The Coagulation System

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Disclosures: Gerald A Soff MD

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  - Amgen
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  - Dova Pharmaceuticals
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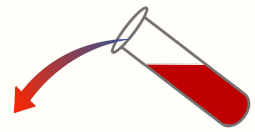

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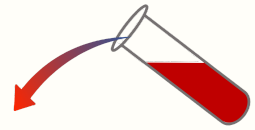
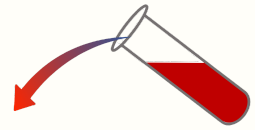
What We'll Cover

1. Overview of Hemostasis and Coagulation
2. The "Classic Coagulation Cascade"
3. The Structure of the Coagulation Factors
4. Two Paths To Initiate Coagulation: Intrinsic and Extrinsic Systems
5. Overview of the Contact Phase:
   Initiation of Intrinsic Pathway
6. Fibrinogen: Fibrin
7. Limitations of the Classic Coagulation Cascade
8. Cell-Based Coagulation Model:
   Assembly Of Enzyme/Cofactor/Substrate Complex On Phospholipid Surface
9. "Cross-Over" of Extrinsic and Intrinsic Pathways
10. Activation of Factors V, VIII, XI, XIII by Thrombin: Thrombin Burst
11. Physiologic Anticoagulant Processes

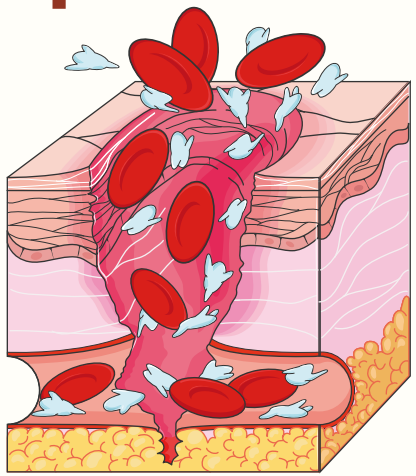


Overview of Hemostasis and Coagulation

- Hemostasis: The processes of keeping the blood liquid in the vasculature.
  - Prevention of hemorrhage following vascular injury.
  - Prevention of excessive clotting (thrombosis) in the vasculature.
- Primary Hemostasis
  - Vascular forces (vasoconstriction) and platelet plug formation.
- Secondary Hemostasis
  - The coagulation factors leading to fibrin clot.
- Physiologic Anticoagulation processes
  - Neutralize activated factors where vessels are intact.
  - Fibrinolysis



Major Components of Hemostasis



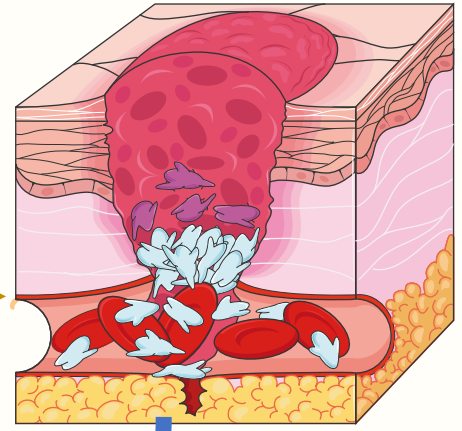
Vascular Injury

Hemostasis

Vasoconstriction

Platelet Plug Formation

Coagulation Cascade



Primary Hemostasis

Secondary Hemostasis

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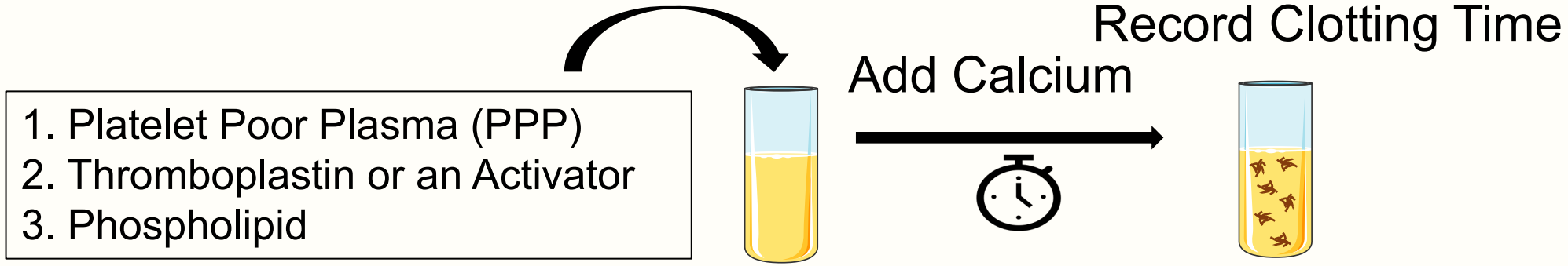
The "Classic Coagulation Cascade" Early Understanding of Coagulation

- The first description of coagulation factors is attributed to Dr. Paul Morawitz in 1905.
- Factor I – Fibrinogen
- Factor II – Prothrombin
- Factor III – Thromboplastin Factor
  - (Tissue extract with Tissues Factor)
- Factor IV – Calcium

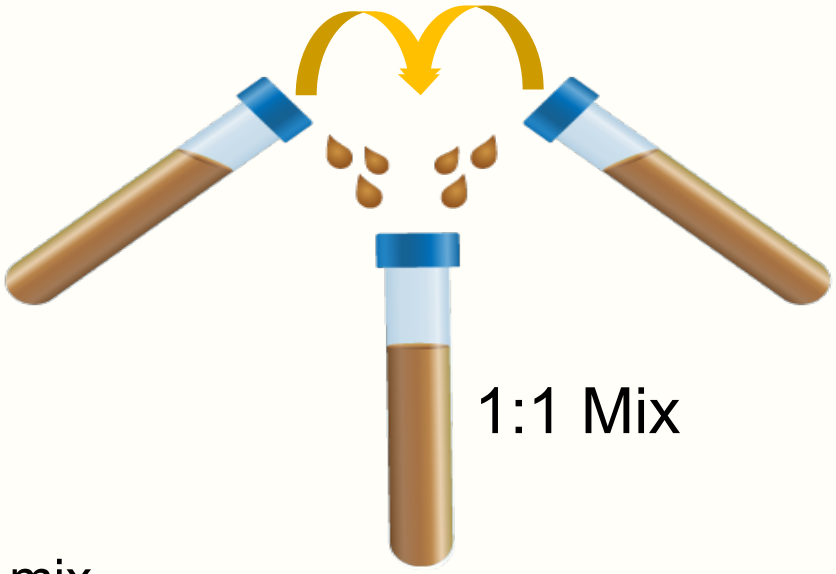
Assay of Coagulation Factors

- In vitro assays developed to test clotting times *in vitro.*
- The prothrombin time in 1935.
- Partial Thromboplastin Time in 1953.
- Led to identification of different coagulation factors.
- Plasma from a patient (and affected family members) with a hereditary bleeding disorder results in slower clotting *in vitro* in one or both assays.
  - The deficient factor was typically named with the family name and Roman numeral.
  - (i.e. Hageman Factor = Factor XII)



Mixing patient plasma with plasma of known deficiency used to determine if the factor deficiency was known or novel.

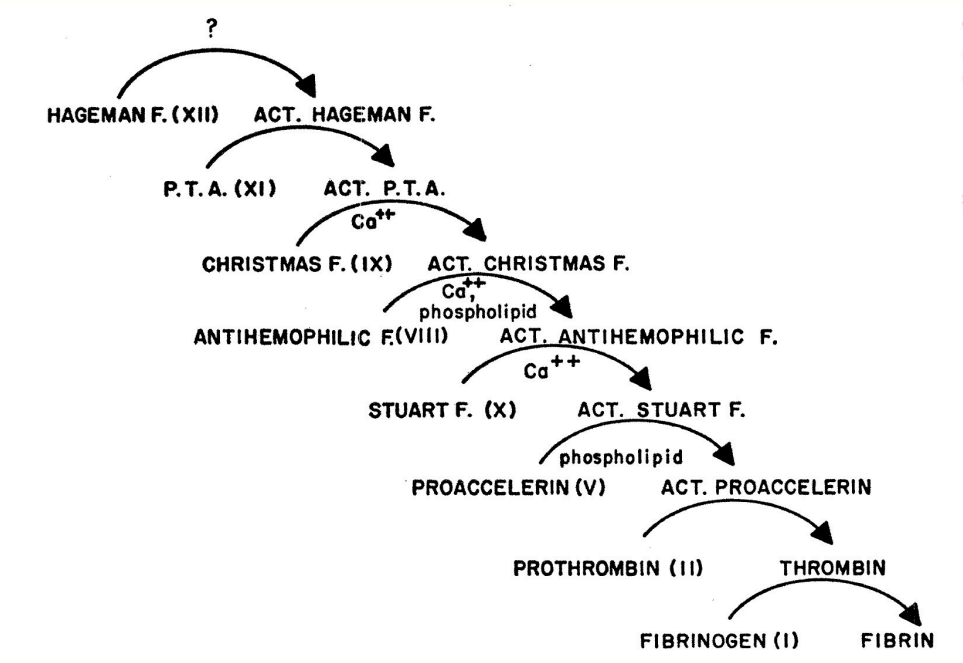
Plasma from patient with unknown bleeding disorder

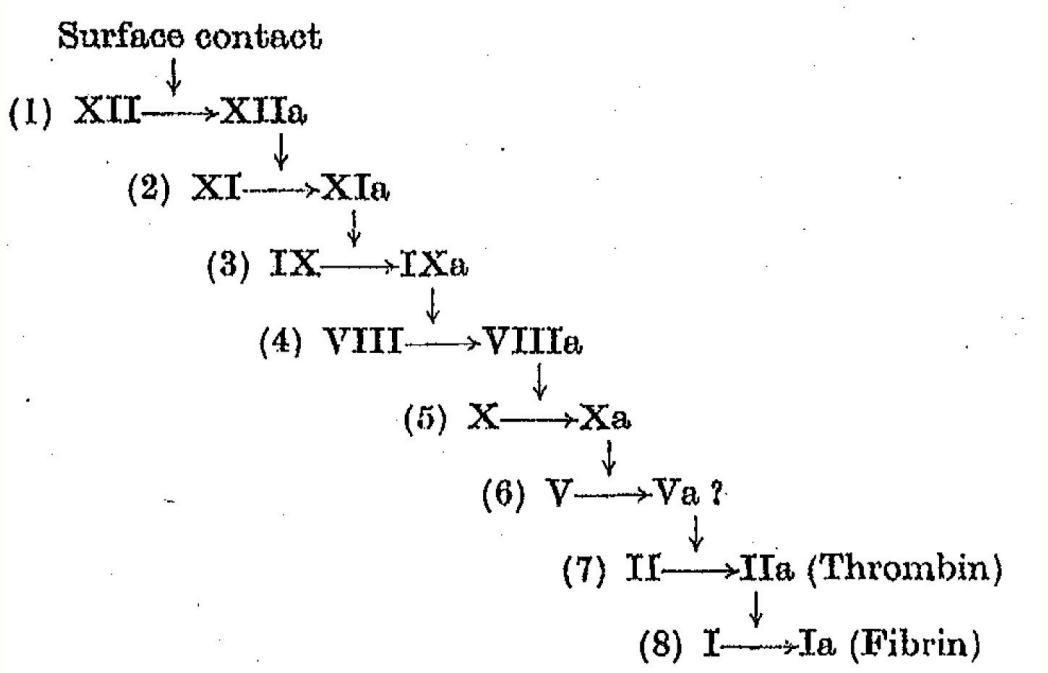


Plasma from patient with known bleeding disorder (i.e. Hemophilia A)

- Perform Clotting Time on 1:1 mix.
- If the 1:1 mix "corrects," then the unknown sample has a different deficiency than the known deficient.
- If the 1:1 mix remains prolonged, then the unknown sample has the same deficiency as the known deficient.
- From the 1930s through the 1950s, most of the factors were identified in this way.
- Limitations: Inhibitors will interfere.

Original Publications Of Coagulation Cascade



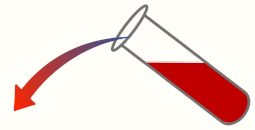


Davie, E. W., and Ratnoff, O. D. "Waterfall sequence for intrinsic blood clotting." Science 1964: 145, 1310–1312

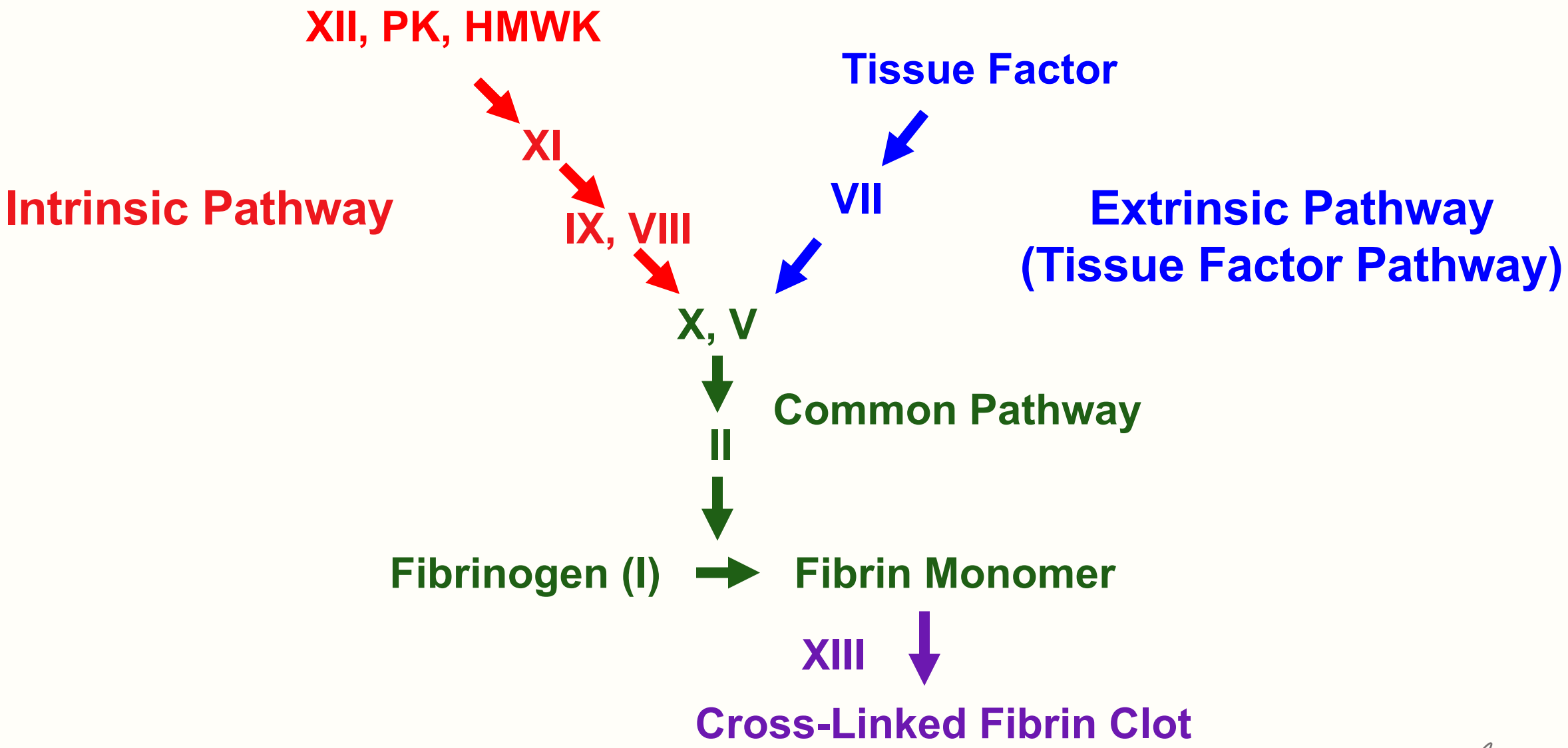
MacFarlane RG. "An enzyme cascade in the blood clotting mechanism, and its function as a biological amplifier." Nature 1964; 202: 498-9

Original Publications Of Coagulation Cascade

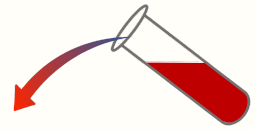
1. Coagulation involves a sequence of reactions.
2. Factors circulate in non-activated forms.
   a. Zymogens or pro-enzymes
   b. Pro-cofactors
3. Factors are activated by proteolytic cleavage by an “upstream factor” and in turn activate a ”downstream factor).
4. Terminology:
   a. Subscript “a” designates activated factor.
   b. Example Factor VIII or FVIII → Factor VIIIa or FVIIIa.
5. A number of gaps or open questions. (To be discussed below).

