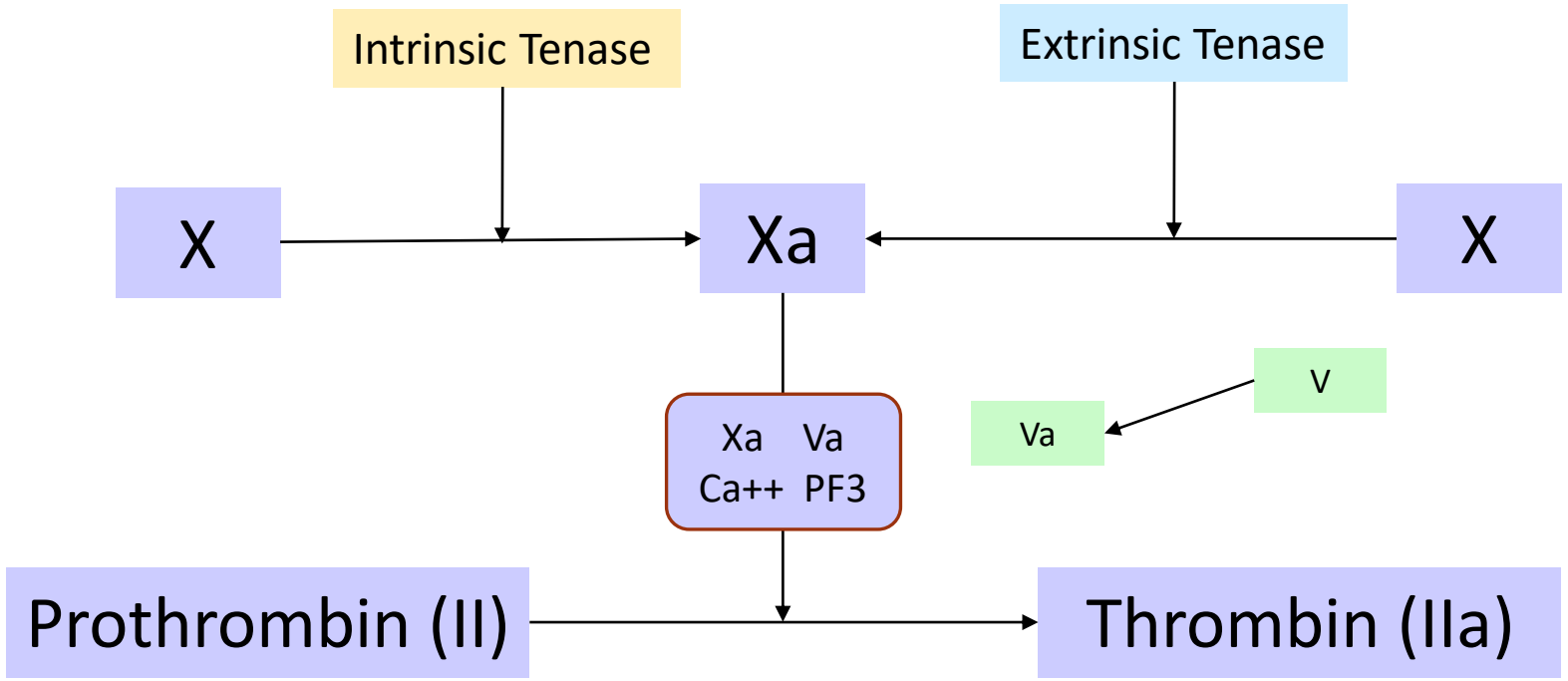
Extrinsic Tenase

- Consists of
  - VIIa
  - TF
  - Ca^{2+}

Common Pathway



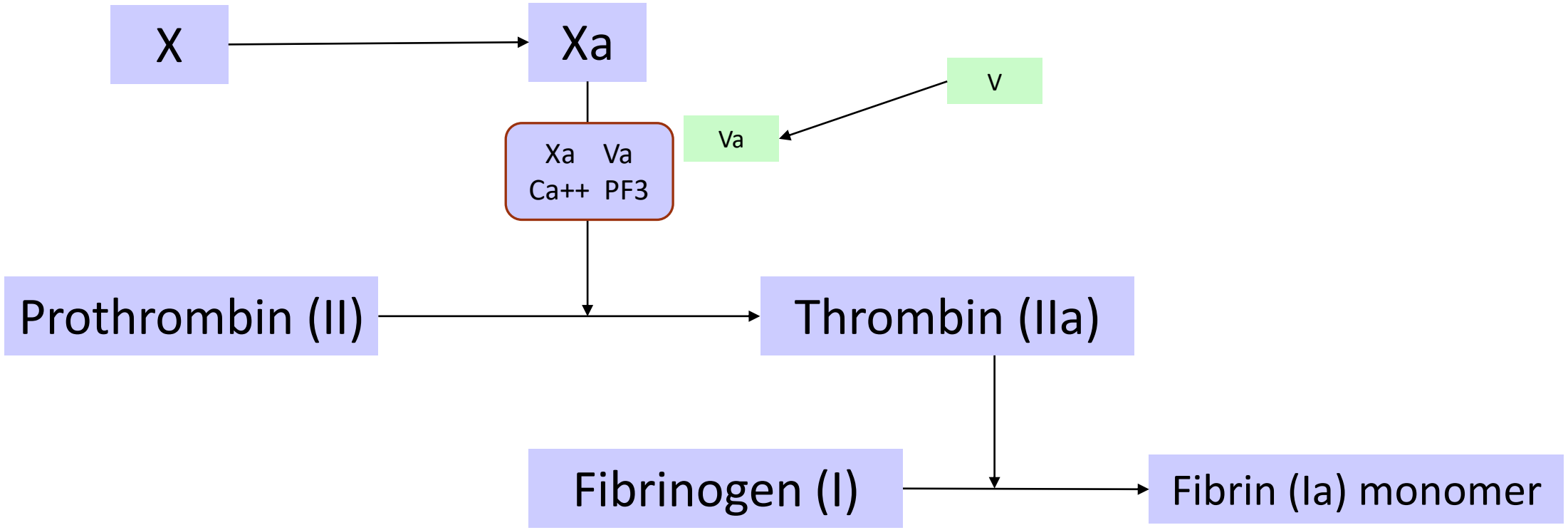
Common Pathway



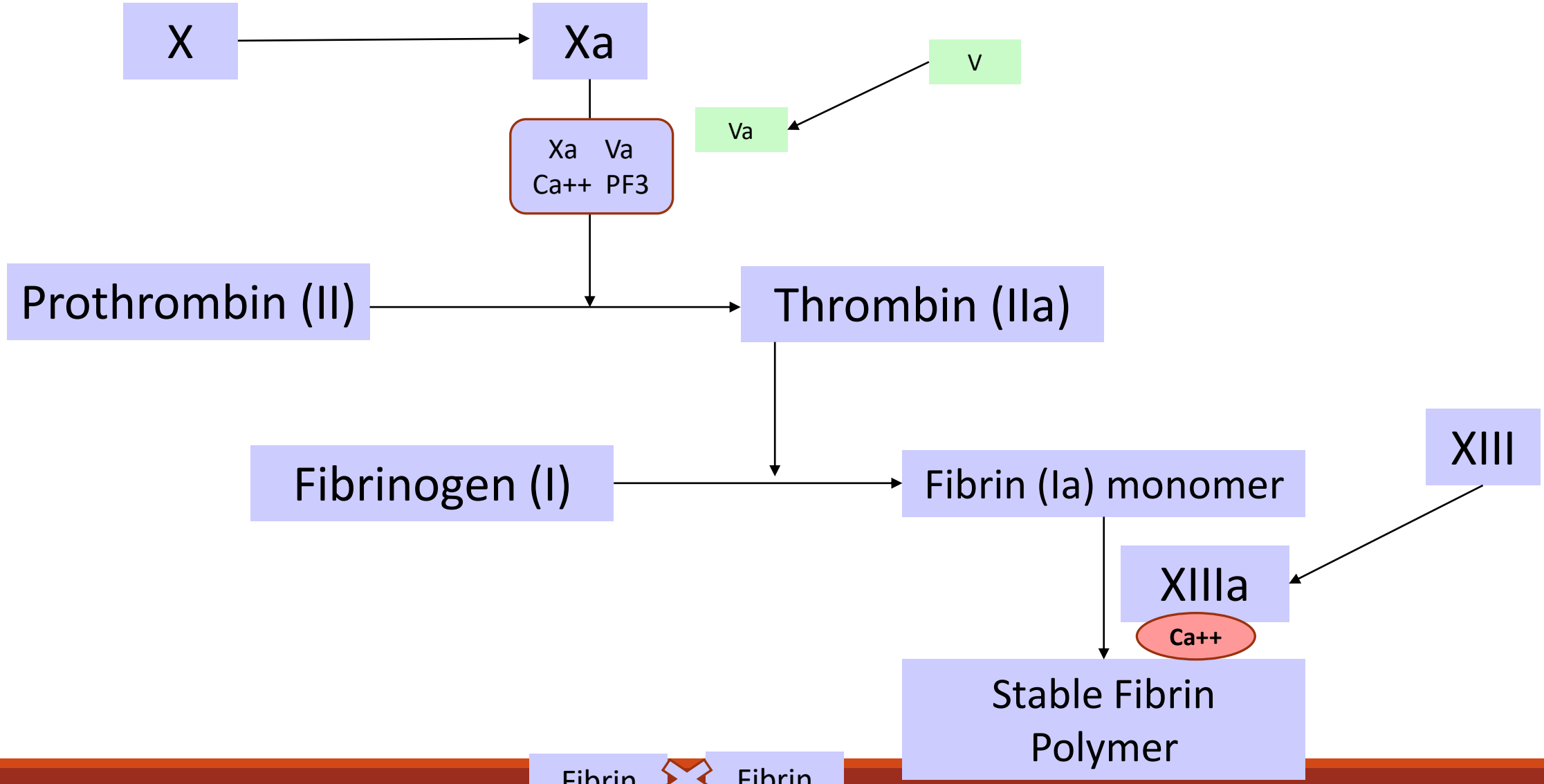
Prothrombinase:

- Forms on the surface of the platelet
- Consists of:
  - Xa
  - Va
  - Ca^{2+}
  - PF_3

Common Pathway



Common Pathway



Fibrinogen Structure

- Thrombin converts soluble fibrinogen → insoluble fibrin to produce a clot
- Fibrinogen essential for platelet aggregation
  - Links activated platelets using GP IIb/IIIa platelet fibrinogen receptor
- Released by platelet α granules
- Molecule
  - Mirror-image dimer

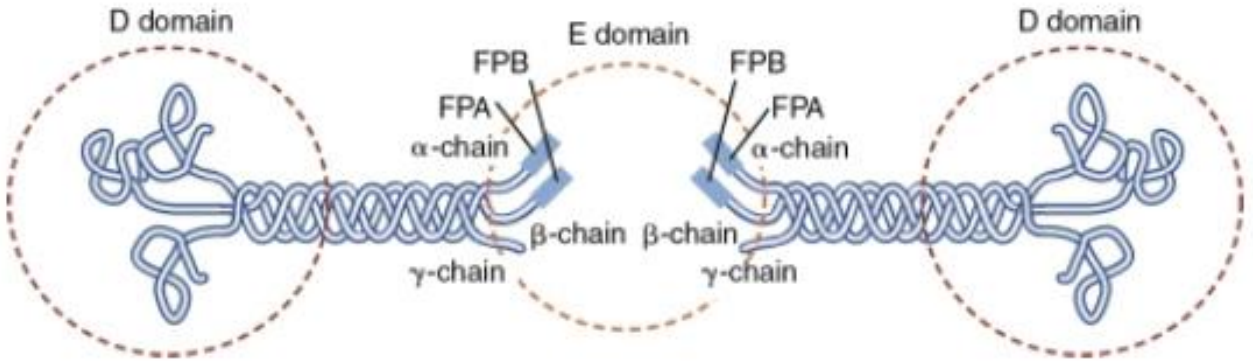


FIGURE 35.8 Structure of Fibrinogen. Fibrinogen has three domains. The central E domain and two terminal D domain nodules at the carboxyl ends of the molecule. The D and E domains are joined together by supercoiled α-helix regions. This trinodular structure is composed of three pairs of disulfide-bonded polypeptides, two each of the Aα, Bβ, and γ chains. *FPA*, Fibrinopeptide A; *FPB*, fibrinopeptide B.

