The Coagulation System

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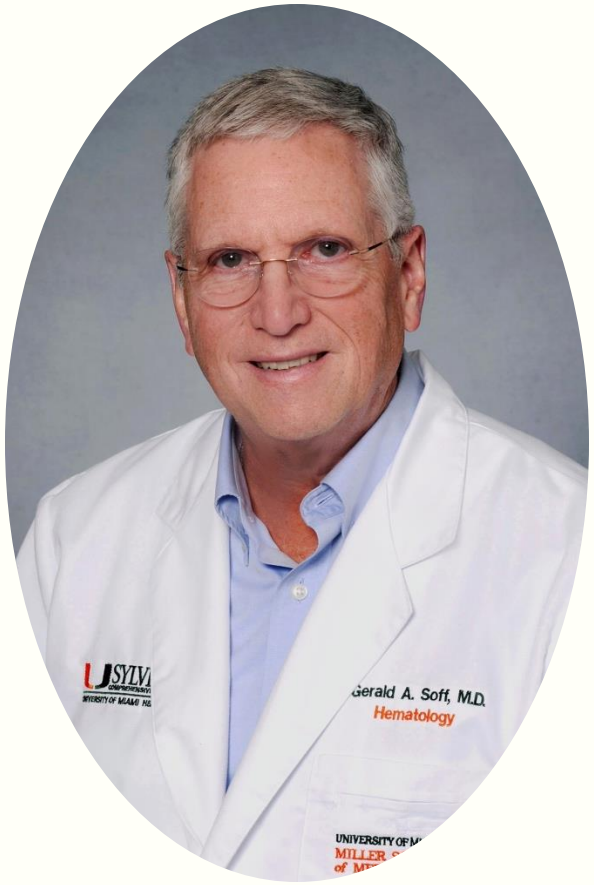

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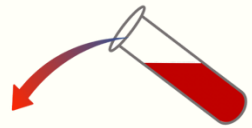
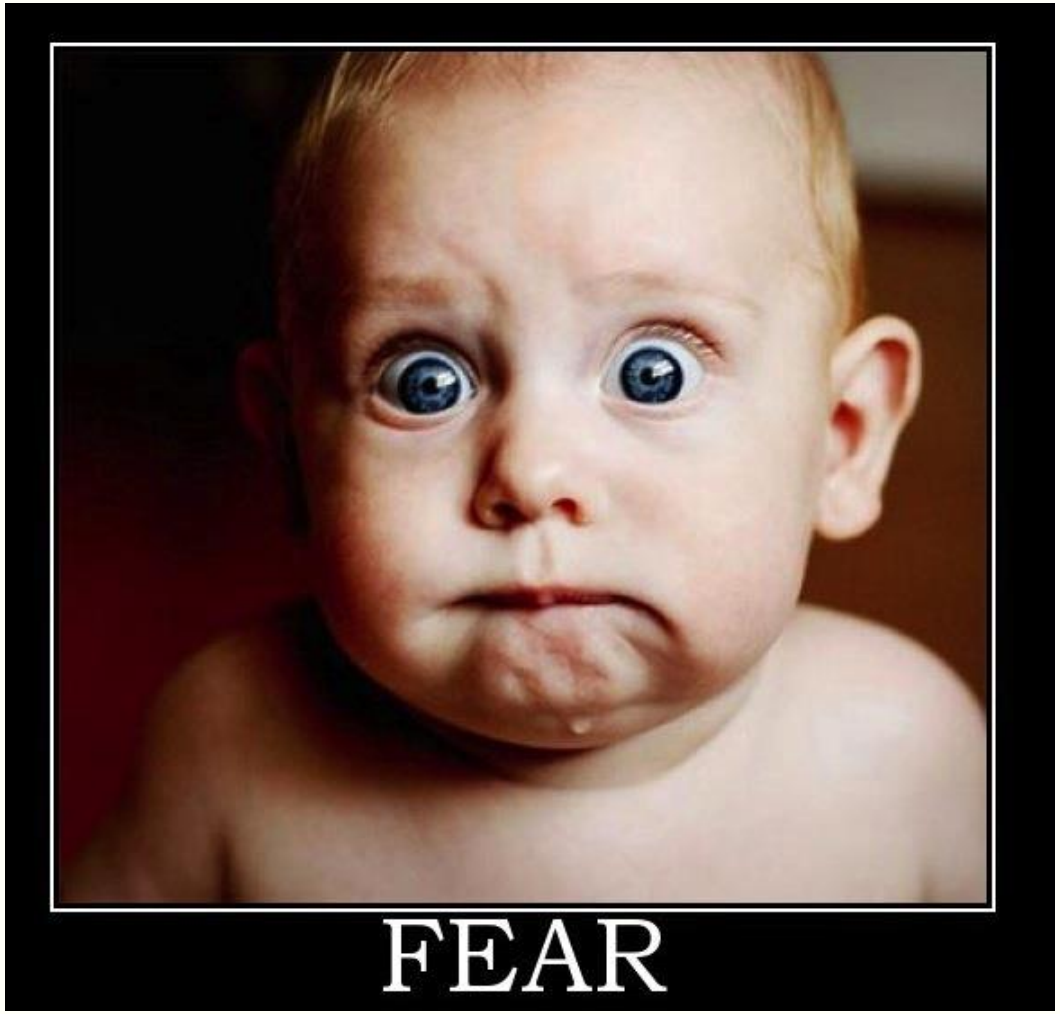
Disclosures