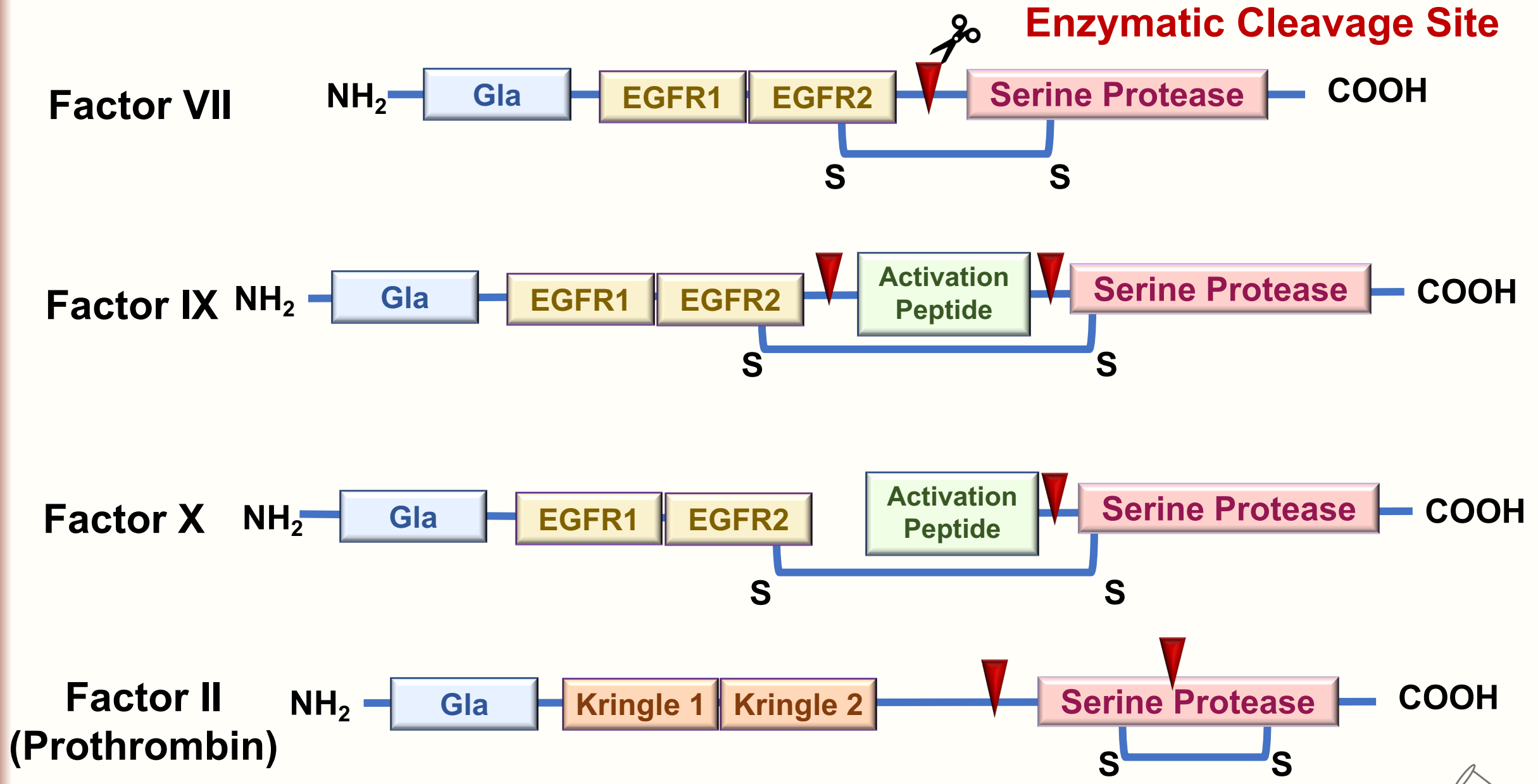


Contemporary Representation of the Coagulation Cascade

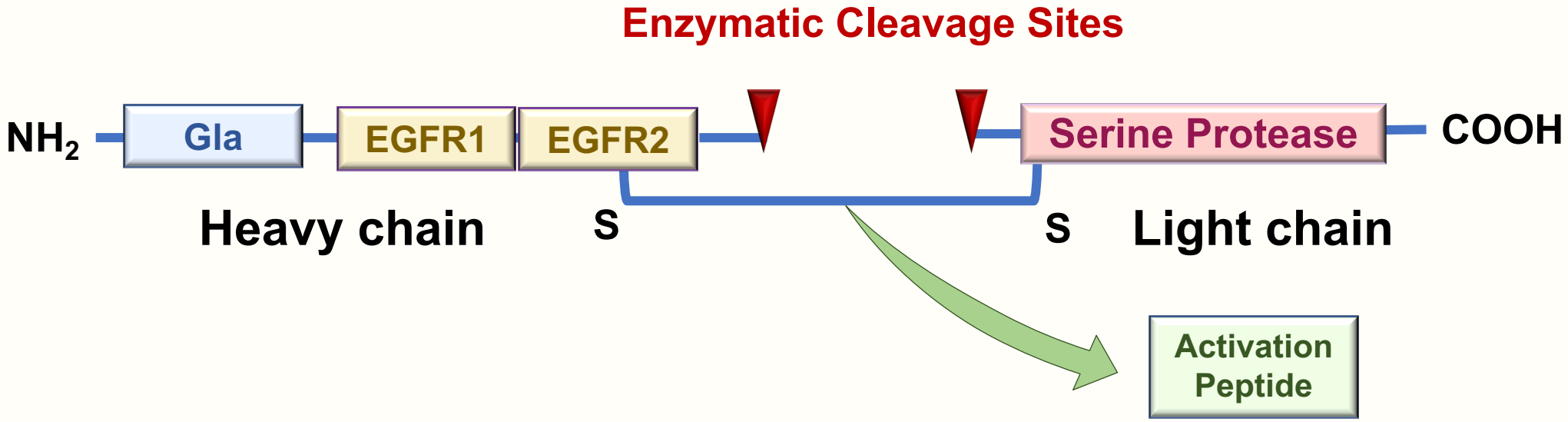


The Structure of the Coagulation Factors



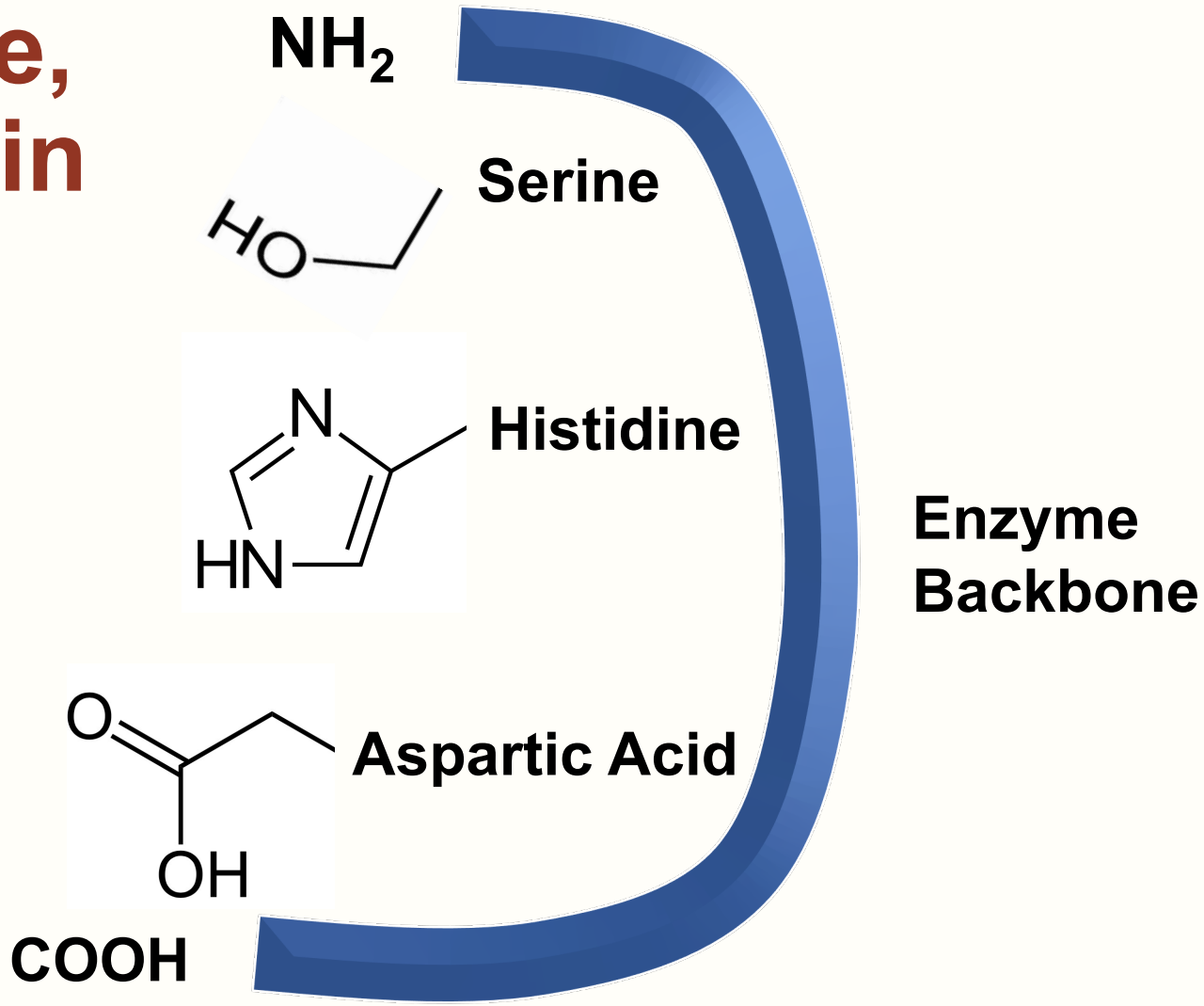

Enzymatic Cleavage Site
Factor VII
NH2
Gla
EGFR1
EGFR2
Serine Protease
COOH
S
S
Factor IX
NH2
Gla
EGFR1
EGFR2
Activation Peptide
Serine Protease
COOH
S
S
Factor X
NH2
Gla
EGFR1
EGFR2
Activation Peptide
Serine Protease
COOH
S
S
Factor II (Prothrombin)
NH2
Gla
Kringle 1
Kringle 2
Serine Protease
COOH
S
S

Factor IX Activation: Two Step Enzymatic Cleavage Site



- After activation, heavy and light chains remain covalently bound by disulfide bonds.
- Heavy chain facilitates binding to substrate.
- Gamma-Carboxyglutamic Acid (Gla) domain is in Heavy Chain.
- Light Chain contains the serine protease enzymatic domain.
- Substrate specificity determined by Heavy Chain binding and structure of the serine protease domain.
  - **Emsley et al. Blood 2010;115:2569-2577**

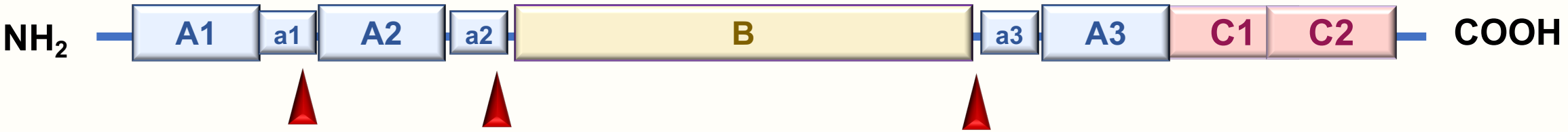
Serine Protease, Catalytic Domain



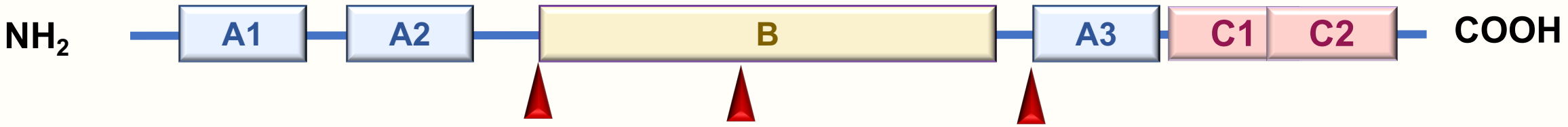
Serine, Histidine and Aspartic acid; amino acids in catalytic domain.