Fibrin Formation

- Thrombin cleaves fibrinopeptides A and B from each of the two α and β chains of fibrinogen
  - Creates the fibrin monomer
- Exposed α and β chains ends (E domain) have an affinity for D domain of neighboring monomers
- FXIIIa cross-links fibrin polymers to form a stable insoluble fibrin clot
  - Incorporate fibronectin and α_2-antiplasmin making it resistant to fibrinolysis

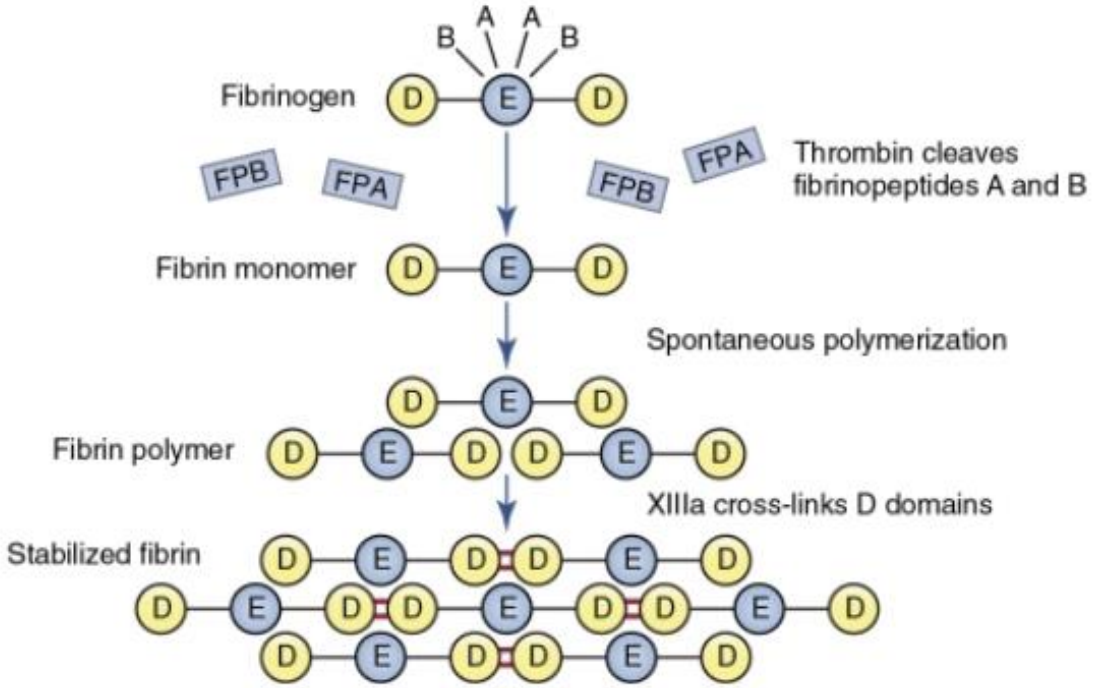
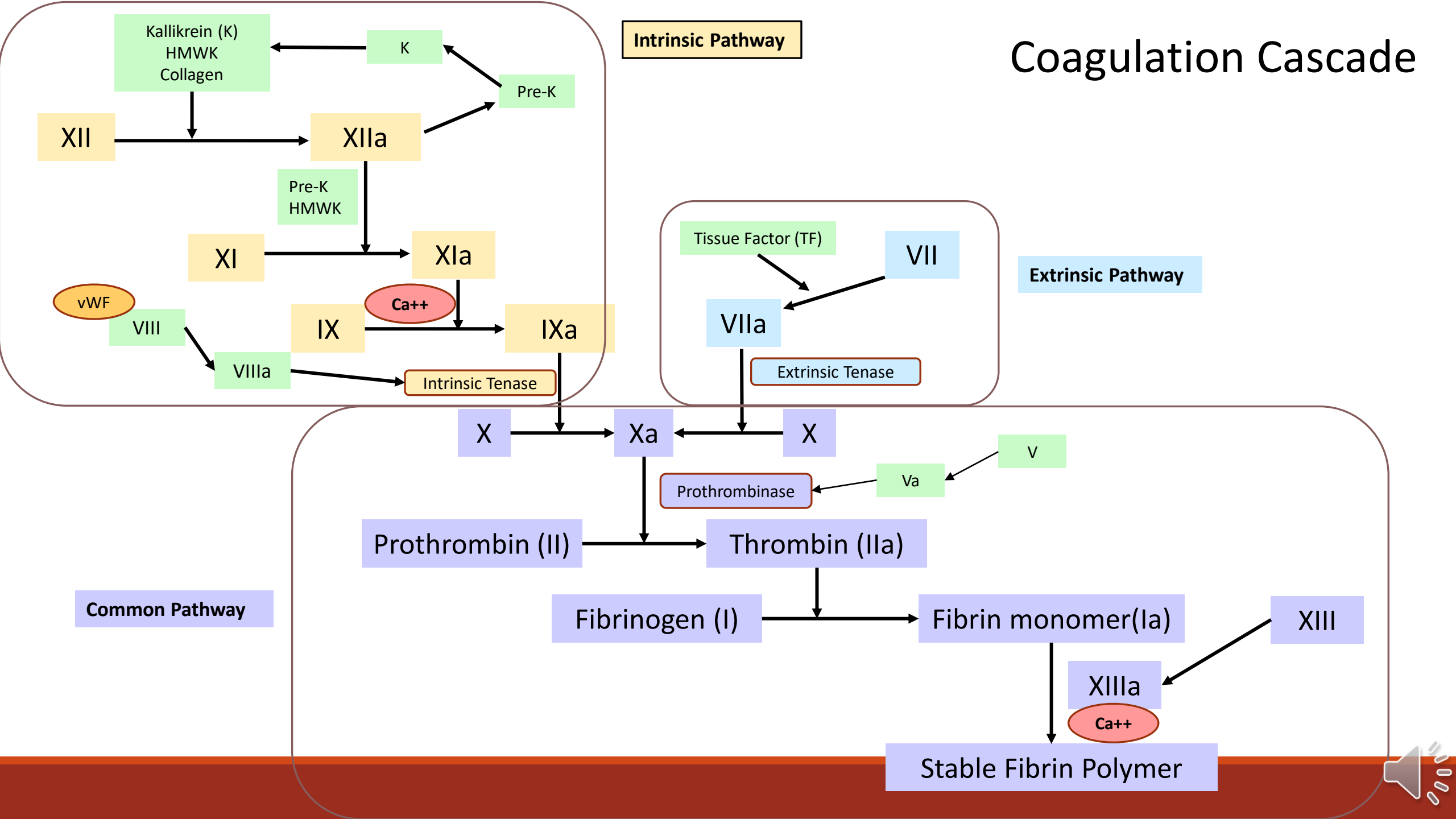
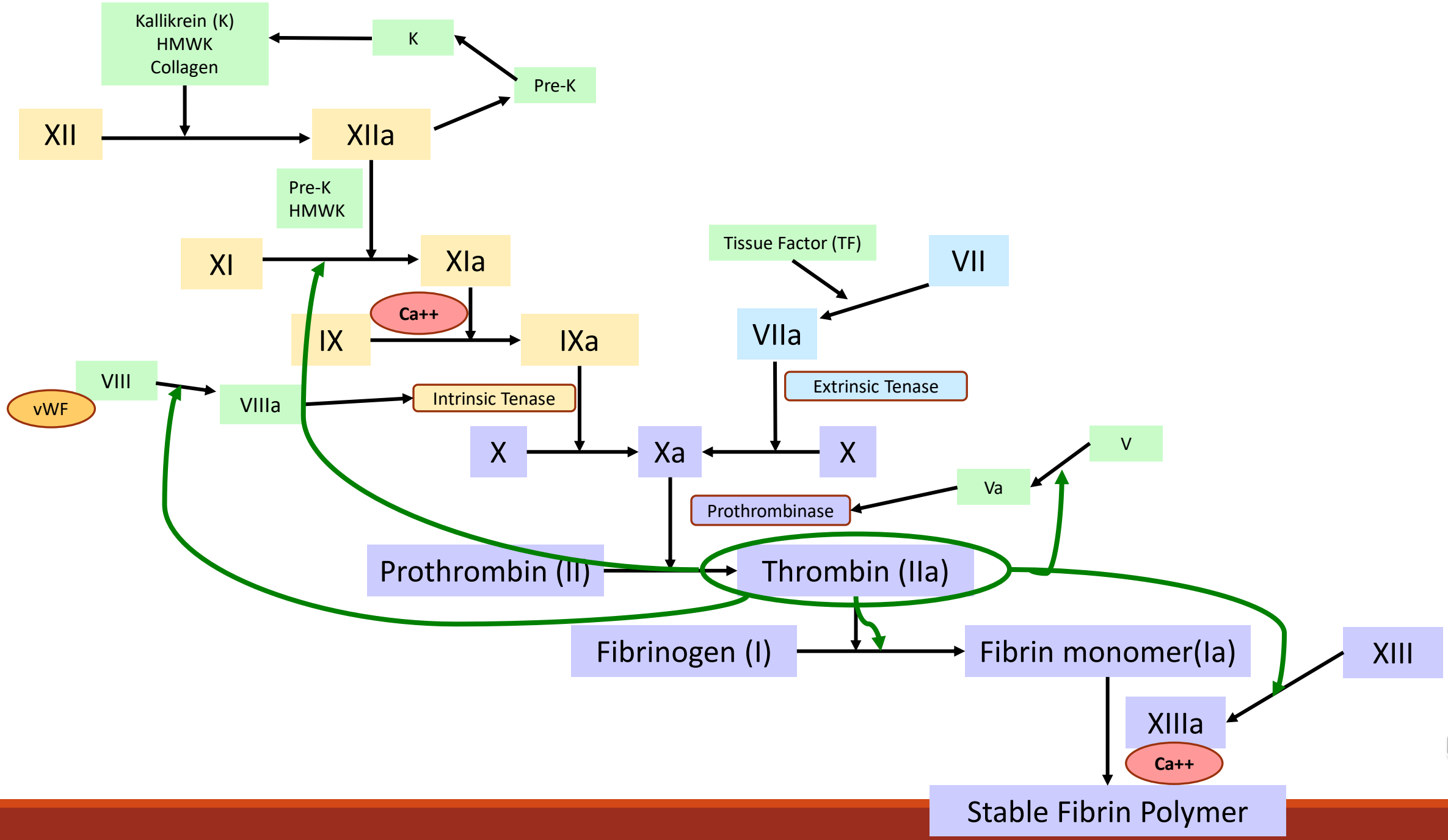
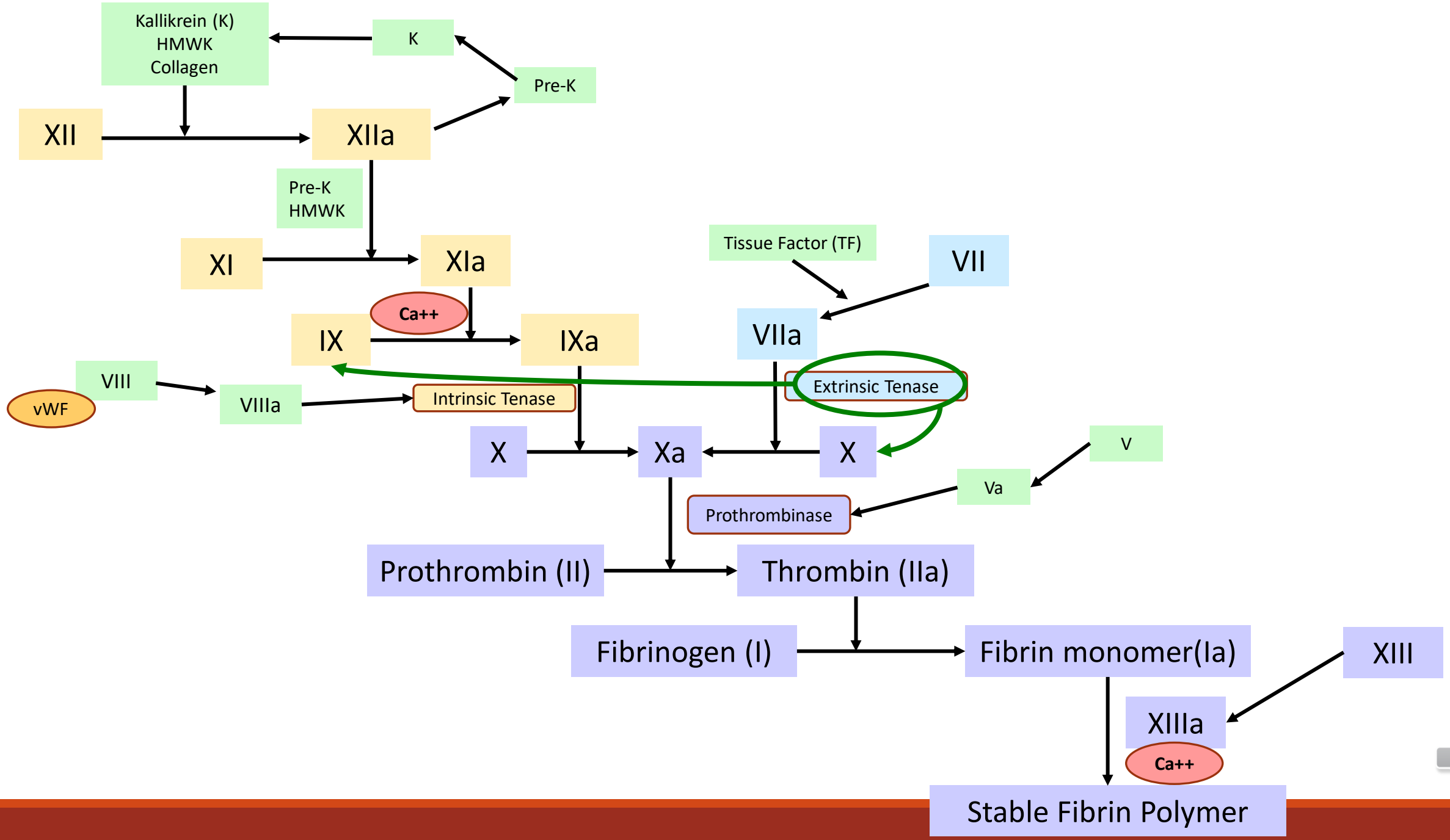