- Research Support (Past 2 years):
  - Amgen
  - Sobi/Dova Pharmaceuticals
  - Anthos Therapeutics
- Data Safety Monitoring Committee
  - Alpine Immune Sciences
- Advisory Boards (Past 2 years)
  - Sanofi
  - Novartis

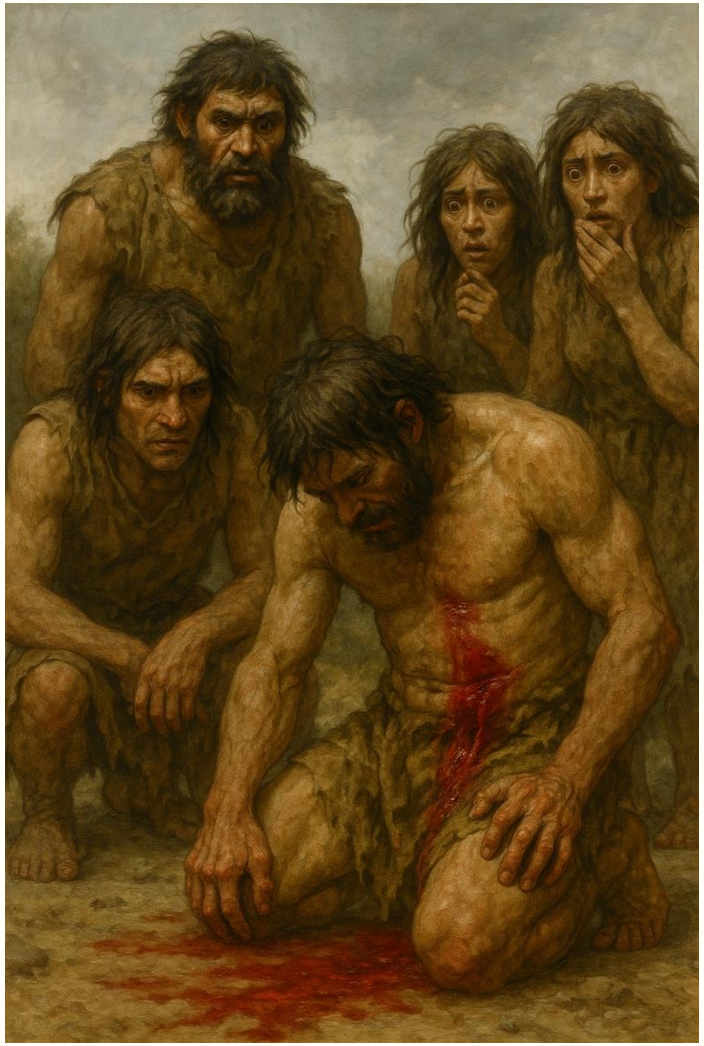


Coagulation System

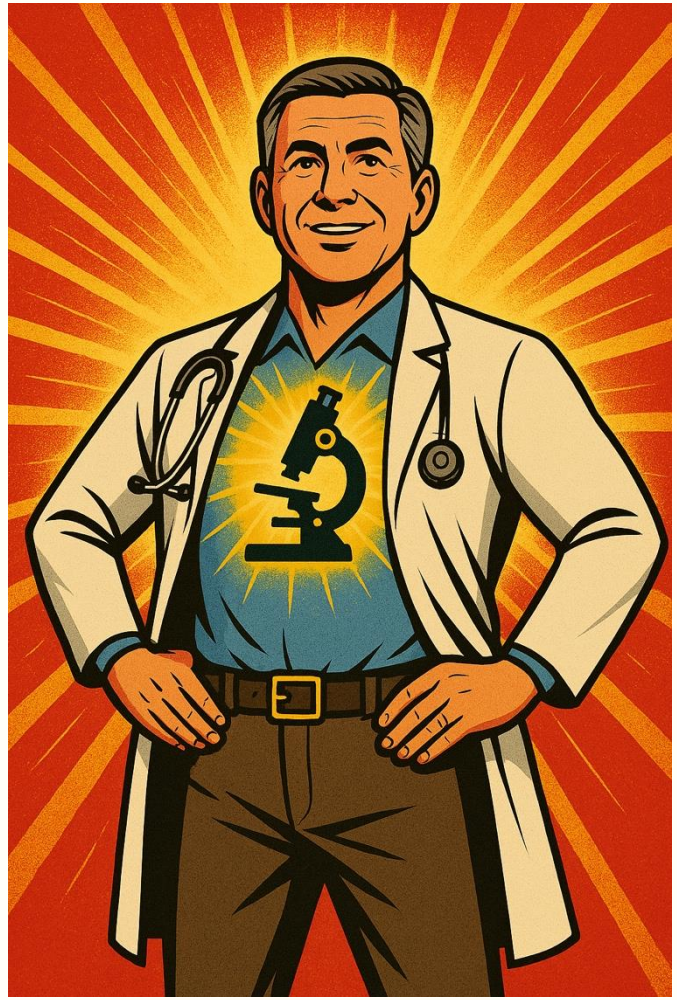


https://kidsfirstpediatrics.com
/babies-separation-anxiety/

One of the most primitive emotions is the terror and fear of hemorrhage.

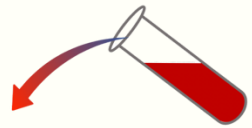


I'm a hematologist! I can stop your bleeding! What is your superpower?



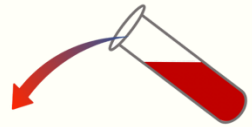
What We'll Cover

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