Cofactors

Factor VIII

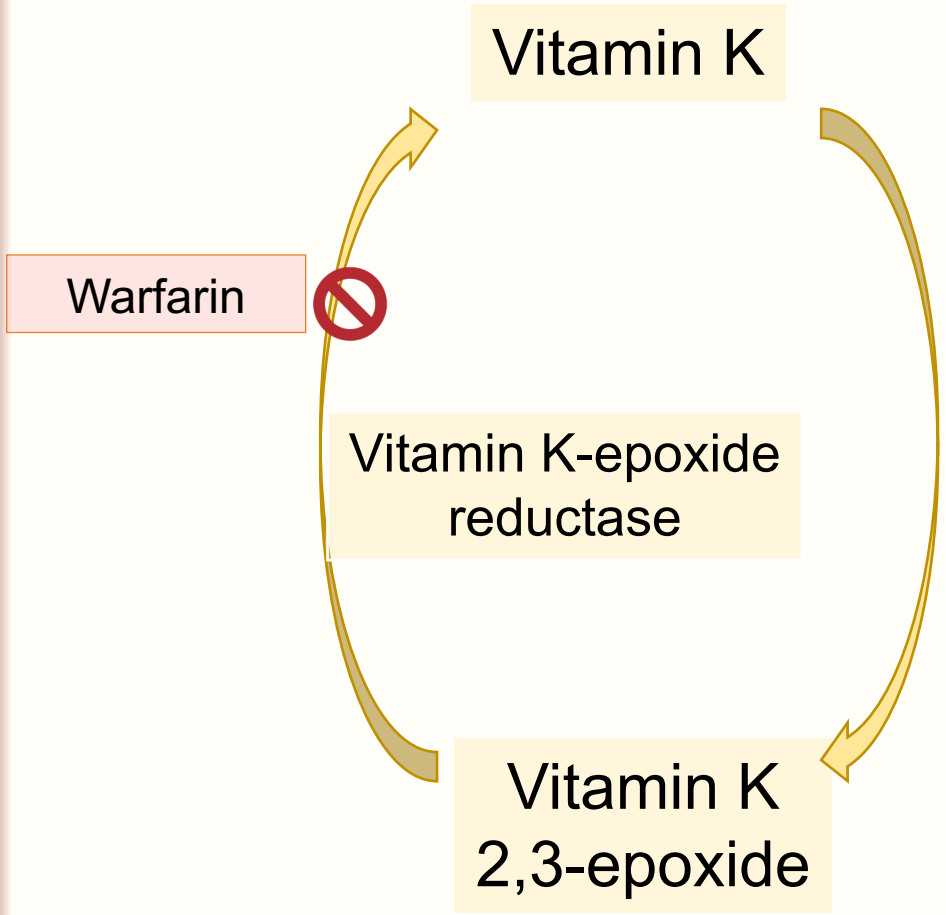


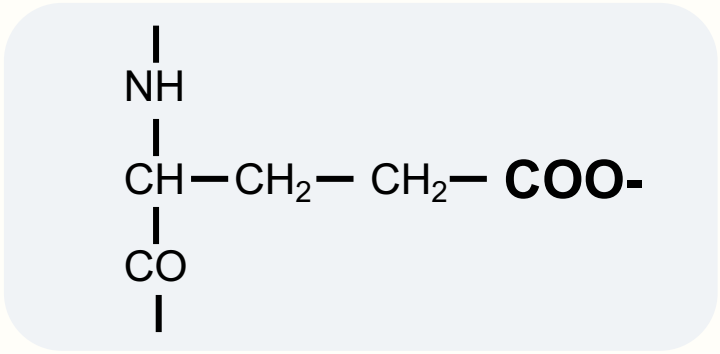
Factor V



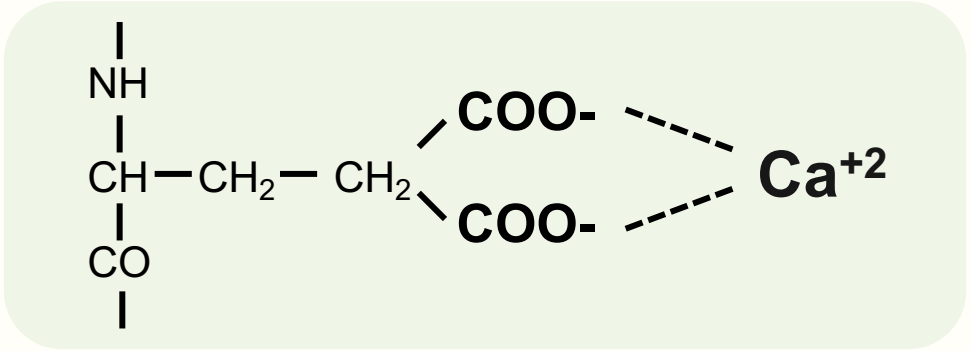
Dahlback B. JTH 15: 1241-1250, 2017
Camire & Bos. JTH, 7: 1951–1961, 2009

Vitamin K Mediated γ-Carboxylation of Glutamic Acid





Glutamic Acid:
Single negative charge

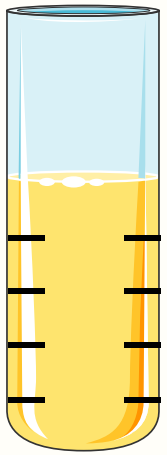


Gamma-Carboxyglutamic Acid (Gla):
Divalent negative charge. Can bind calcium

There Are Two Ways to Initiate Coagulation System *in Vitro*

Intrinsic Pathway:

Initiated by Contact with a Negatively Charged Surface



XII, PK, HMWK

↓

XIIa

Extrinsic Pathway:

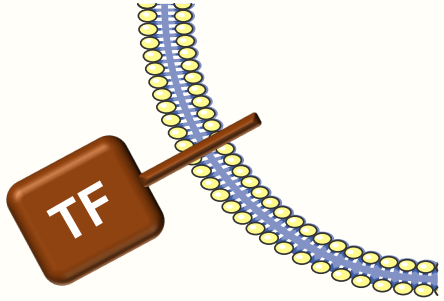
Initiated by addition of Tissue Thromboplastin (Tissue Factor and Phospholipid)

TF:VII

Auto-Activation

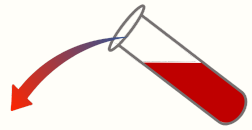
↓

VIIa



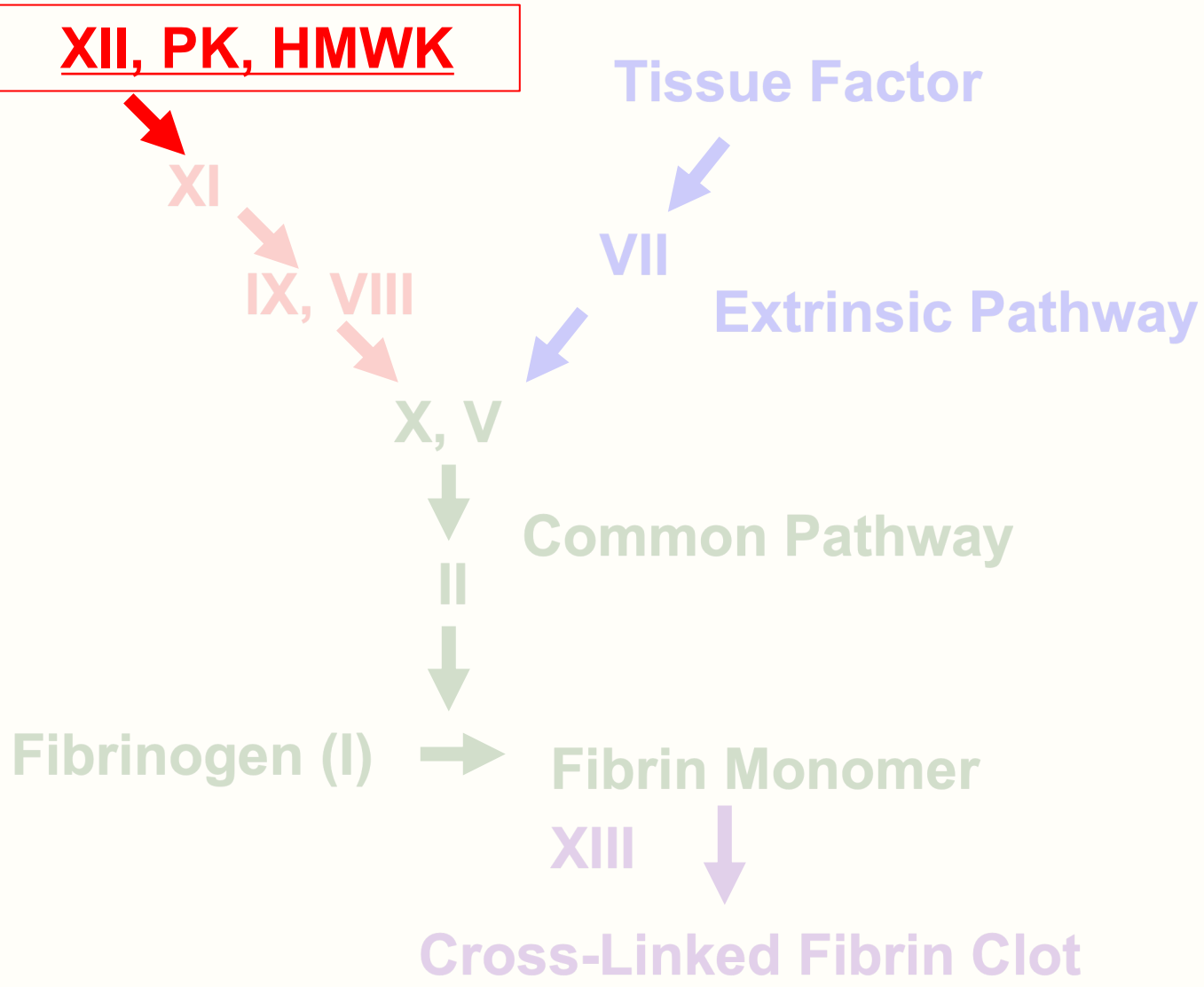
TF bound to cell surface

Overview of the Contact Phase: Initiation of Intrinsic Pathway

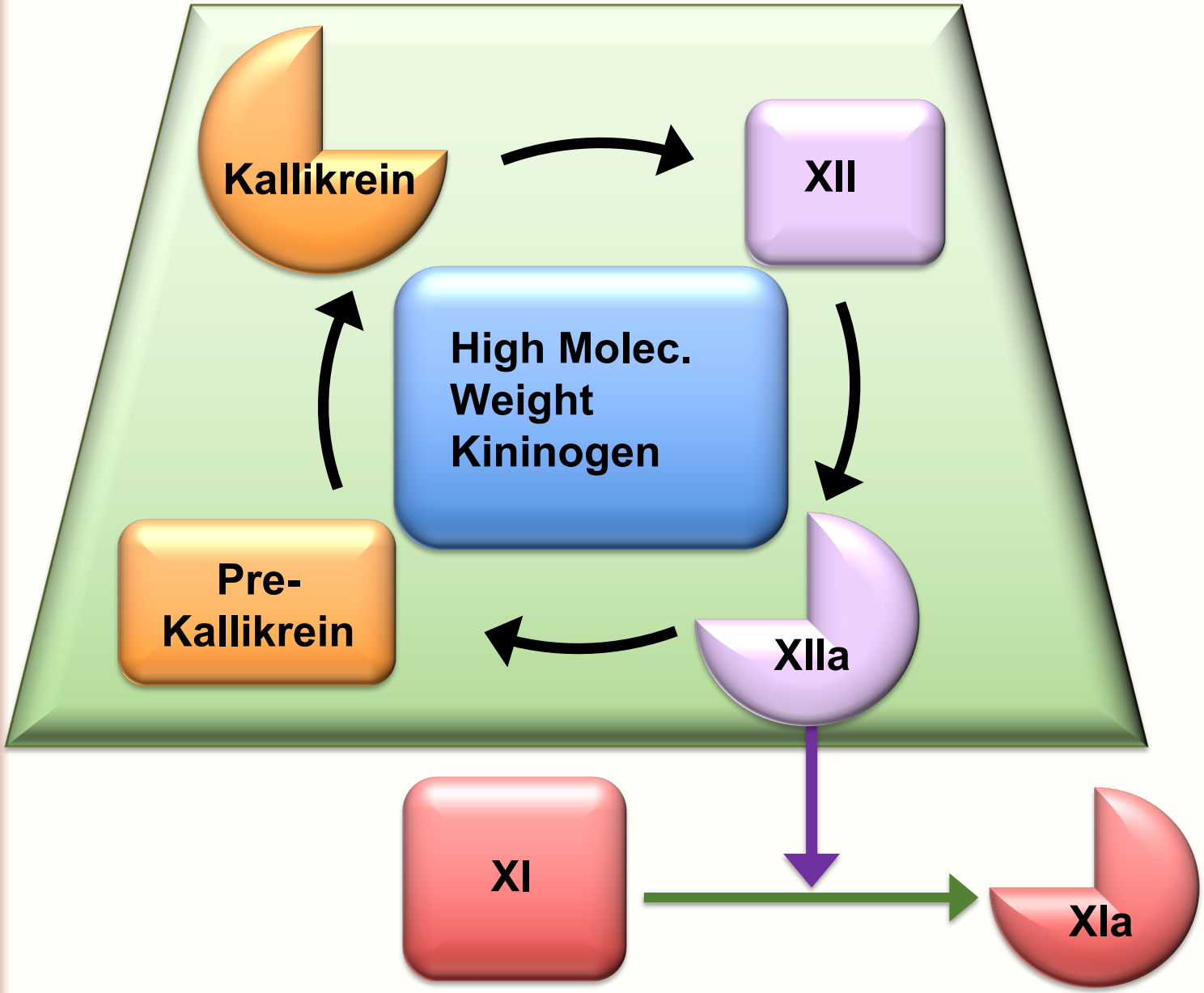


Contact System
(Activated by binding to a negatively charged surface)

Intrinsic Pathway



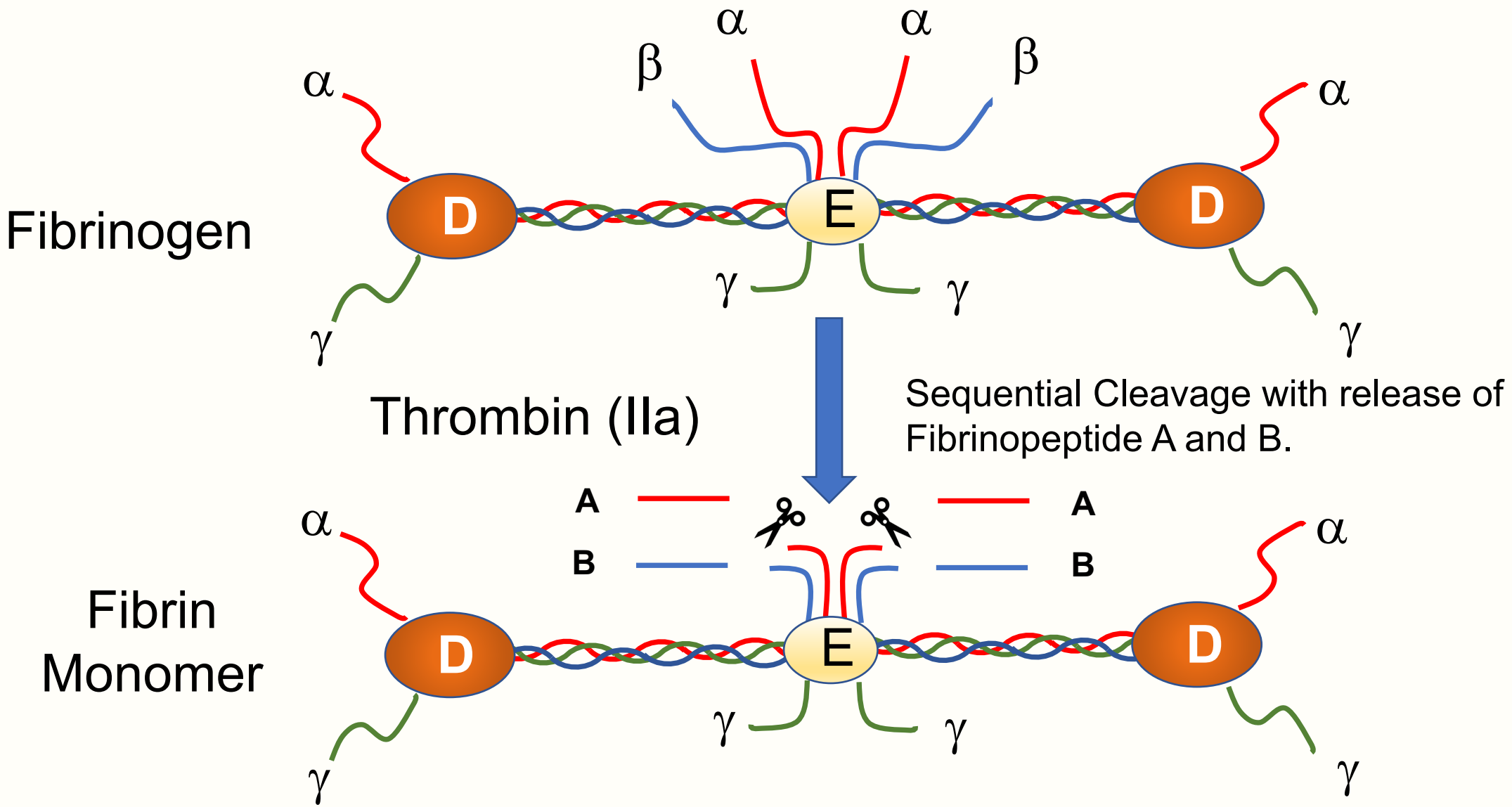
Contact System



- Factor XII
- Prekallikrein
- High Molecular Weight Kininogen

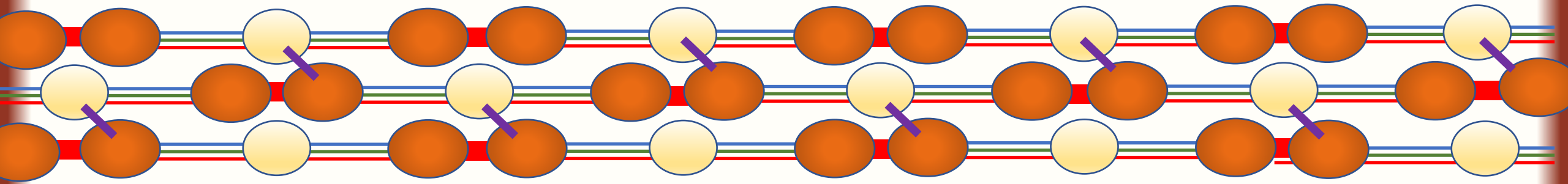
- Minimal contribution to hemostasis in most situations, although FXIIa can activate Factor XI to XIa.
- Deficiencies of the Contact Factors are not associated with bleeding tendency.
- Bradykinin (Derived from HMWK)
- Role in inflammation, vascular tone.