FIGURE 35.9 Formation of a Stabilized Fibrin Clot. Thrombin cleaves a small portion of the α and β chains in the central E domain node releasing free peptides, fibrinopeptide A *(FPA)* and fibrinopeptide B *(FPB)*. This forms fibrin monomer. Fibrin monomers spontaneously polymerize due to the affinity of thrombin-cleaved positively charged E domains for negatively charged D domains of other monomers. This forms the fibrin polymer. Factor XIIIa catalyzes the covalent cross-linking of γ chains of adjacent D domains (double red line) to form an insoluble stable fibrin clot.

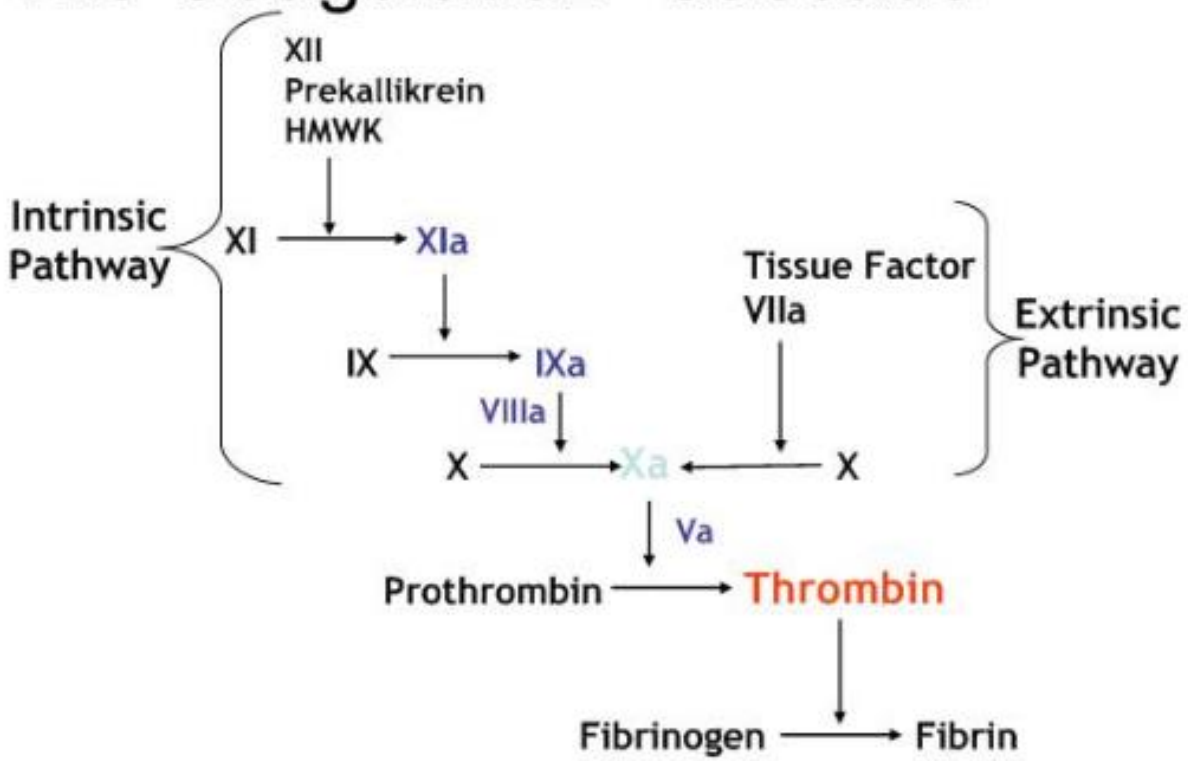
Coagulation Cascade

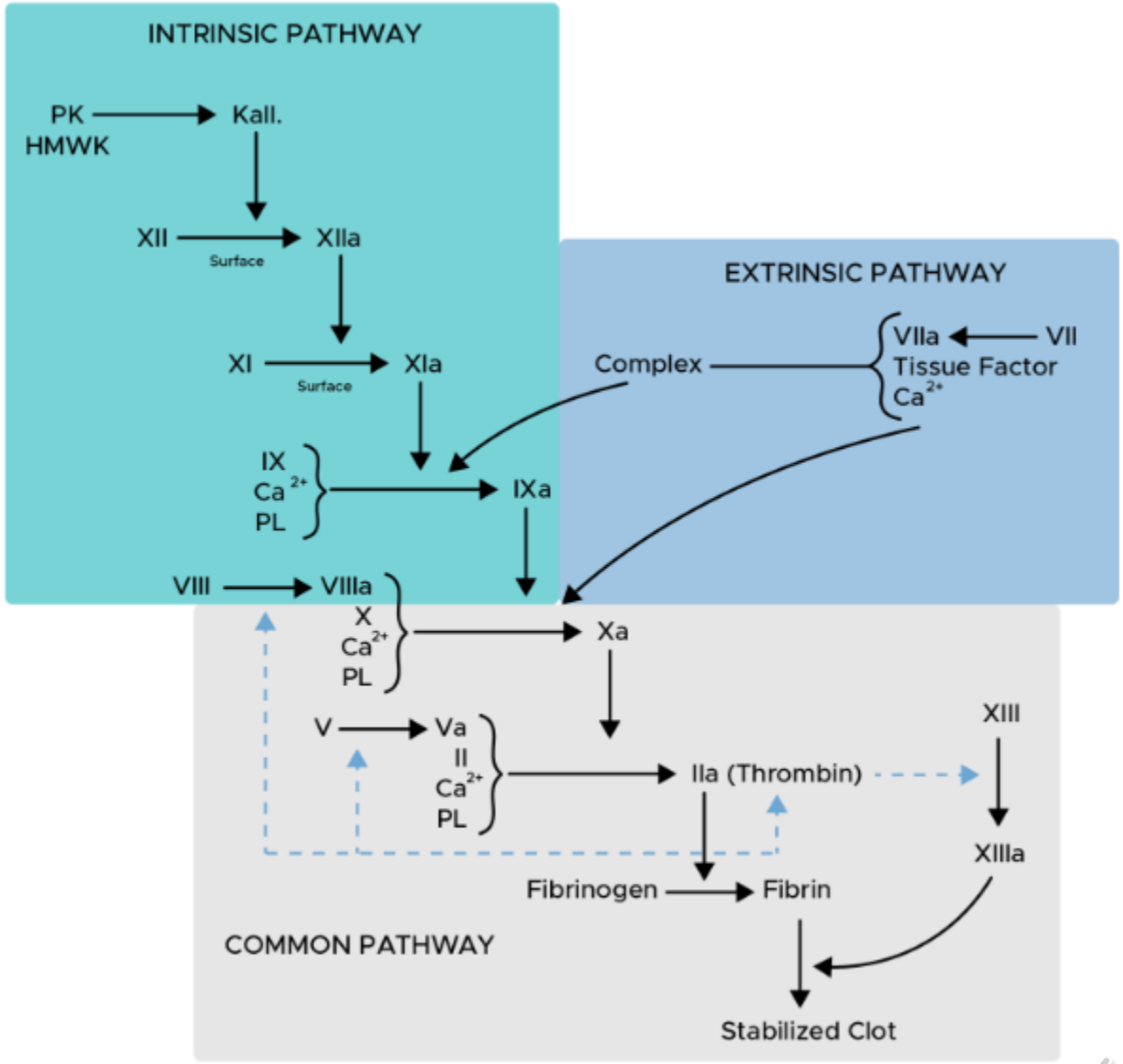



Kallikrein (K)
HMWK
Collagen
K
Pre-K
XII
XIIa
Pre-K
HMWK
XI
XIa
Ca++
IX
IXa
Tissue Factor (TF)
VII
VIIa
VIII
vWF
VIIIa
Intrinsic Tenase
Extrinsic Tenase
X
Xa
X
V
Va
Prothrombinase
Prothrombin (II)
Thrombin (IIa)
Fibrinogen (I)
Fibrin monomer(Ia)
XIII
XIIIa
Ca++
Stable Fibrin Polymer