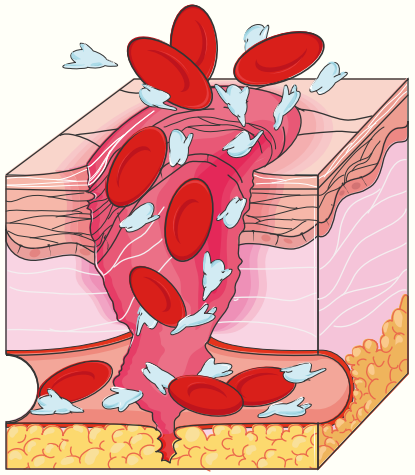


Overview of Hemostasis and Coagulation

- 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 and inhibit platelet function where vessels are intact.
  - Fibrinolysis

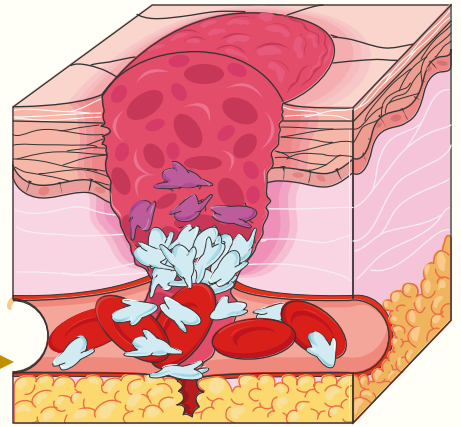


Major Components of Hemostasis



Vascular Injury

Hemostasis



Vasoconstriction

Platelet Plug Formation

Coagulation "Cascade"

Primary Hemostasis

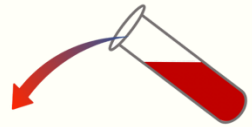
Secondary Hemostasis

The Dawn of *In Vitro* Studies

- Contact Activation:
  - Described by Joseph Lister in 1863
  - Blood would clot when brought in contact with a surface other than vascular endothelium.