Fibrinogen: Fibrin



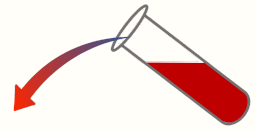
Factor XIIIa (Transglutaminase): Cross-Link Fibrin

XIIIa: Cross-Links Fibrin Clot



Limitations of The Classic Coagulation Cascade

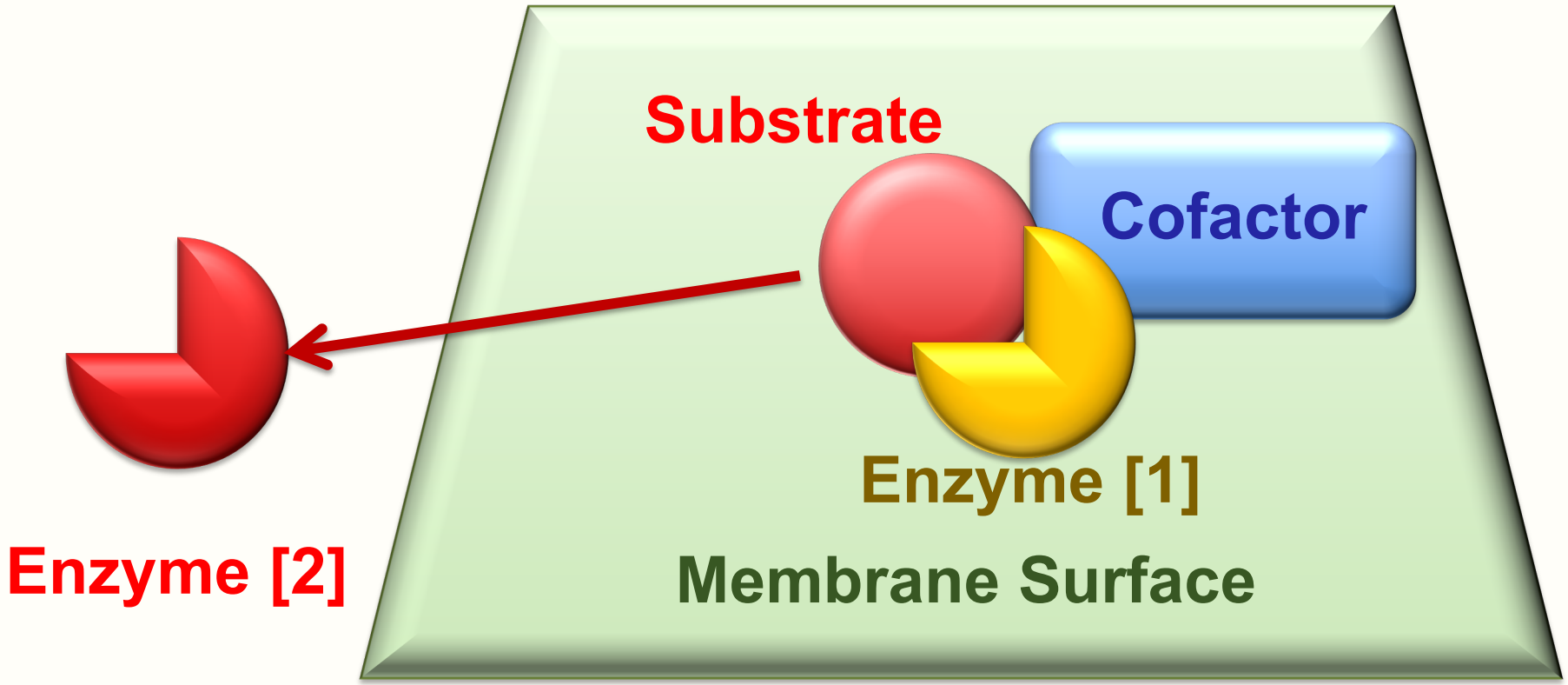
1) For years we have recognized the inconsistencies within these pathways to truly inform us of a patient's hemostatic system.
2) There are markedly different clinical manifestations of deficiencies of different factors, particularly within the Intrinsic Pathway.
   - Why do some deficiencies of the Intrinsic Pathway lead to severe bleeding, while other deficiencies do not cause bleeding?
3) The classic understanding that factors are activated in a "cascade," from top to bottom, is known to be incorrect.
4) Some enzymes have multiple substrates, and some factors can be activated by more than one enzyme.
5) In the following material, we will address these points and clarify the current understanding of the coagulation system.



Cell-Based Coagulation Model: Assembly Of Enzyme/Cofactor/Substrate Complex On Phospholipid Surface

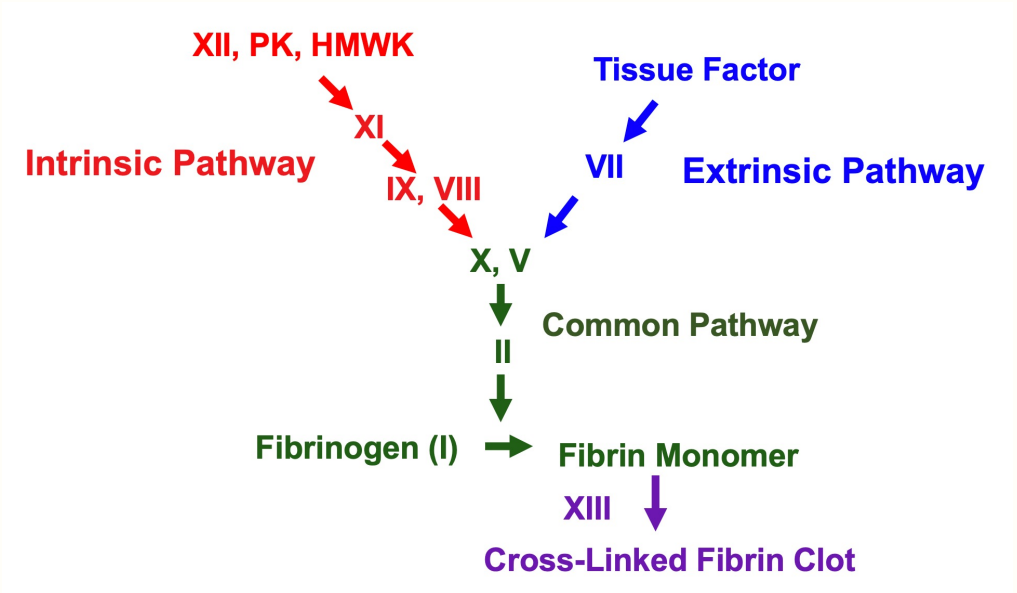
The Cell Based Model Of Coagulation

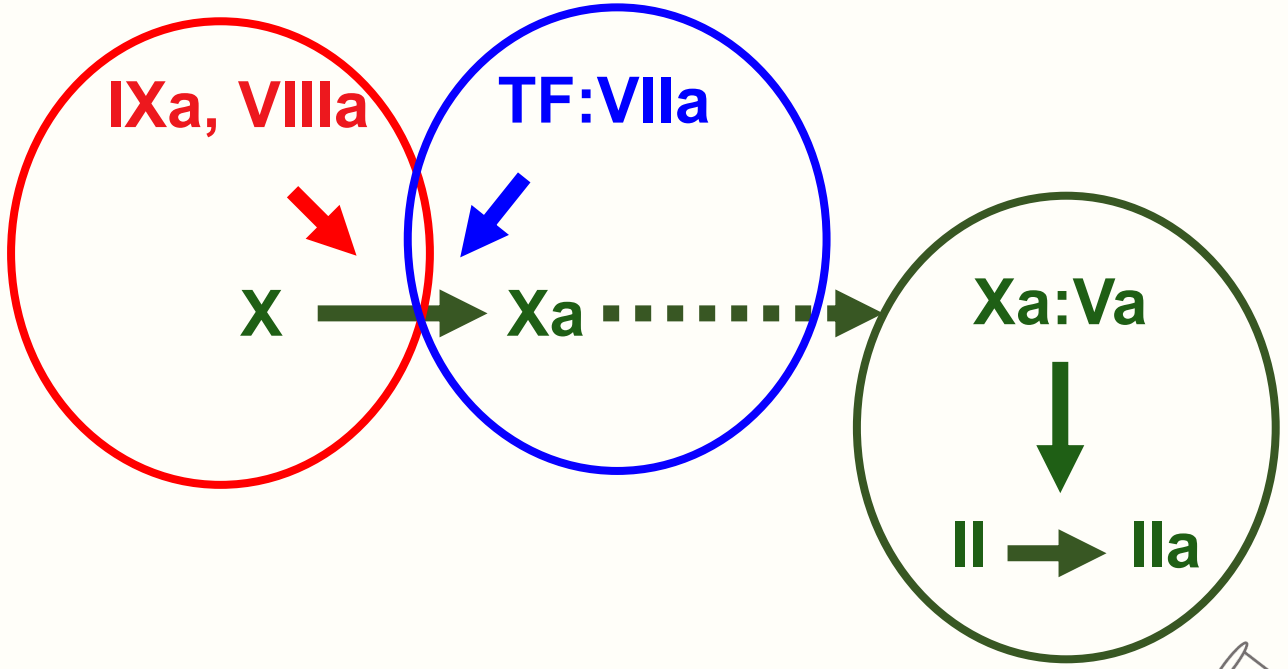
Coagulation is "Best" understood as a series of membrane-bound complexes: enzyme/cofactor/substrate.



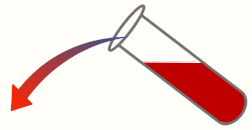
Three Complexes of "Classic Cascade"

Pathway	Complex	Enzyme	Cofactor	Substrate	Product
Extrinsic Pathway	Extrinsic Xase	VII/VIIa	TF	X	Xa
Intrinsic Pathway	Intrinsic Xase	IXa	VIIIa	X	Xa
Common Pathway	Prothrombinase	Xa	Va	II	IIa