Kallikrein (K)
HMWK
Collagen
K
Pre-K
XII
XIIa
Pre-K
HMWK
XI
XIa
Ca++
IX
IXa
Tissue Factor (TF)
VII
VIIa
VIII
vWF
VIIIa
Intrinsic Tenase
Extrinsic Tenase
X
Xa
X
V
Va
Prothrombinase
Prothrombin (II)
Thrombin (IIa)
Fibrinogen (I)
Fibrin monomer(Ia)
XIII
XIIIa
Ca++
Stable Fibrin Polymer

The Coagulation "Cascade"





MediaLab- Antiplatelet and Anticoagulation Pharmacology for the Laboratory Professional

Cell Based Model of Coagulation

Cell Based Model

- Proposed to replace the traditional cascade
- It occurs in 3 overlapping stages
  - **Initiation**
    - Occurs on TF bearing cell
      - Interaction of TF and FVII
  - **Amplification**
    - Platelets and cofactors are activated to set the stage for thrombin (FII) generation
      - Platelets, FV, FVIII, and FXI
  - **Propagation**
    - Thrombin is generated on the platelet surface
      - Production of a temporary plug at the site of injury
- Platelets and thrombin are central to the process

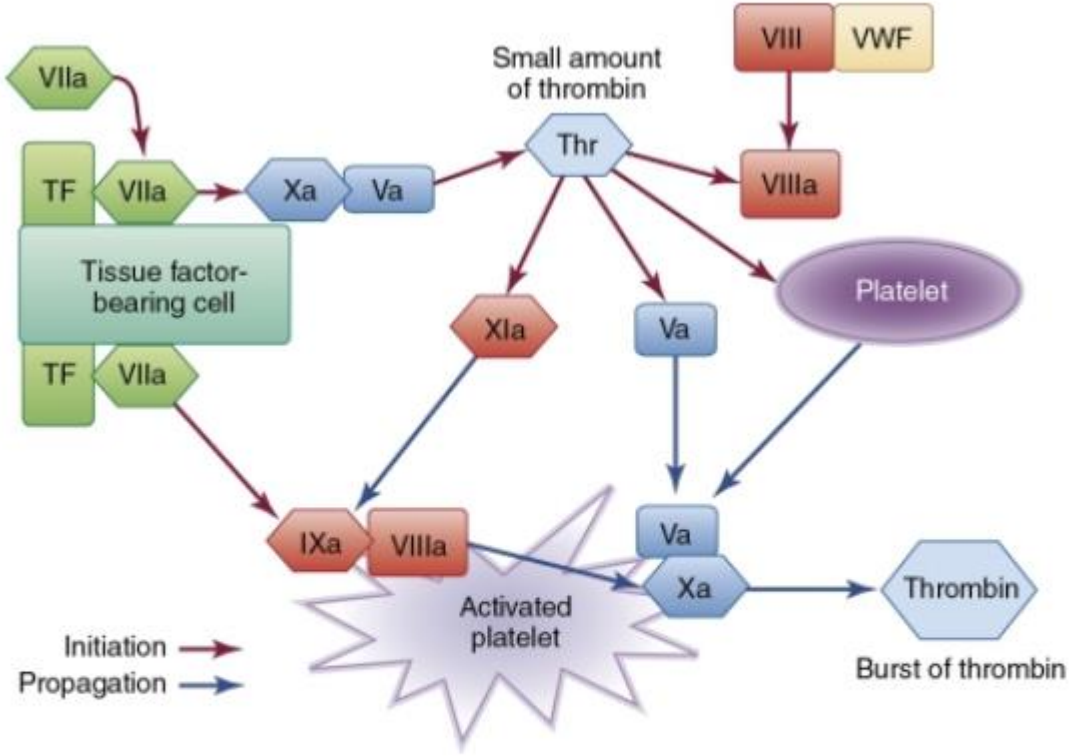


FIGURE 35.11 **Cell-Based Coagulation.** VIIa binds to cell-exposed tissue factor *(TF)* and activates both factors X and IX. Cell-bound factor Xa combines with Va and generates a small amount of thrombin *(Thr)*, which activates platelets, V, VIII, and XI and begins fibrin formation. Factor IXa, activated by both TF:VIIa and XIa, combines with factor VIIIa on the platelet surface to activate X, which binds with Va to form the prothrombinase complex (Xa:Va) and produces a burst of thrombin. *VWF*, Von Willebrand factor.

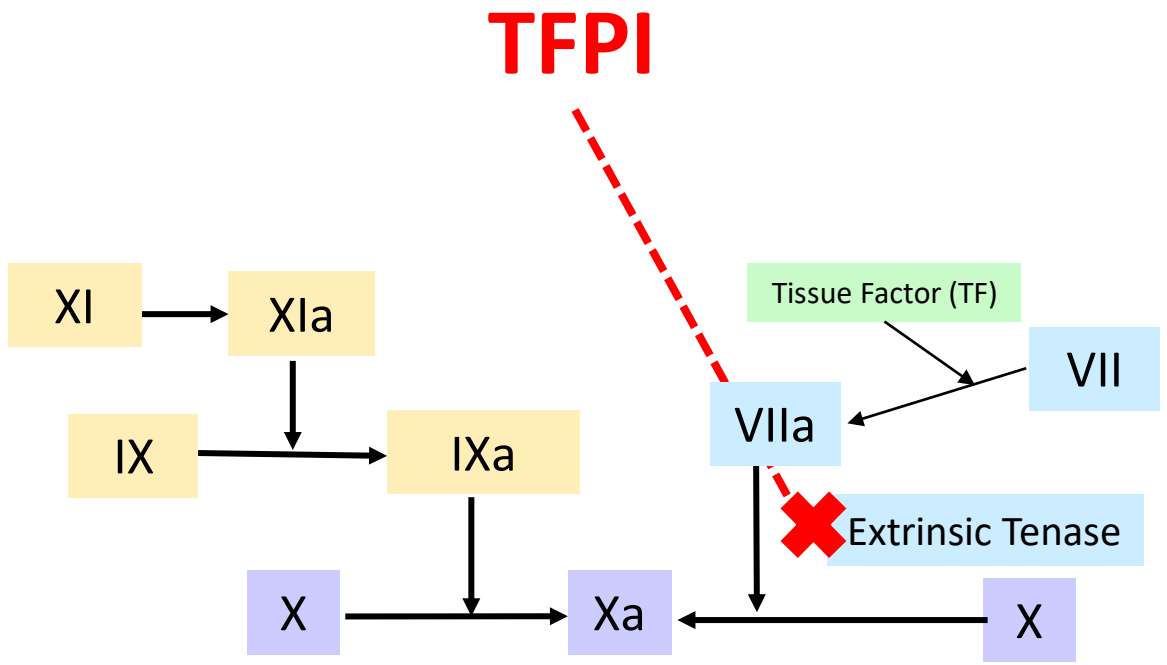
Coagulation Regulatory Mechanism

Regulatory Mechanisms

- Inhibitors or natural anticoagulants
- Function to slow the activation of procoagulants and suppress thrombin production
- Ensure coagulation is localized and not a systemic response
- Principal regulators
  - Tissue Factor Pathway Inhibitor (TFPI)
  - Antithrombin (AT)
  - Activated protein C (APC)

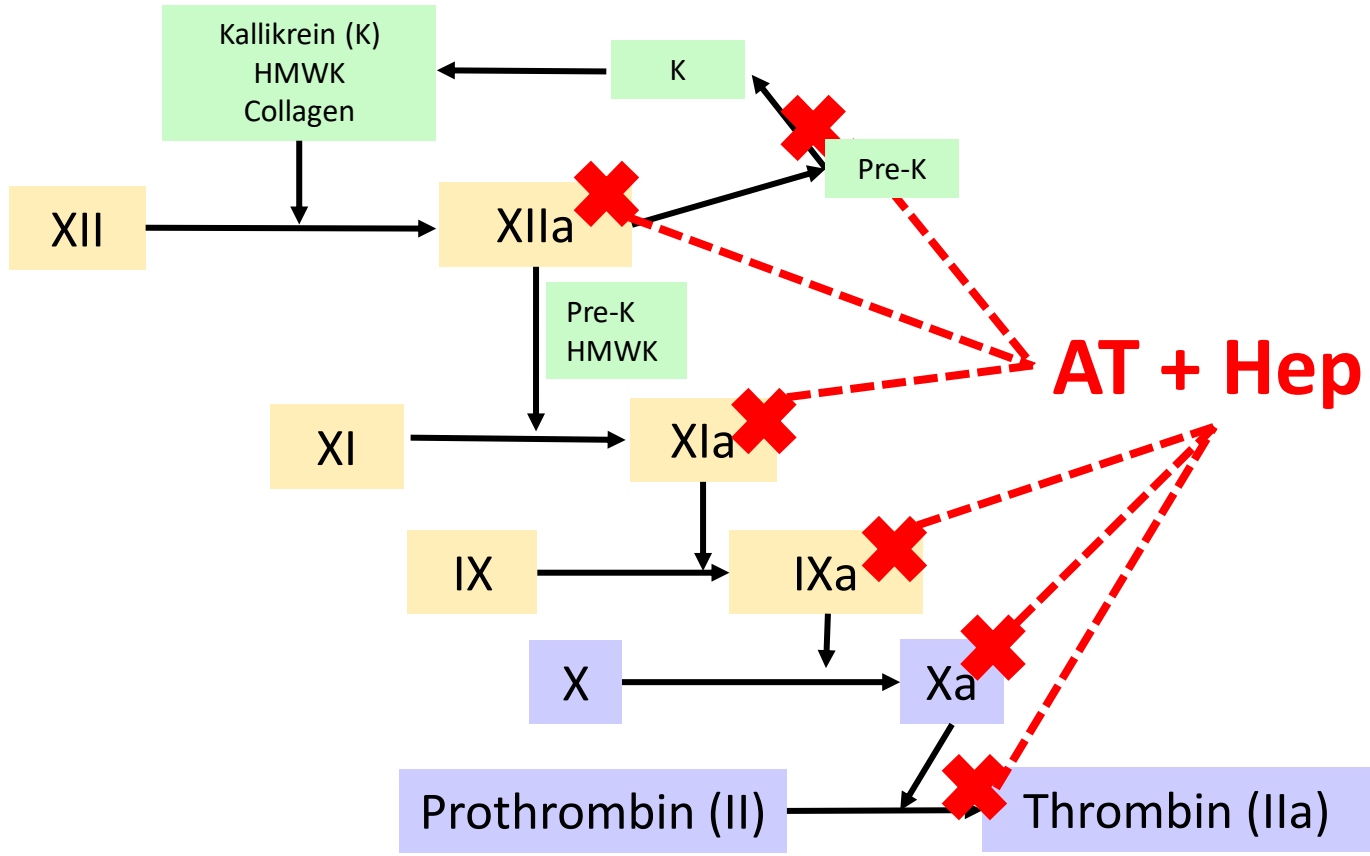
Tissue Factor Pathway Inhibitor (TFPI)

- Inhibits the extrinsic pathway
- Blocks TF from activation of FX and FIX
- TFPI is synthesized by ECs and expressed on platelets
- Feedback inhibition
  - Actively engaged when FX is activated