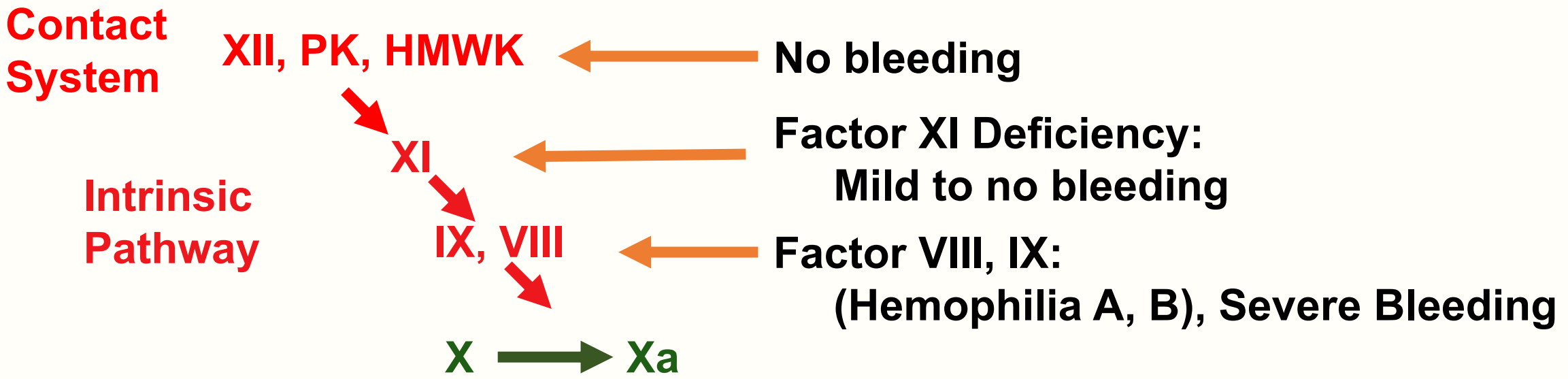




"Cross-Over" of Extrinsic and Intrinsic Pathways



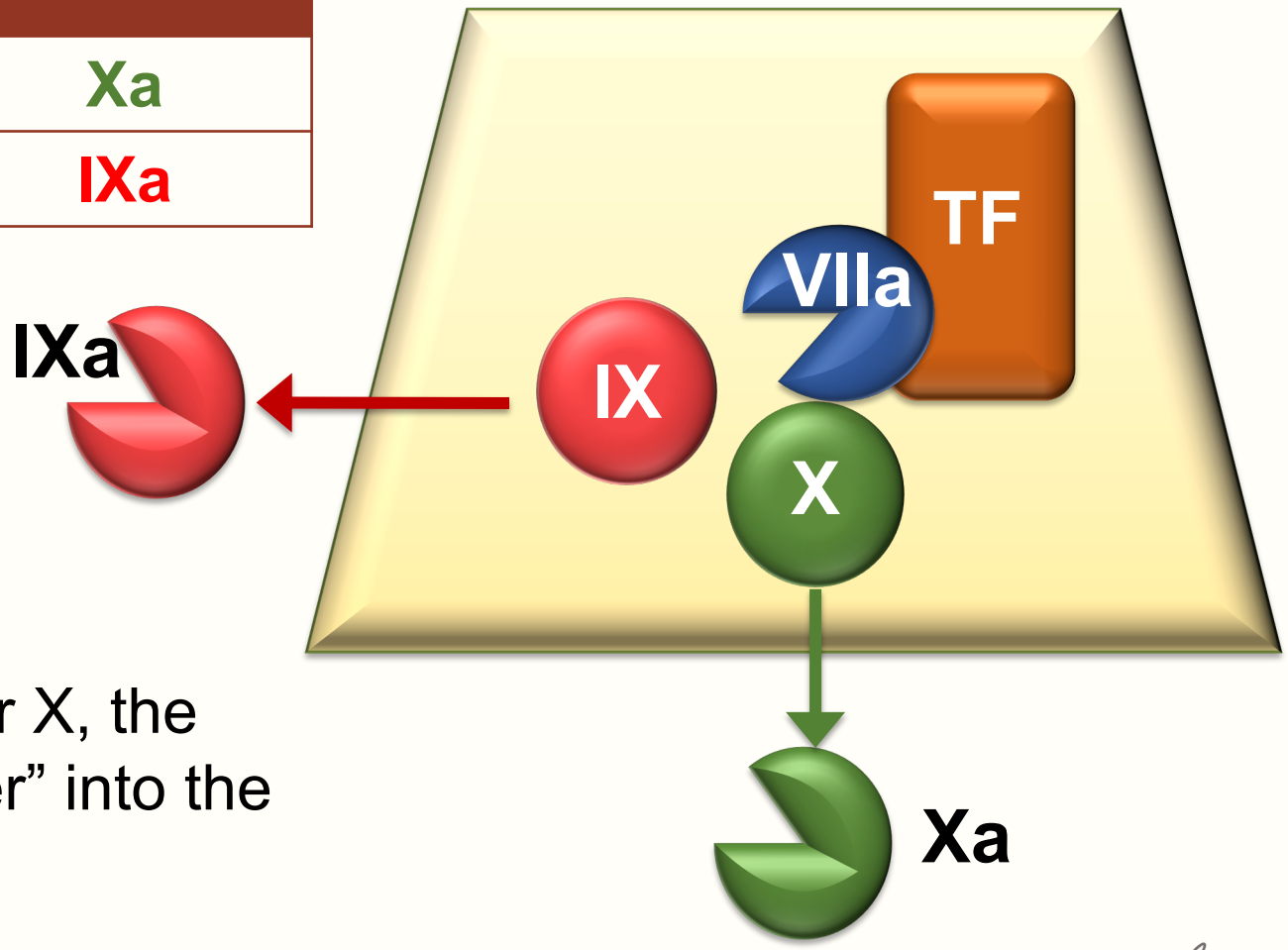
Clinical Manifestation Of Deficiencies of Factors Within the Intrinsic Pathway



- If this is a single pathway, why are different factor deficiencies associated with marked differences in clinical manifestations?
- This indicates our classic coagulation cascade is not the full story.

There Are Two Alternative Substrates Of TF:VIIa

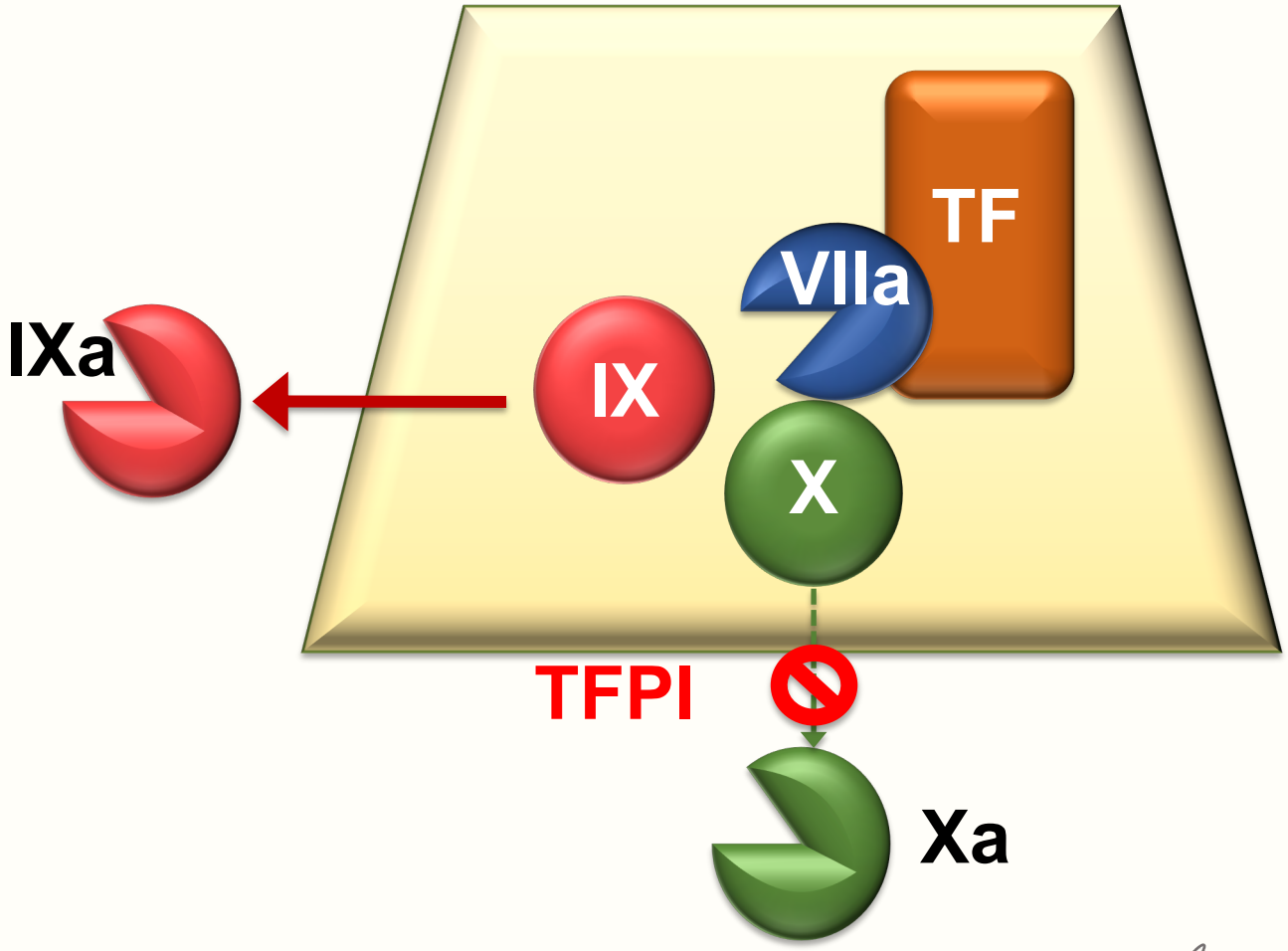
Enzyme	Cofactor	Substrate	Product
VII/VIIa	Tissue Factor	X	Xa
		IX	IXa



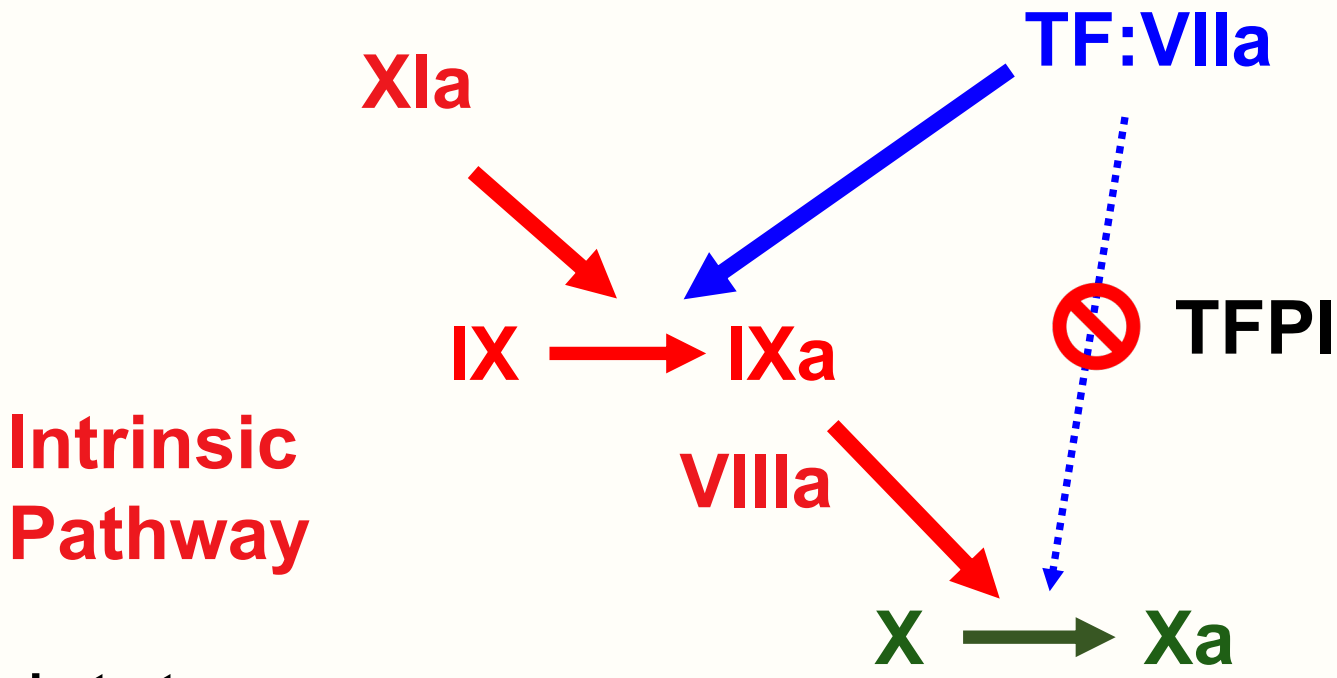
- In addition to activation of Factor X, the Extrinsic Pathway “Crosses Over” into the Intrinsic Pathway.

Tissue Factor Pathway Inhibitor (TFPI)

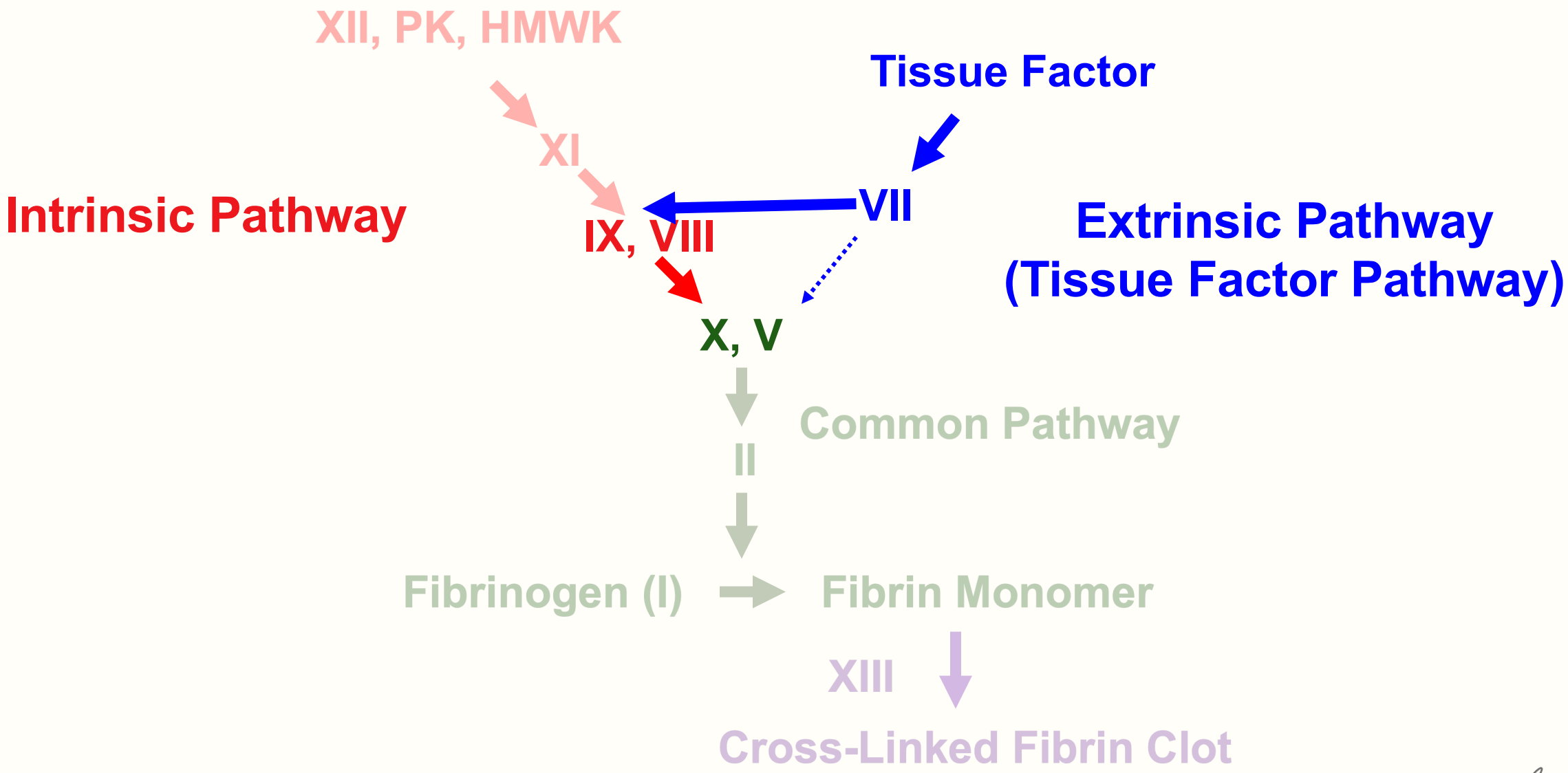
- TFPI inhibits activation of Factor X by TF:VIIa.
- Therefore, *In Vivo, the primary substrate of FVIIa is F IX.*



Factor IX Can Be Activated By TF:VIIa or XIa



- TF:VIIa has two substrates
- FIX can be activated by two different enzymes
- The concept of a simple "cascade," with an ordered process of one factor activating the next, is not the full picture.
- *In vivo, the Common Pathway starts with F VIII and F IX.*

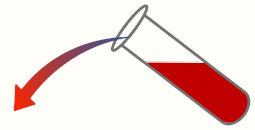

XII, PK, HMWK
Tissue Factor
XI
Intrinsic Pathway
VII
Extrinsic Pathway
(Tissue Factor Pathway)
IX, VIII
X, V
Common Pathway
II
Fibrinogen (I)
Fibrin Monomer
XIII
Cross-Linked Fibrin Clot

The Thrombin Burst:
Activation of Factors V, VIII, XI, XIII by Thrombin:

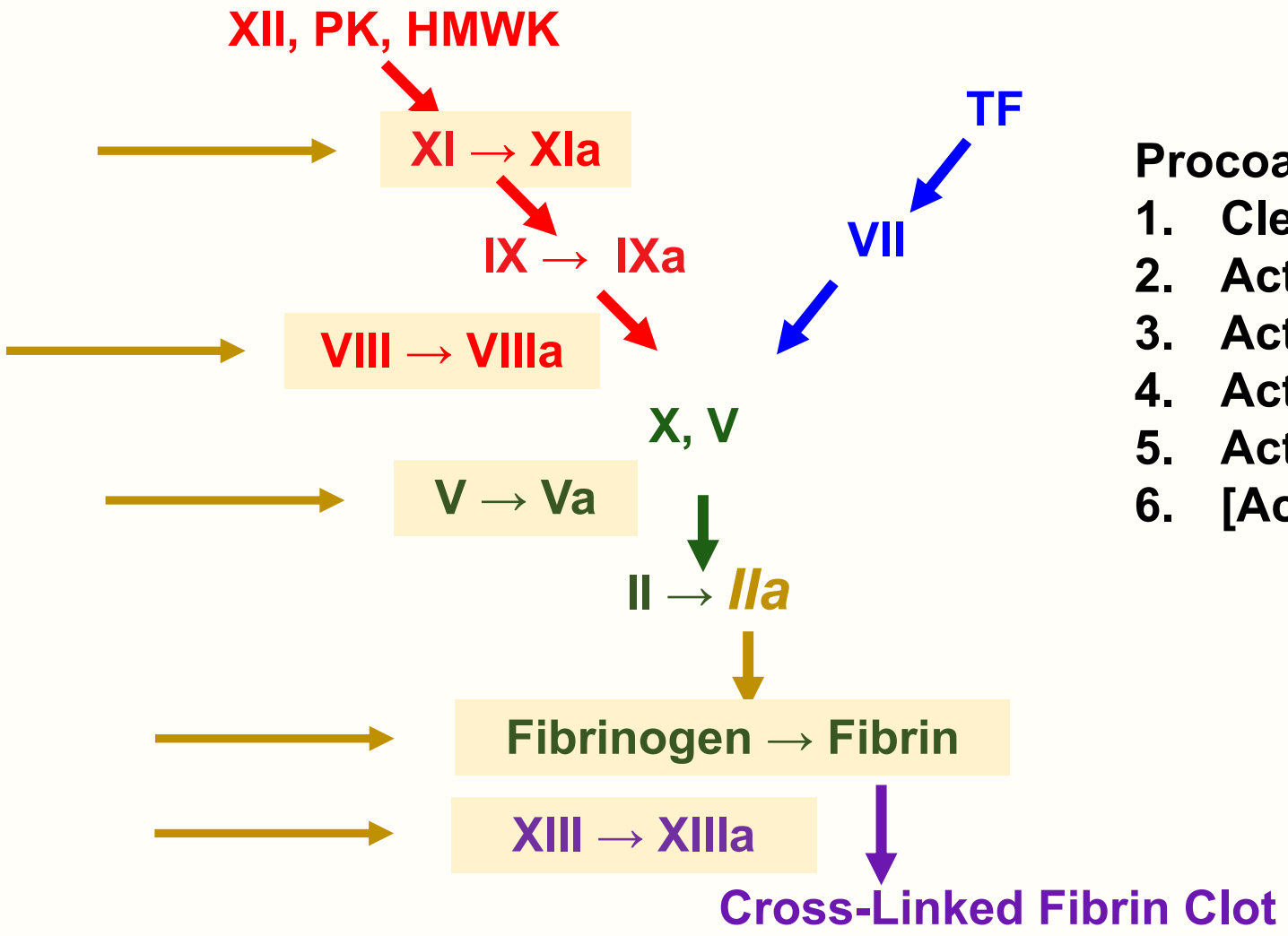
https://commons.wikimedia.org/wiki/File:Most_distant_Gamma-ray_burst.jpg

Thrombin: Multiple Roles In Coagulation

- How are Factors V and VIII activated?
- How is Factor XIII activated?
- Concept of Thrombin Burst: There are several steps within the coagulation cascade where thrombin participates in positive feedback processes, to greatly amplify the pro-coagulant state.



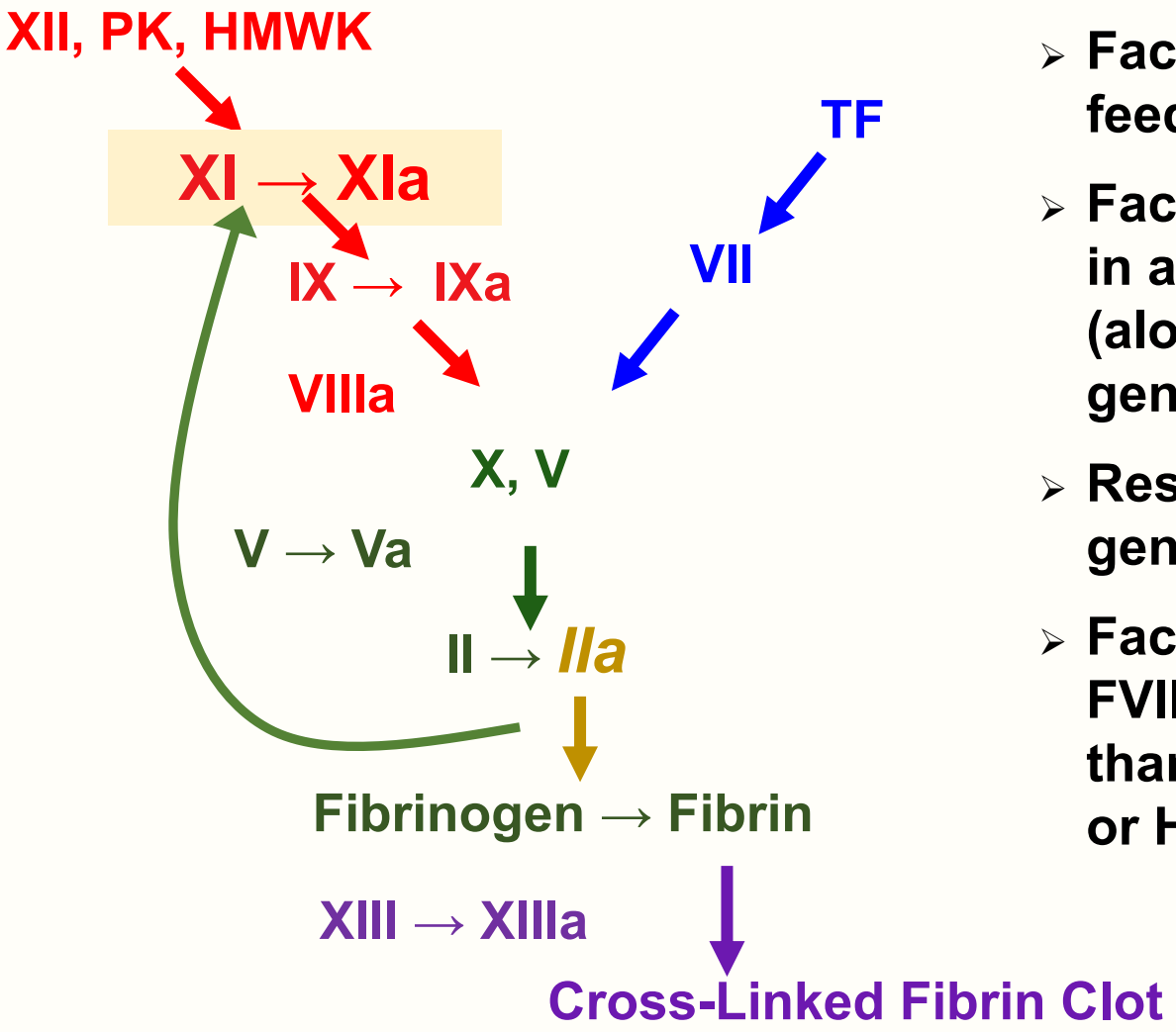
Thrombin Feedback;
Activation of Factors V, VIII, XI, XIII



Procoagulant Activities of Thrombin

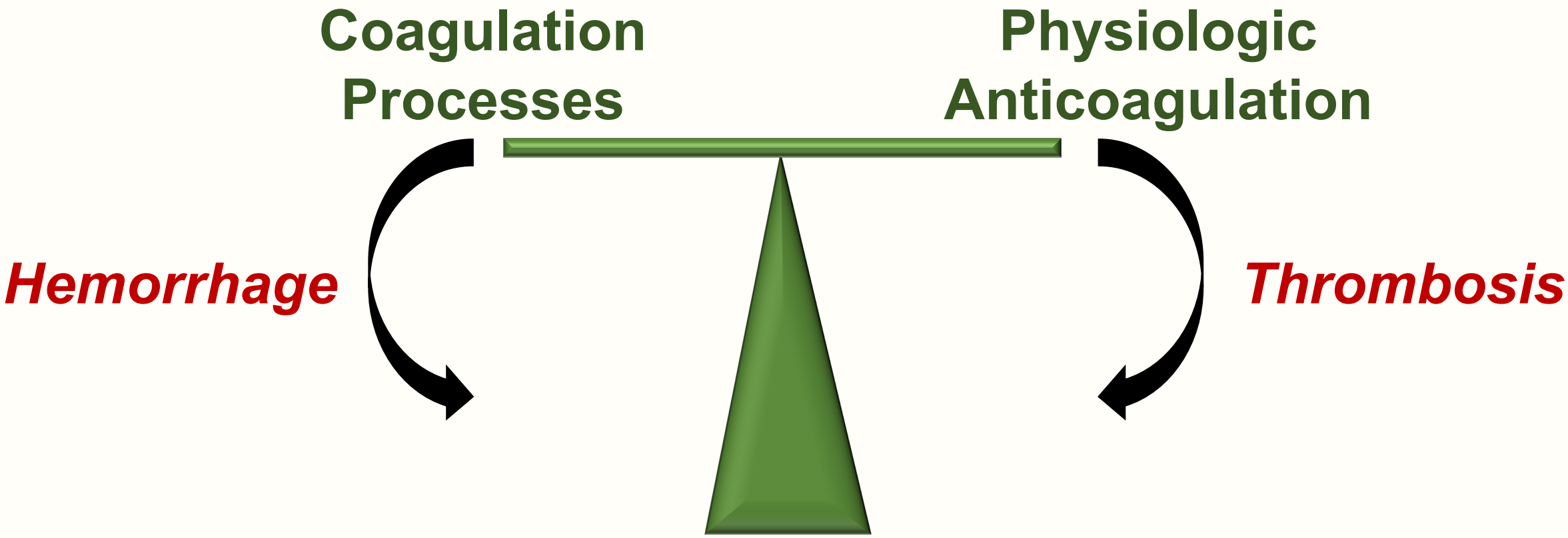
1. Cleavage of Fibrinogen
2. Activation of Factor V
3. Activation of Factor VIII
4. Activation of Factor XI
5. Activation of Factor XIII
6. [Activation of Platelets]

Role of Factor XI

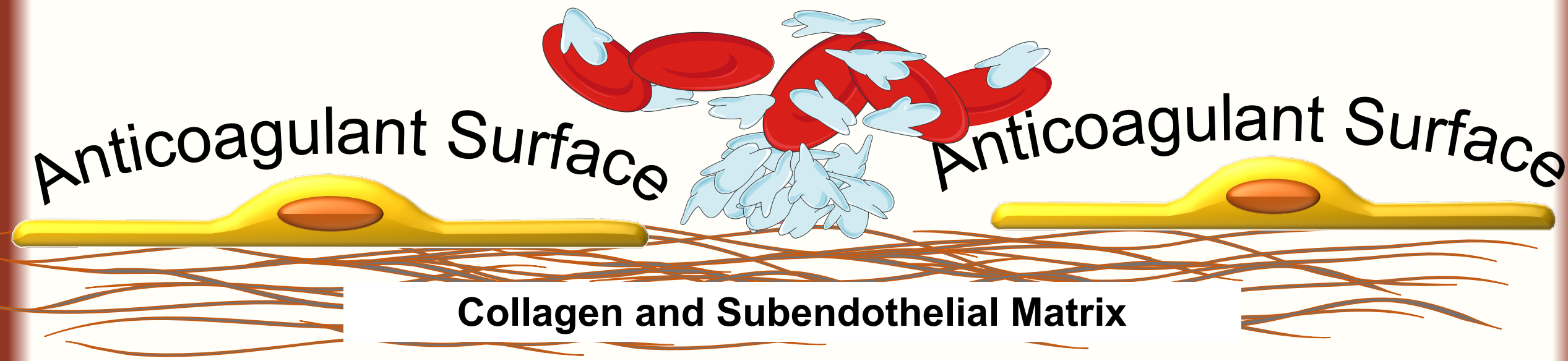


- Factor XI is a component of a positive feedback loop.
- Factor XI can be activated by FXIIa, but in addition, thrombin activates FXI (along with V, VIII, and XIII), which helps generate more thrombin.
- Results in augmentation of fibrin generation.
- Factor XI deficiency is not as severe as FVIII or FIX, but more clinically relevant than deficiencies of FXII, Prekallikrein, or High Molecular Weight Kininogen.

The Hemostatic Balance:
Physiologic Anticoagulation Processes



Vascular Endothelial Cells Present Anticoagulant Surface



Physiologic Anticoagulation Processes on Endothelial Cells

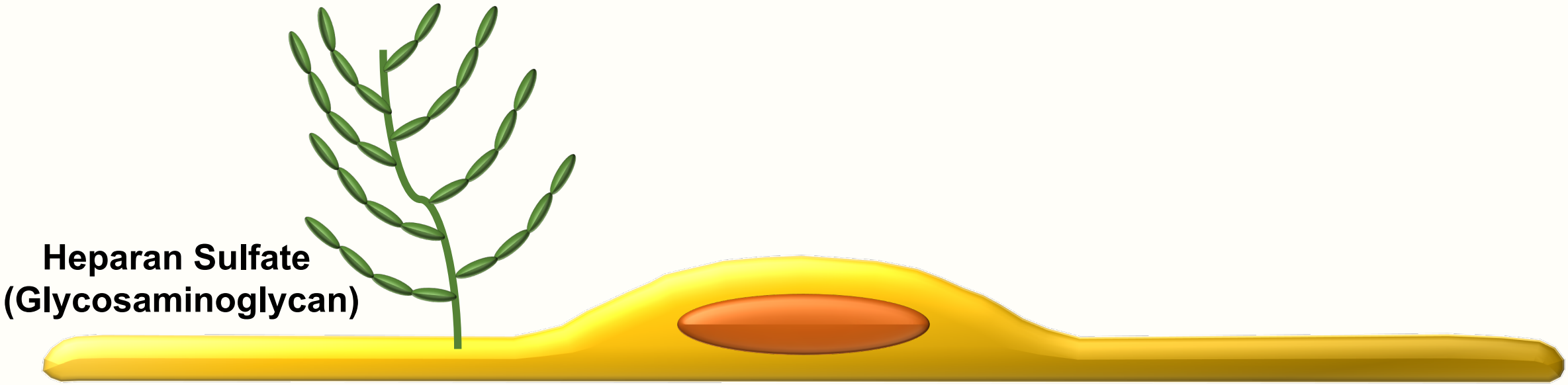
Pathway	Activity	Effect
CD39-Ecto ADPase	Degrades ADP	Reduced ADP, reduced platelet activation
NO Synthase	Synthesis of Nitric Oxide	Relaxes smooth muscle and inhibits platelet activation
Cyclooxygenase 2	Synthesis of Prostacyclin (PGI2)	Relaxes smooth muscle and inhibits platelet activation
Heparan Sulfate (Glycosaminoglycan)	Heparan binds Antithrombin	Heparan:AT complex neutralizes coagulation enzymes
Thrombomodulin & Endothelial Protein C Receptor	Thrombomodulin binds Thrombin EPCR binds protein C	Thrombin:TM complex has reduced procoagulant activity. Activates protein C which inactivates Cofactors
Tissue Factor Pathway Inhibitor	TFPI inhibits direct activation of Factor X by TF:VIIa complex	