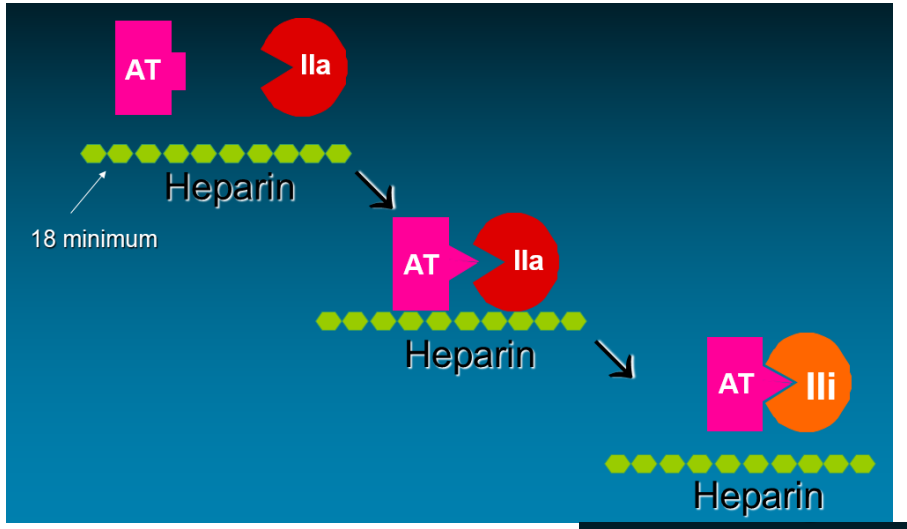
Antithrombin (AT)

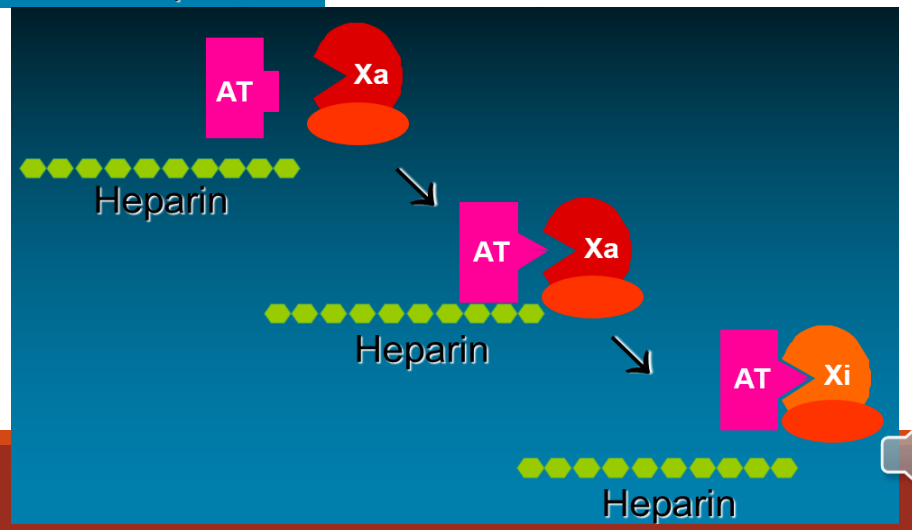
- Binds and neutralizes serine proteases
  - Thrombin (IIa)
  - FIXa
  - FXa
  - FXIa
  - FXIIa
  - Prekallikrein
  - Plasmin
- Requires heparin or heparan for effective anticoagulant activity



Anticoagulant Activity of Antithrombin (AT)

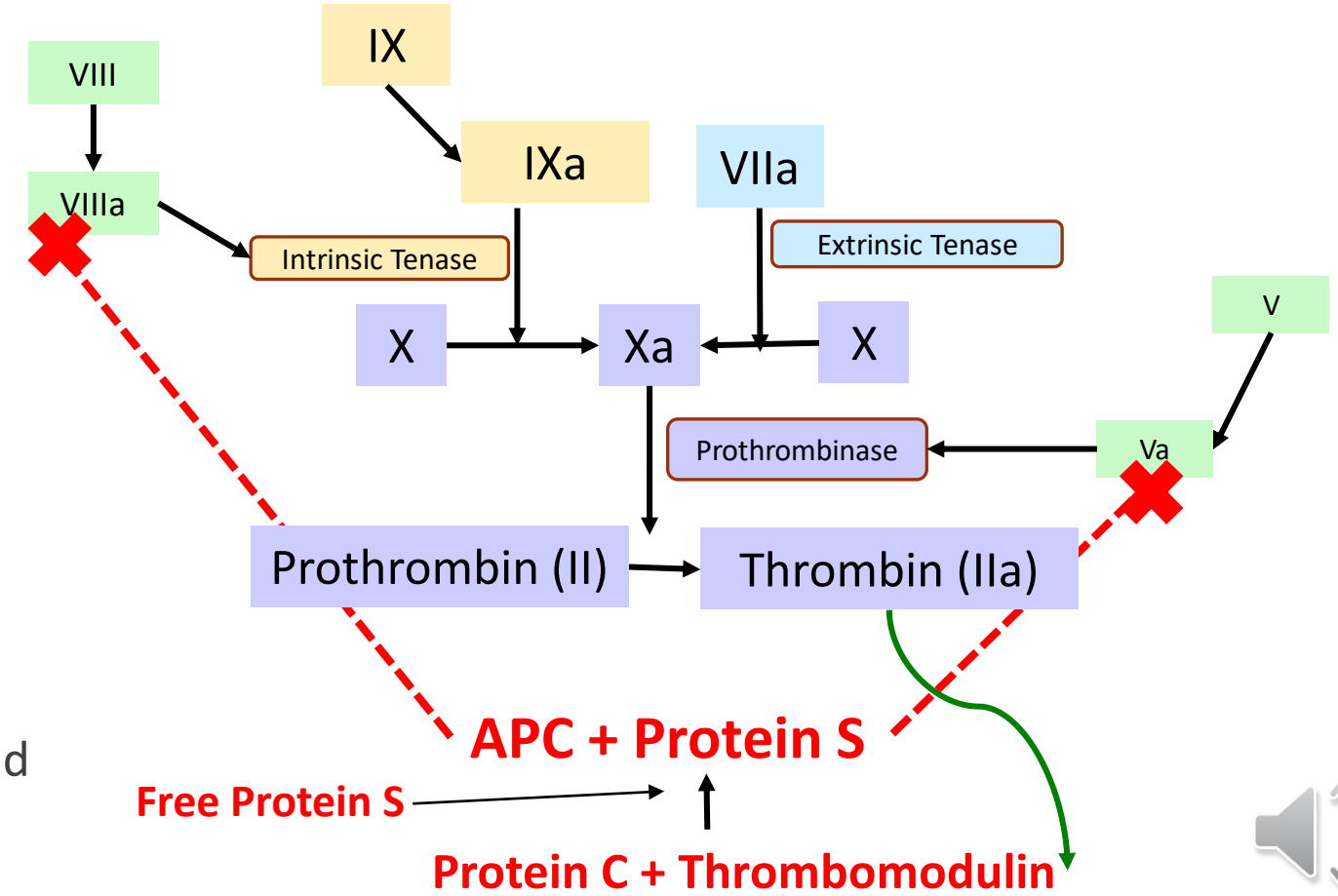
- Heparin+ AT causes a conformational change in AT so it will inactivate IIa
- Dependent on the length of the heparin (minimum of 18)
  - Unfractionated heparin
- Unfractionated heparin will inactivate IIa and Xa
- If shorter strands (>18) of heparin
  - Interaction of heparin with AT will be more selective for Xa only
  - Called LMWH



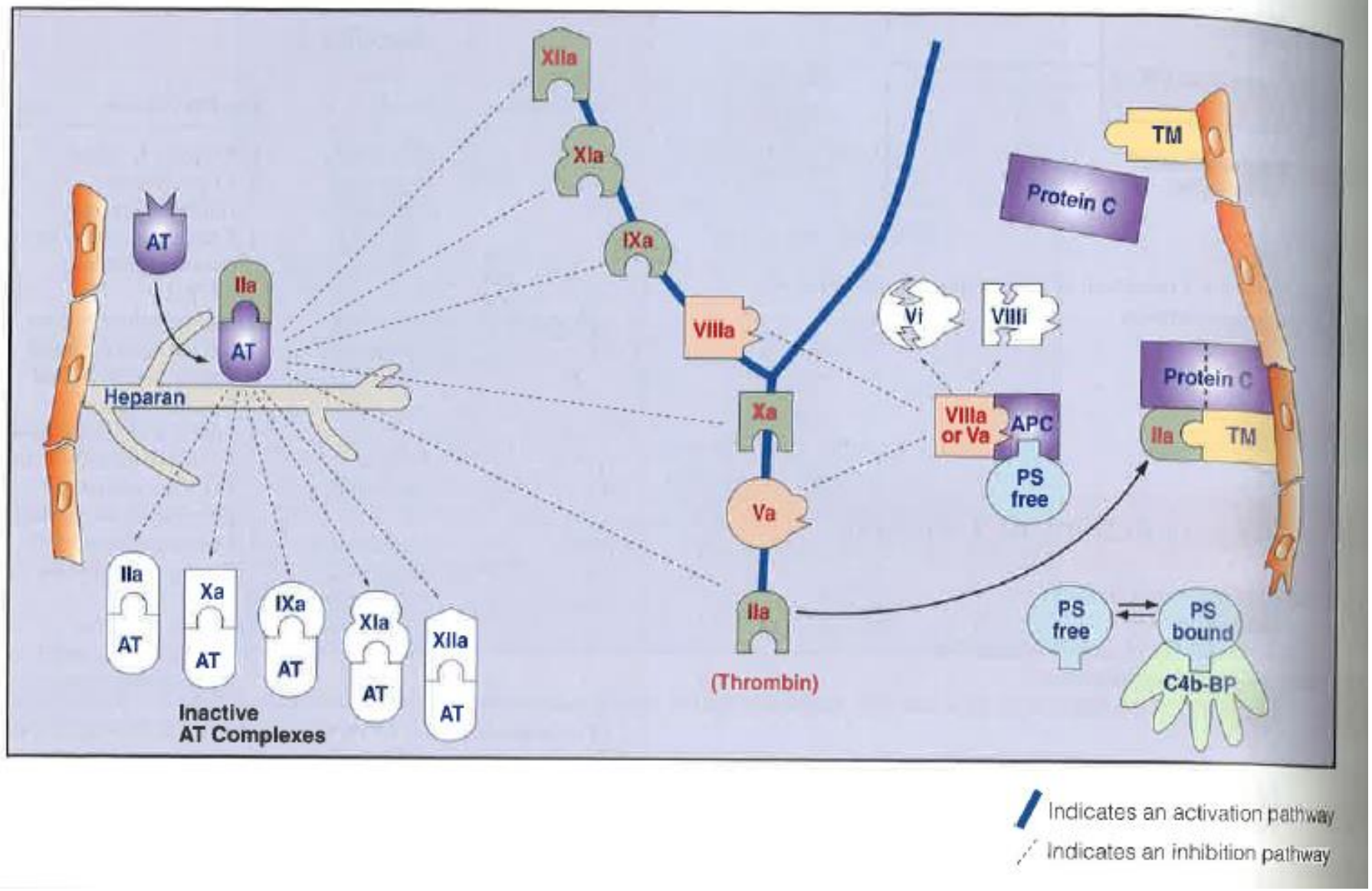


Activated Protein C (APC)