Antithrombin: Glycosaminoglycan

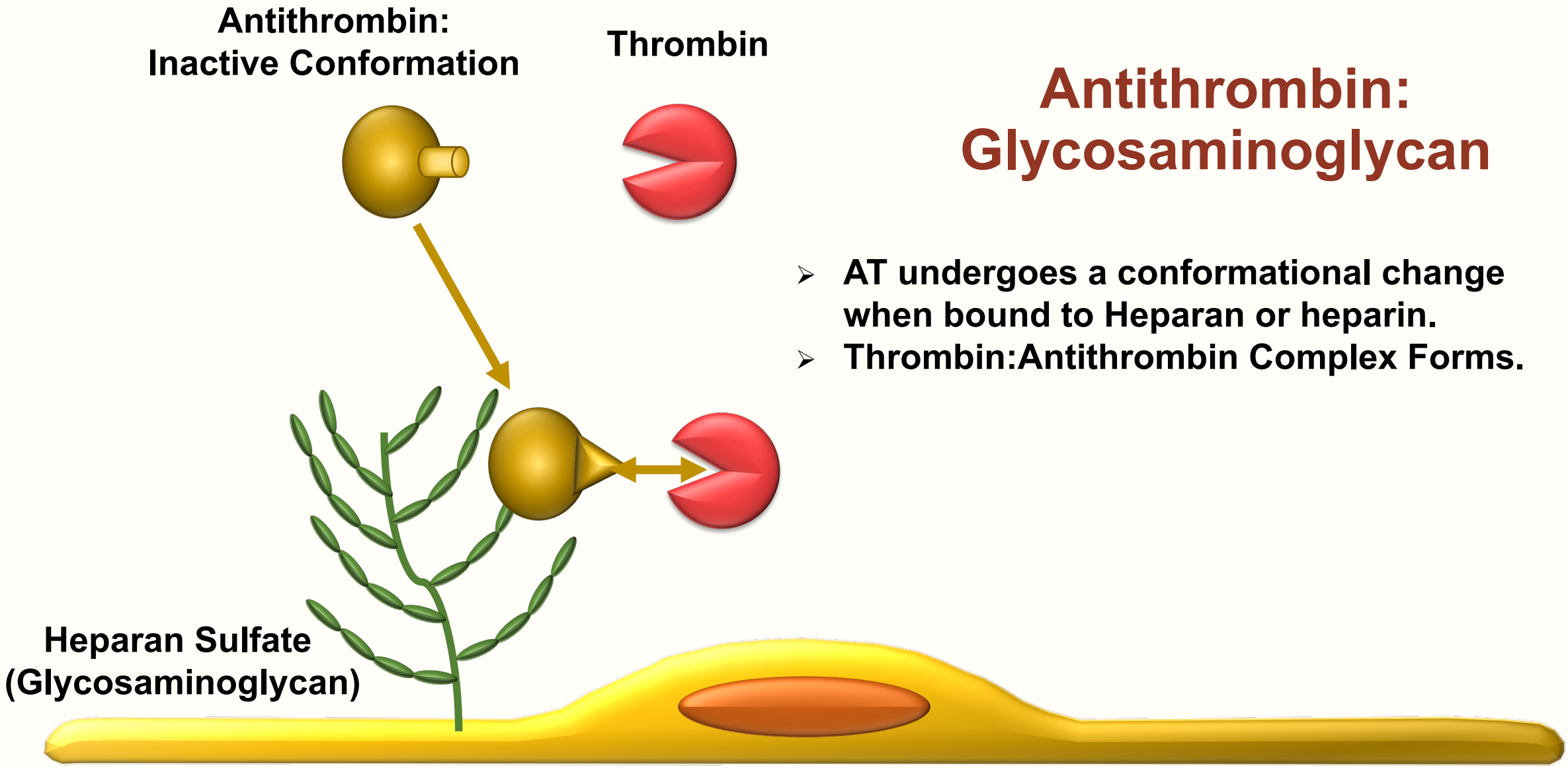
Antithrombin:
Inactive Conformation

Thrombin

Antithrombin, in fluid phase is unable to bind thrombin or other enzymes.



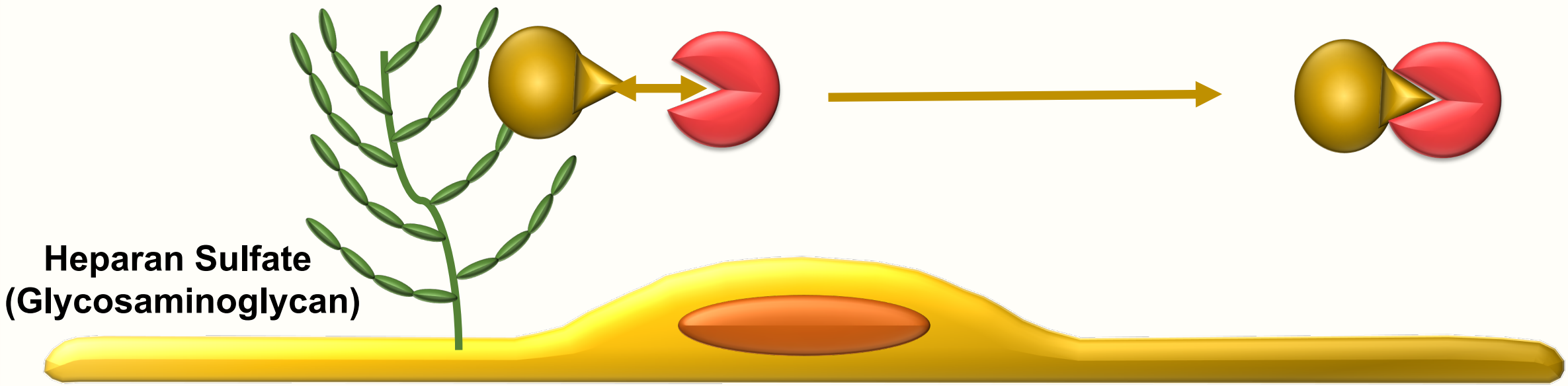
Antithrombin: Glycosaminoglycan



- AT undergoes a conformational change when bound to Heparan or heparin.
- Thrombin:Antithrombin Complex Forms.

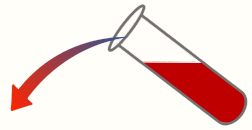
Antithrombin: Glycosaminoglycan

Thrombin:Antithrombin Complex Dissociates from Glycosaminoglycan

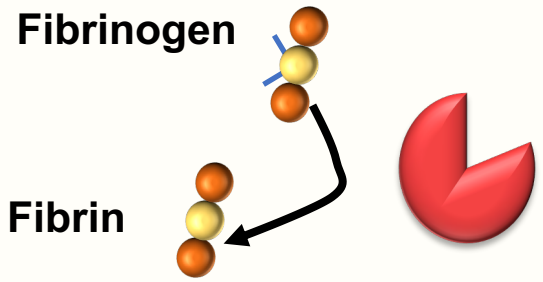


Protein C/Protein S/Thrombomodulin System

- Constituents:
  - Protein C
  - Protein S
  - Thrombomodulin
  - Endothelial cell protein C receptor (EPCR)
- Activated Protein C (With cofactor Protein S) inactivates FVa and FVIIIa, the cofactors of the cascade.
- EPCR localizes Protein C/Ca to endothelial cell surface.
  - May have non-coagulation roles.

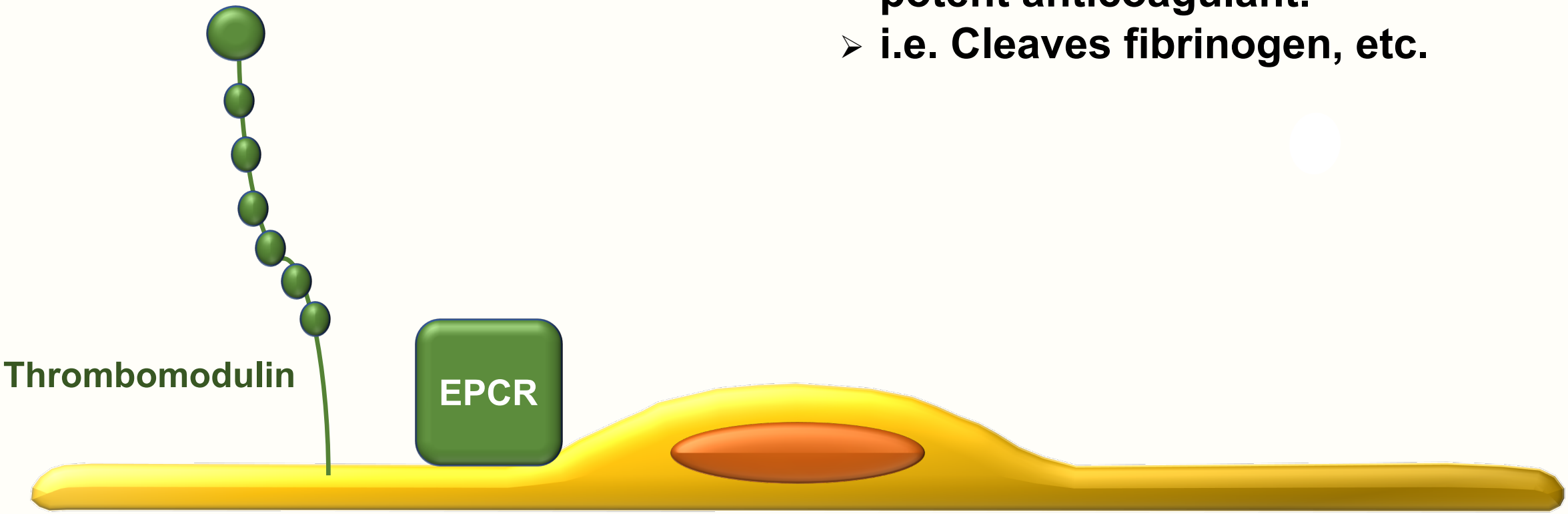


Protein C/Protein S/Thrombomodulin



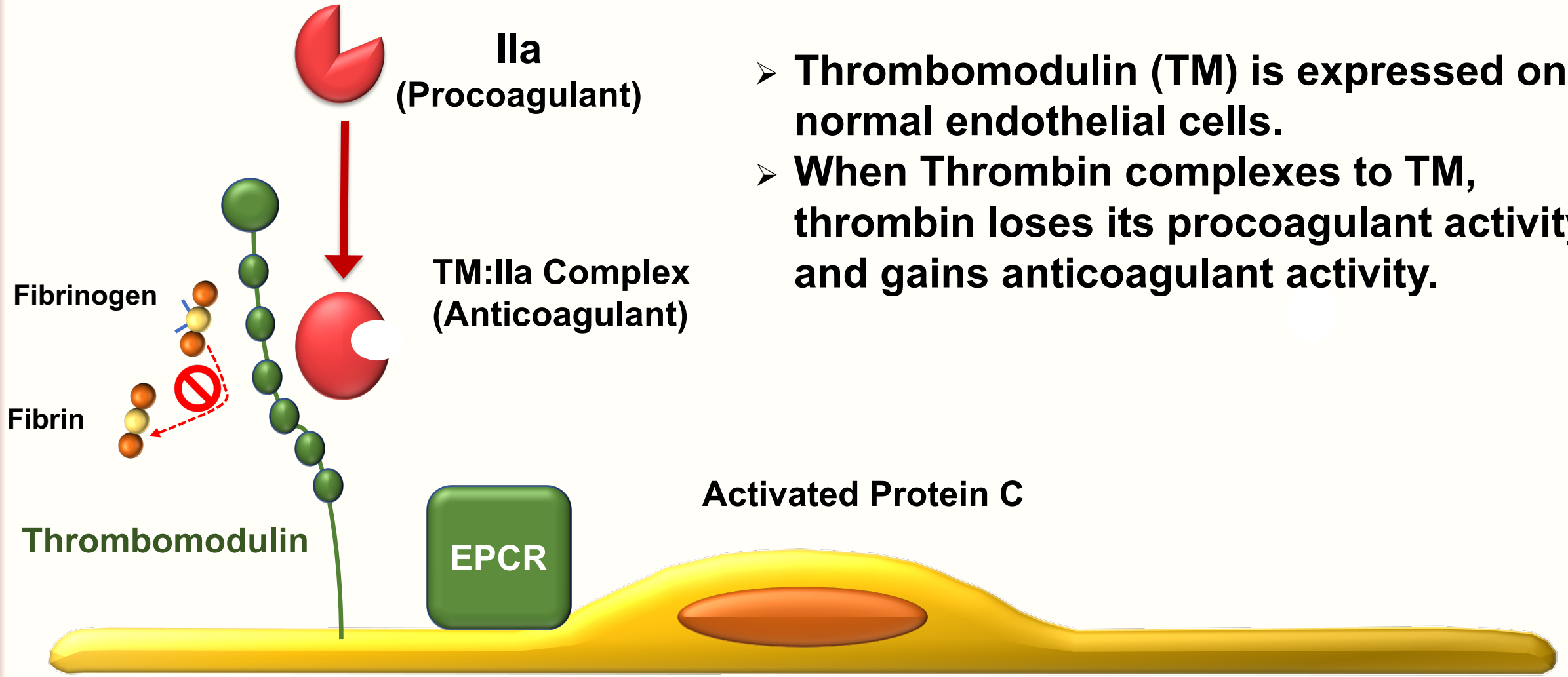
IIa
(Procoagulant)

- **Thrombin, in fluid phase is a potent anticoagulant.**
- **i.e. Cleaves fibrinogen, etc.**



EPCR: Endothelial Cell Protein C Receptor

Protein C/Protein S/Thrombomodulin

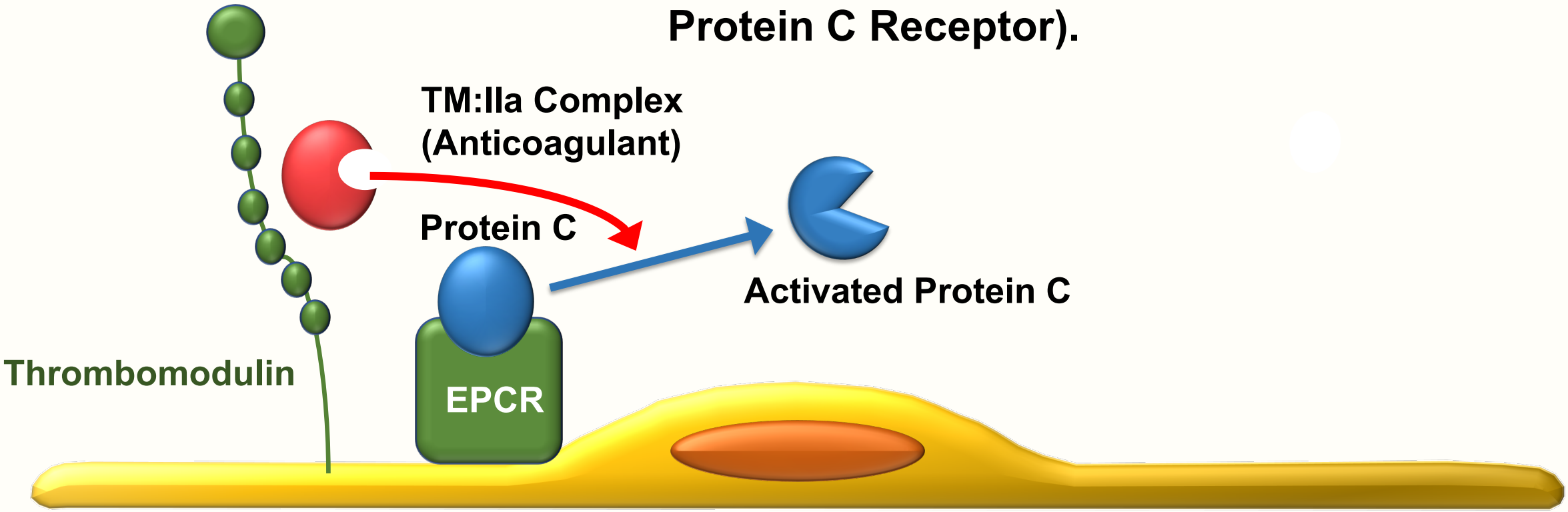


- Thrombomodulin (TM) is expressed on normal endothelial cells.
- When Thrombin complexes to TM, thrombin loses its procoagulant activity and gains anticoagulant activity.

EPCR: Endothelial Cell Protein C Receptor

Protein C/Protein S/Thrombomodulin

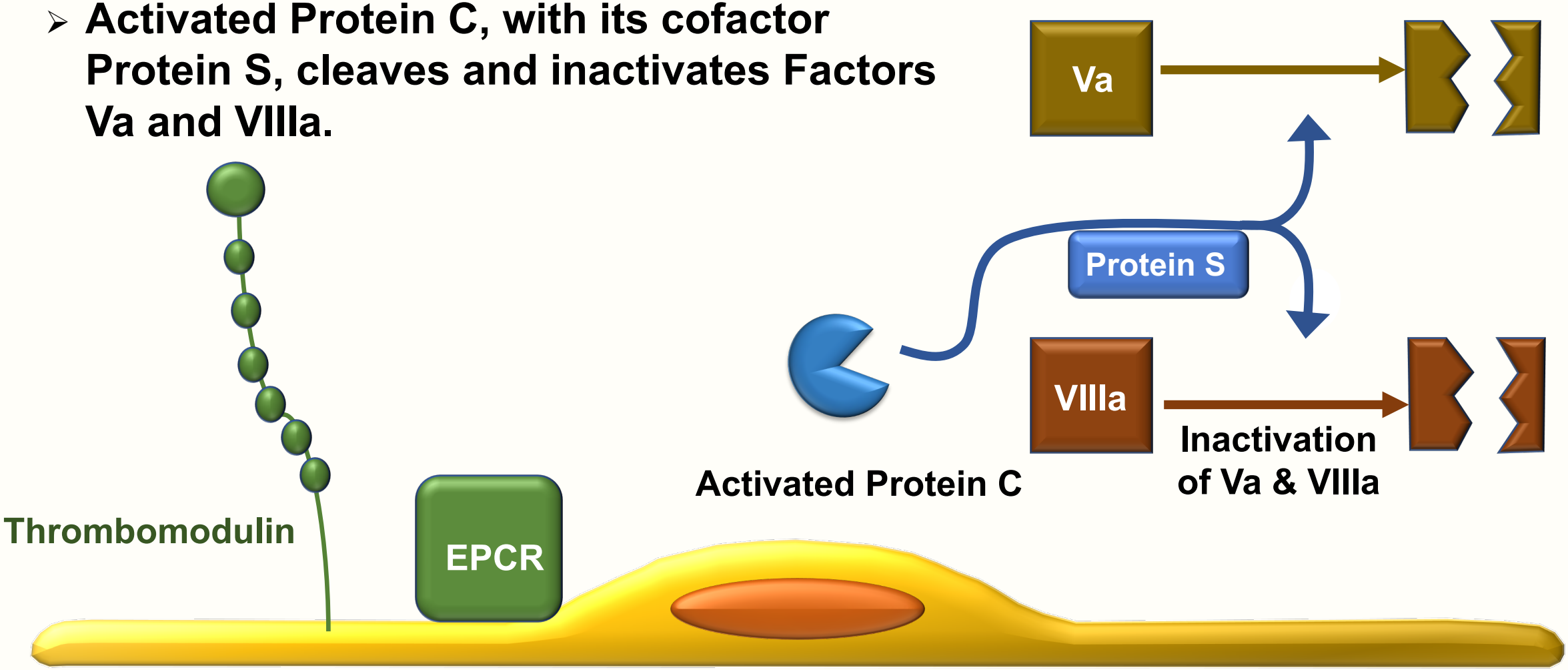
- Thrombin:Thrombomodulin complex cleaves and activates Protein C.
- (Protein C localizes to Endothelial Cell Protein C Receptor).



EPCR: Endothelial Cell Protein C Receptor

Protein C/Protein S/Thrombomodulin

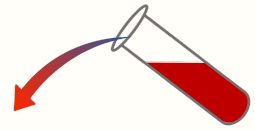
- **Activated Protein C, with its cofactor Protein S, cleaves and inactivates Factors Va and VIIIa.**



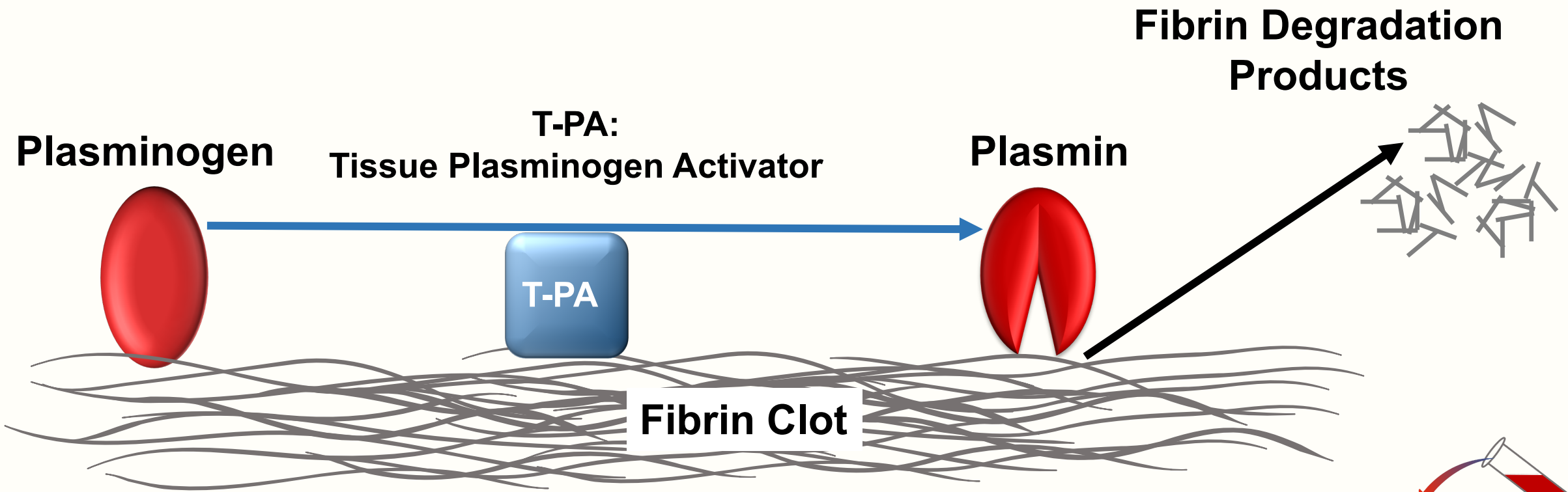
EPCR: Endothelial Cell Protein C Receptor

Fibrinolytic Pathway

- Plasminogen
  - Activated to Plasmin (a serine proteinase)
  - Plasmin proteolyzes fibrin and fibrinogen
- Plasminogen Activators
  - t-PA (Tissue-Plasminogen Activator)
    - Localizes to fibrin clot
  - u-PA (Urokinase-Plasminogen Activator)
    - Localizes to cell membrane uPA receptor.
  - Released by endothelial cells.
- Inhibitors/Serpins
  - PAI-1, PAI-2; Plasminogen Activator Inhibitors
  - α2-Antiplasmin.

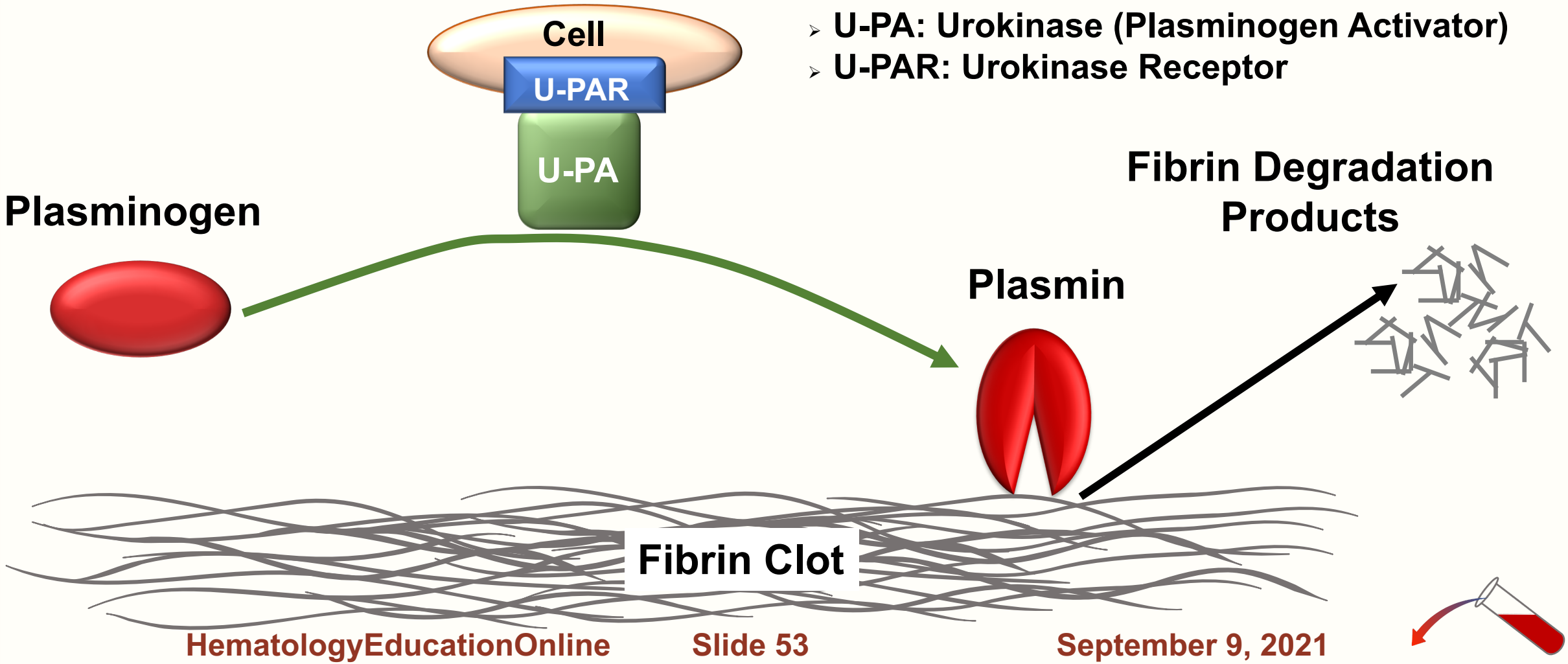


Fibrinolytic Pathway: T-PA, Fibrin Clot Based Activation

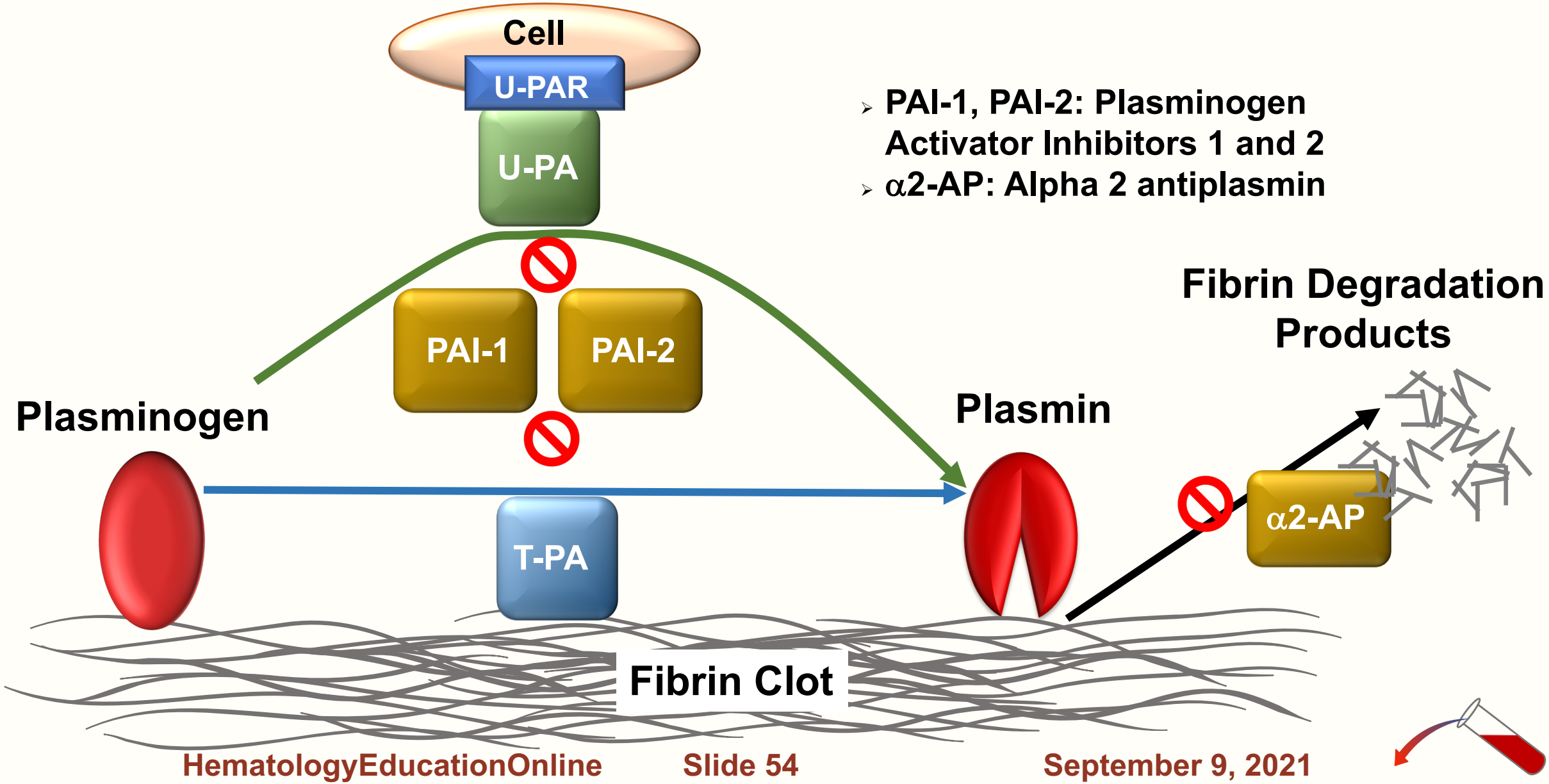


Fibrinolytic Pathway: U-PA/U-PAR, Cell Based Activation

- U-PA: Urokinase (Plasminogen Activator)
- U-PAR: Urokinase Receptor



Fibrinolytic Pathway: Inhibitors



- PAI-1, PAI-2: Plasminogen Activator Inhibitors 1 and 2
- α2-AP: Alpha 2 antiplasmin


Thank You!