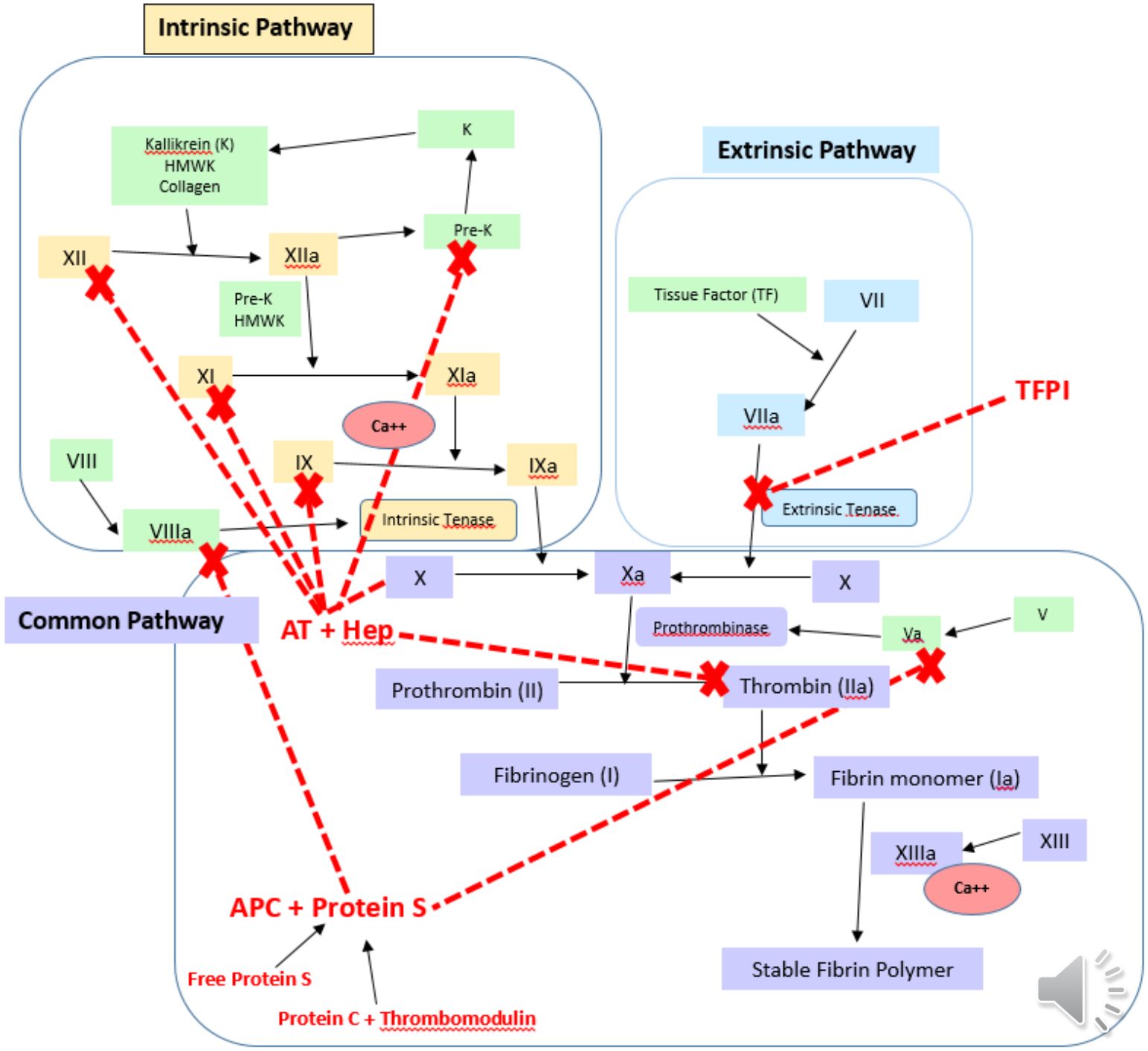
- Will block the cofactors in the cascade
- Activated when Thrombin binds with thrombomodulin which activates the Protein C system
- Activated protein C (APC) dissociates and binds its cofactor- free protein S
- Stabilized APC-protein S complex will inactivate
  - FVa
  - FVIIIa
- Will slow or block thrombin generation and coagulation



Antithrombin (AT) and Protein C Pathways



Cascade with Regulatory Mechanisms



Classification of Factors

Classify by physical properties

Contact Factors
FXII
Prekallikrein
HMWK
FXI

Procoagulant Co-factors
Tissue factor (TF)
FV
FVIII
HMWK

Classify by physical properties

Vitamin K- Dependent Coagulation Factors	
Procoagulants	**Regulatory Proteins**
Prothrombin (II)	Protein C
VII	Protein S
IX	Protein Z
X	

"2 + 7= 9 and then there is 1 more (factor 10)" – Dr. Theil

Fibrinolysis

Fibrinolysis

Final stage of hemostatic activation

Hydrolysis of fibrin by plasmin*

To localize fibrinolysis, fibrinolytic proteins become incorporated into the fibrin clot as it is forming

Begins several hours after fibrin polymerization and cross linking and in response to inflammation and coagulation

The activators of fibrinolysis convert fibrin-bound plasminogen → plasmin

- Tissue Plasminogen Activator (TPA)
- Urokinase Plasminogen Activator (UPA)

Degrades fibrin and restores normal blood flow during vascular repair

Plasminogen and Plasmin

Plasminogen

- Plasma zymogen produced by the liver
- Binds to fibrin molecule during the polymerization process through loops called kringles
  - Essential to fibrinolysis

Fibrin-bound plasminogen → two chain active plasmin when cleaved by neighboring bound TPA and UPA

Plasminogen and Plasmin

Plasmin

- Serine protease
- Digests fibrin polymer by hydrolysis
  - Leads to exposed carboxyl-terminal lysine that will bind additional plasminogen and TPA
    - Further accelerate plasminogen activation

Bound localized plasmin digests clots and restores blood vessel patency

Free plasmin in circulation is capable of digesting plasma fibrinogen, FV, FVIII, and fibronectin

- Inactivated when bound to plasma α_2-antiplasmin

Plasminogen Activators

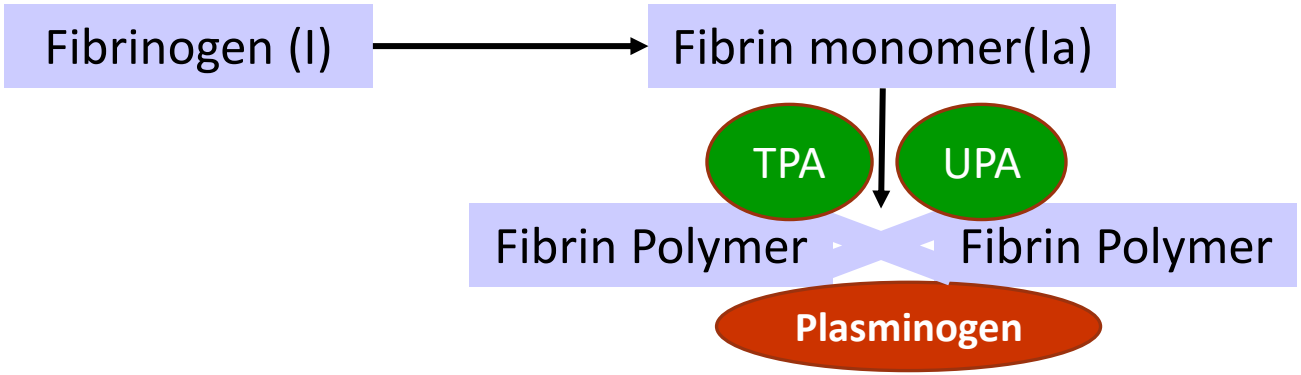
Tissue plasminogen activator (TPA)

- Major plasminogen activator
- Secreted by endothelial cells
- Initiates fibrinolysis
- Hydrolyzes fibrin-bound plasminogen → plasmin
- Binds with fibrin during polymerization with plasminogen
- Circulates bound to inhibitor (PAI-1) *

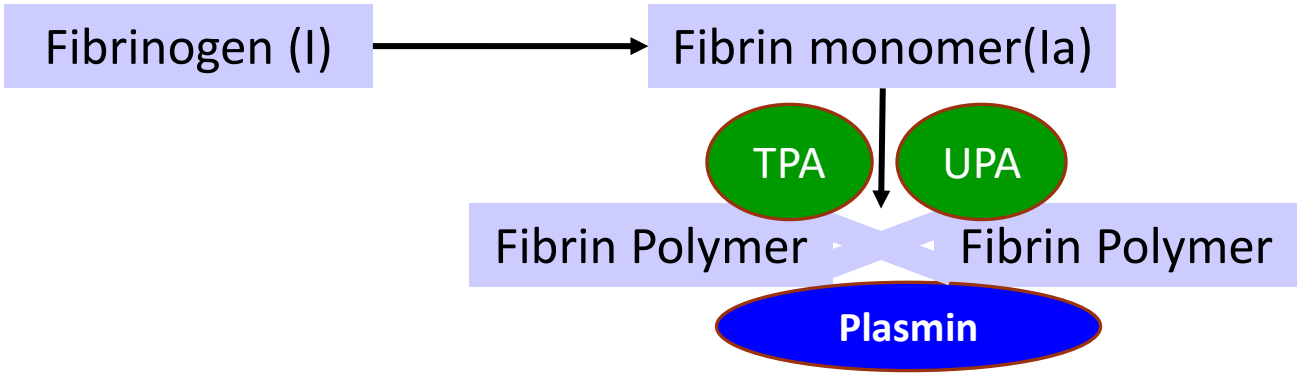
Urokinase plasminogen activator (UPA)

- Secreted by endothelial cells, monocytes, and macrophages
- Circulate in plasma and bind to fibrin, plasminogen, and TPA during clot formation
- Converts plasminogen → plasmin
- Minor physiologic effect

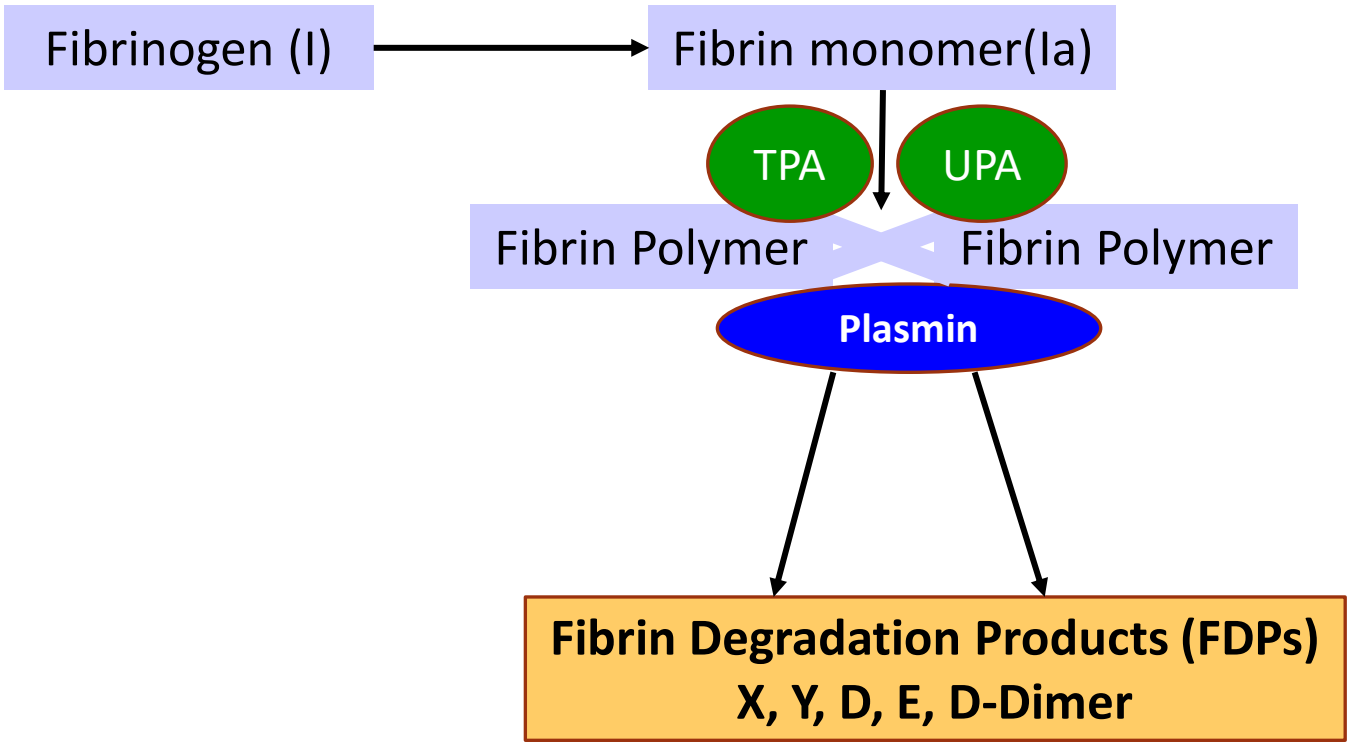
Fibrinolysis



Fibrinolysis



Fibrinolysis



Fibrin Degradation Products (FDPs)

- Fibrin fragments:
  - X, Y, D, E
  - D-D fragment (D-Dimer)
    - 2 D domains from separate fibrin molecules linked by FXIIIa
- Fragments X,Y, D, and E are produced by digestion of either fibrin or fibrinogen by plasmin
- D-dimer is a specific production of digestion of cross-linked fibrin only
  - Marker of thrombosis and fibrinolysis

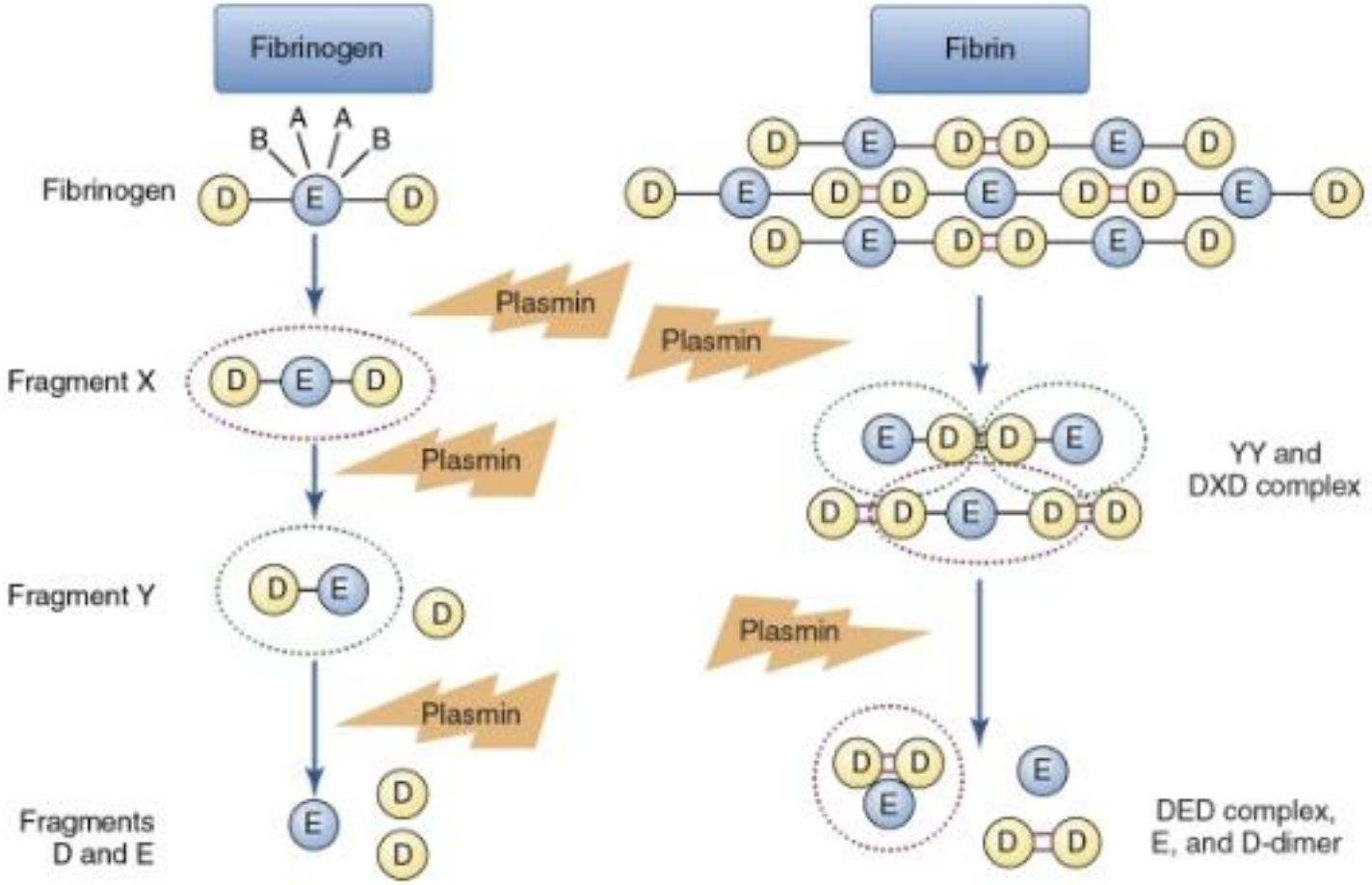


FIGURE 35.18 Fibrinolysis: Degradation of Fibrinogen and Fibrin by Plasmin. Plasmin systematically degrades fibrinogen and fibrin by cleaving off small peptides and digesting D-E domains. From fibrinogen, fragment X consists of a central E domain with two D domains (D-E-D); further cleavage produces fragment Y (D-E), with eventual degradation to D and E domains. From cross-linked, stabilized fibrin (note double red line on the D domains), plasmin digestion produces fragment complexes from one or more monomers. D-dimer consists of two D domains from adjacent monomers that have been cross-linked by factor XIIIa in the process of fibrin formation.

Plasminogen Regulators

3 regulators:

Plasminogen Activator Inhibitor-1 (PAI-1)

- Inactivates both TPA and UPA
- Produced by ECs, megakaryocytes, smooth muscle cells, and other cell types
- Platelets store a pool of PAI-1
- Higher concentration than TPA in circulation

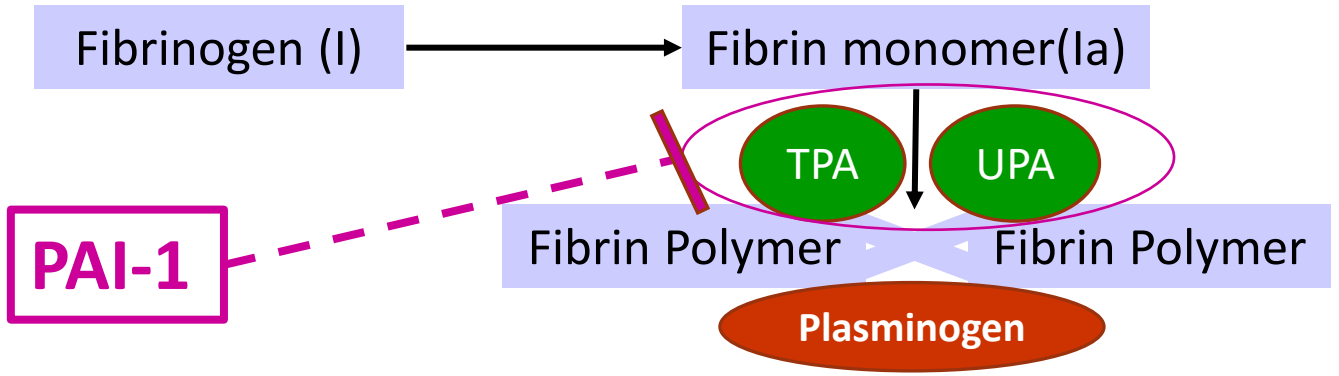
α_2-Antiplasmin

- Synthesized by the liver
- Primary inhibitor of free plasmin

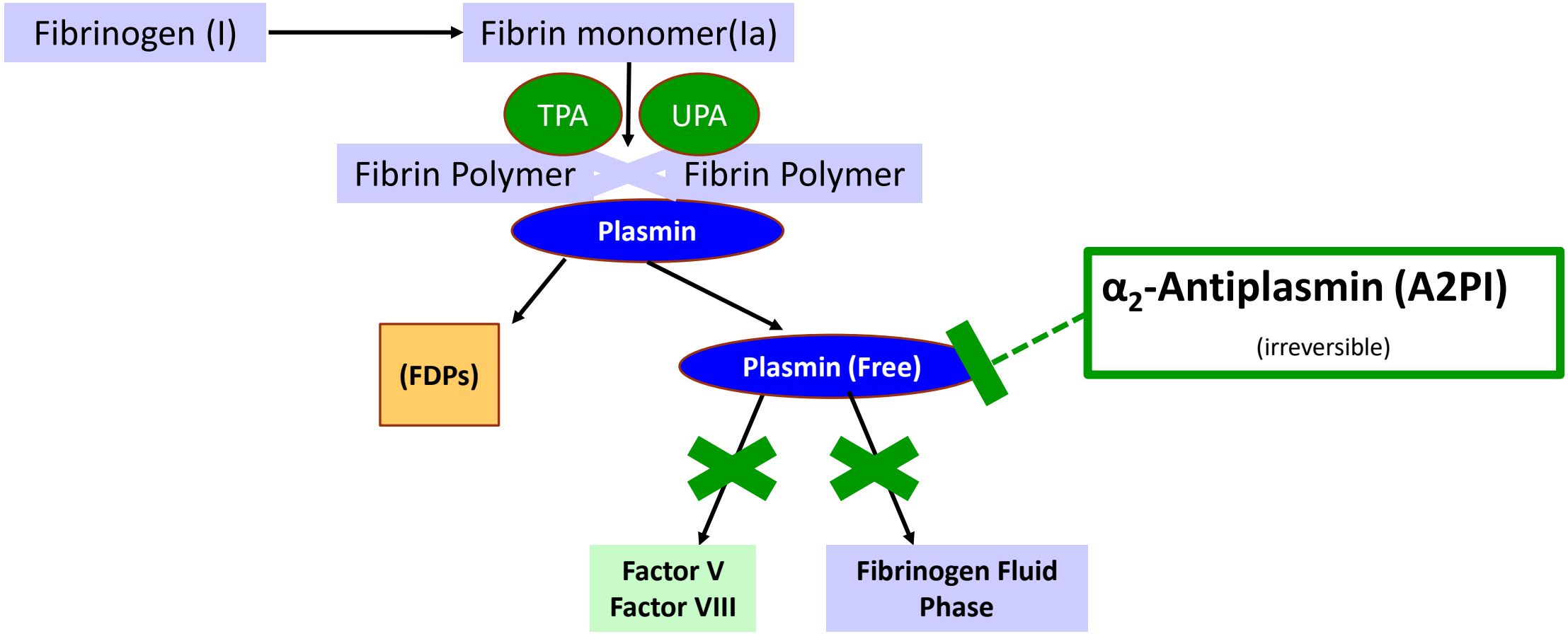
Thrombin Activatable Fibrinolysis Inhibitor (TAFI)

- Synthesized by the liver
- Activated by thrombin-thrombomodulin complex
- Inhibits fibrinolysis by cleaving resides from partially degraded fibrin- prevent binding of TPA and plasminogen

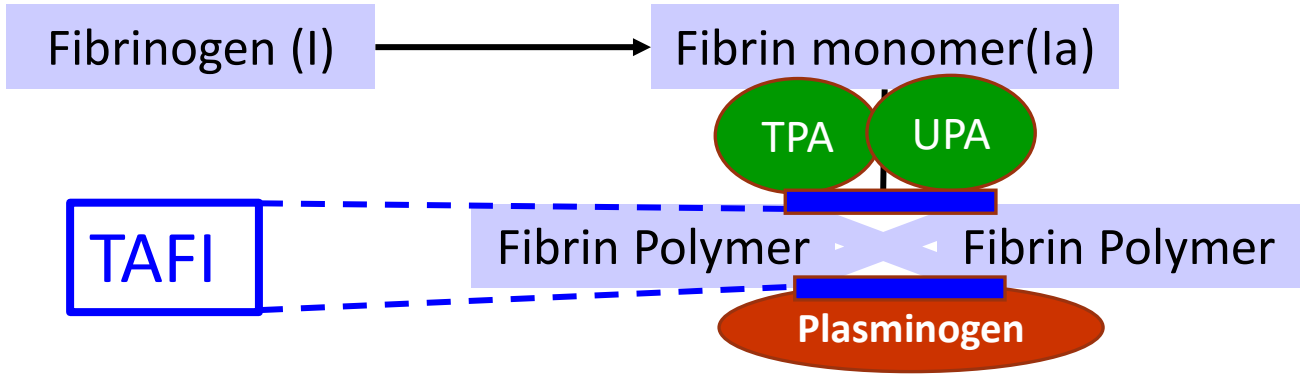
Plasminogen Activator Inhibitor (PAI-1)



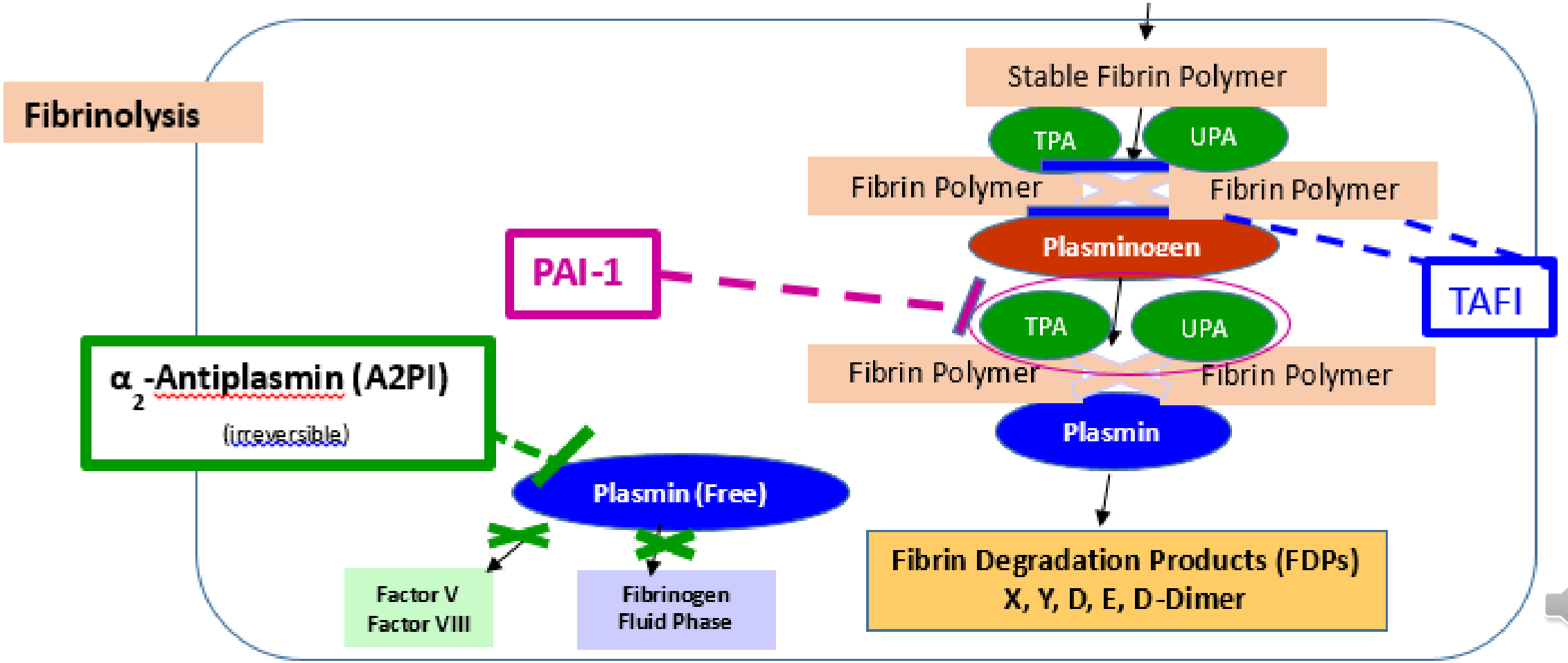
α_2-Antiplasmin (A2PI)



Thrombin Activatable Fibrinolysis Inhibitor



Cascade with Fibrinolysis and Regulators



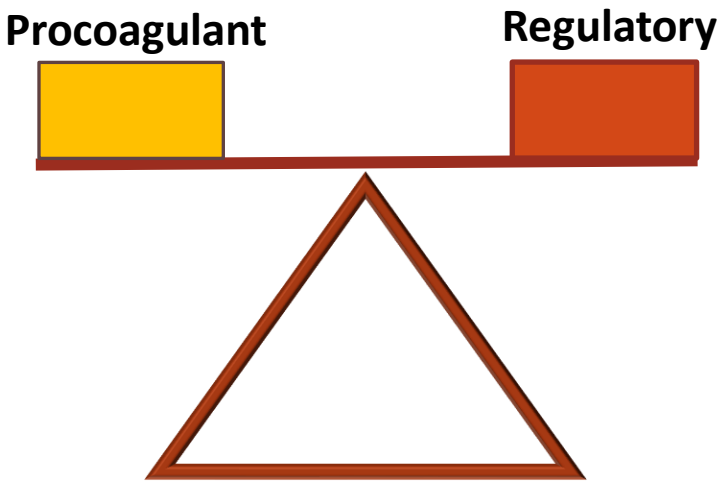
Hemostasis Balance

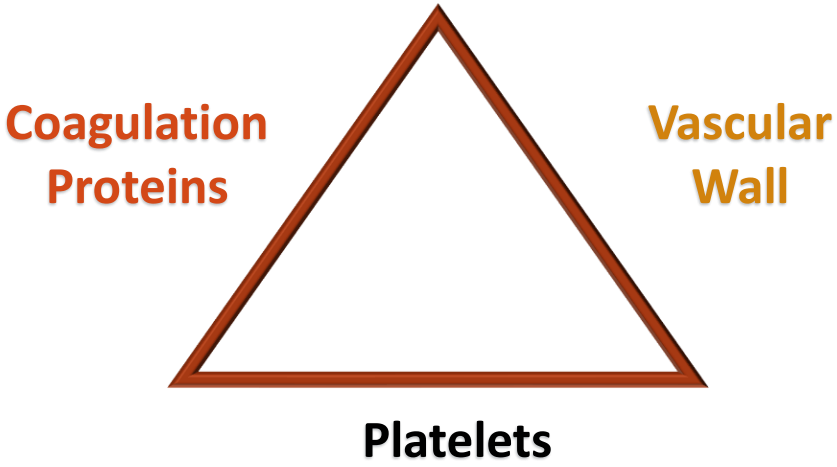
Factors ↓ → ↓ Thrombin Production → ↓ TAFI Activation → ↑ Fibrinolysis **Bleeding**

Factors ↑ → ↑ Thrombin Production → ↑ TAFI Activation → ↓ Fibrinolysis **Thrombosis**

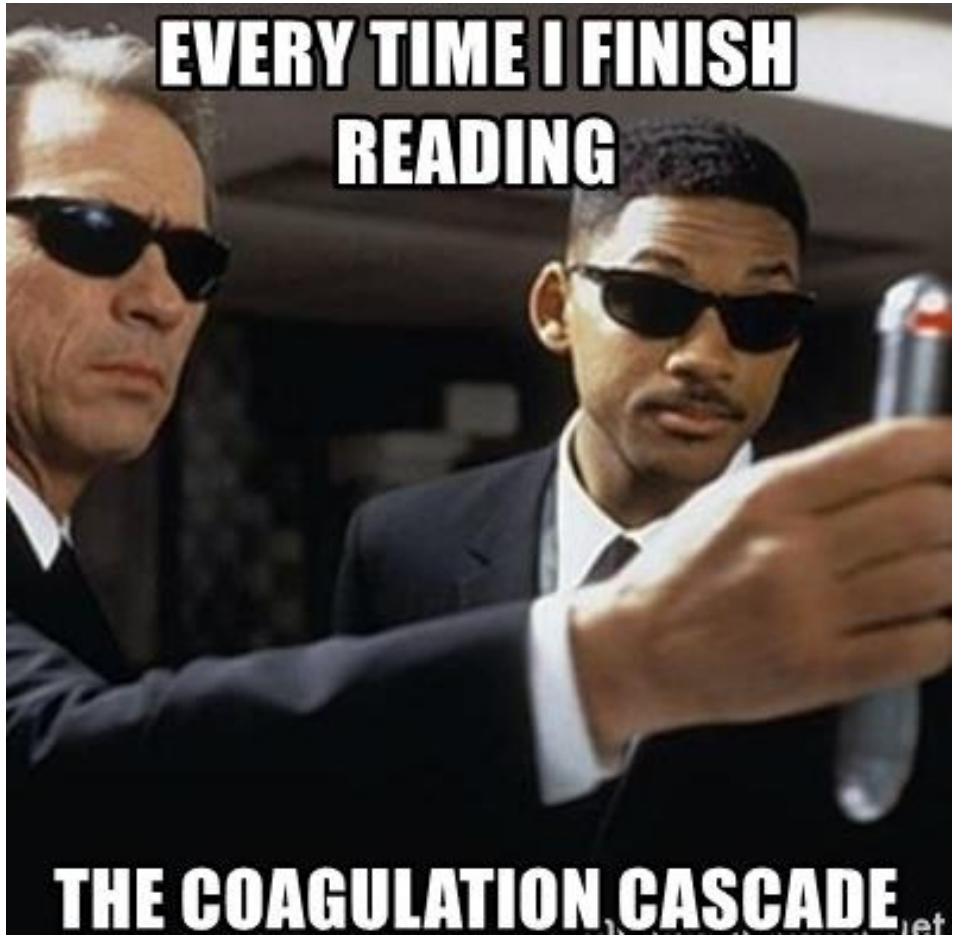
Hemostasis Balance

Ability to maintain blood in a fluid state and prevent loss form sites of vascular damage





FIN



References

Rodak's Hematology 6th edition

Barb Emboden MLS(ASCP) CM