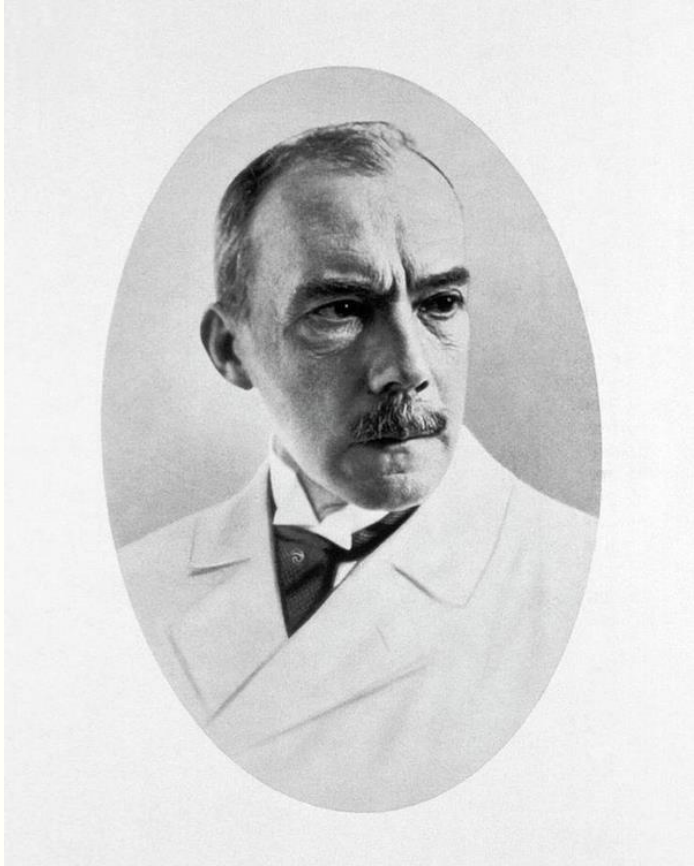
- Tissue Thromboplastin:
  - Tissue extract accelerated blood clotting.
  - Described by Nicolas Maurice Arthus in 1902.

- Calcium Chelation could prevent blood clotting, and recalcification could restore the ability to clot:
  - Maurice Arthus and Calixte Pagès, in 1890



The "Classic Coagulation Cascade" Early Understanding of Coagulation

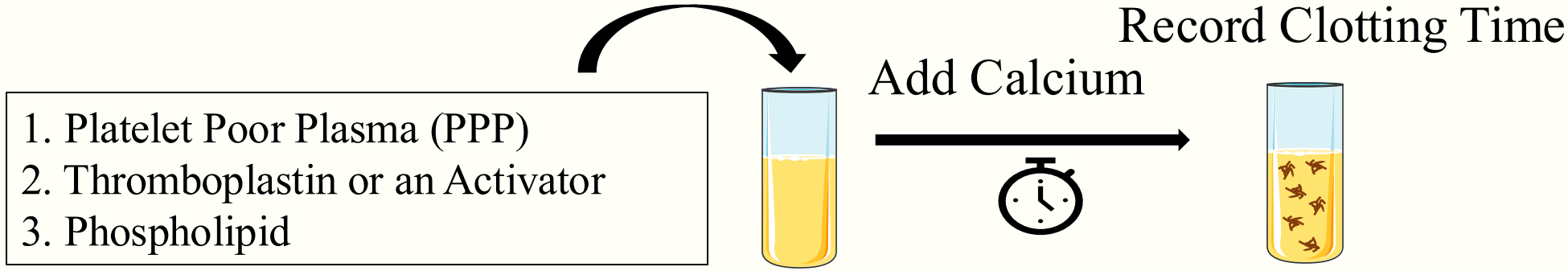
- The first description of coagulation factors is attributed to Dr. Paul Morawitz in 1905.
- Factor I – Fibrinogen
- Factor II – Prothrombin
- Factor III – Thromboplastin Factor
  - (Tissue extract with Tissues Factor)
- Factor IV – Calcium



- (Morawitz P (1905). "Die Chemie der Blutgerinnung". Ergebn Physiol (in German). 4: 307–422. doi:10.1007/BF02321003.)

Assays of Coagulation

- The prothrombin time in 1935.
  - Quick A, et al, Am.J. Med Sci., 1935
- Partial Thromboplastin Time in 1952.
  - Langdell RD, et al, J. Lab. Clin. Med, 1952
- One, or both, of the tests are prolonged in individuals and families, who had bleeding tendency.



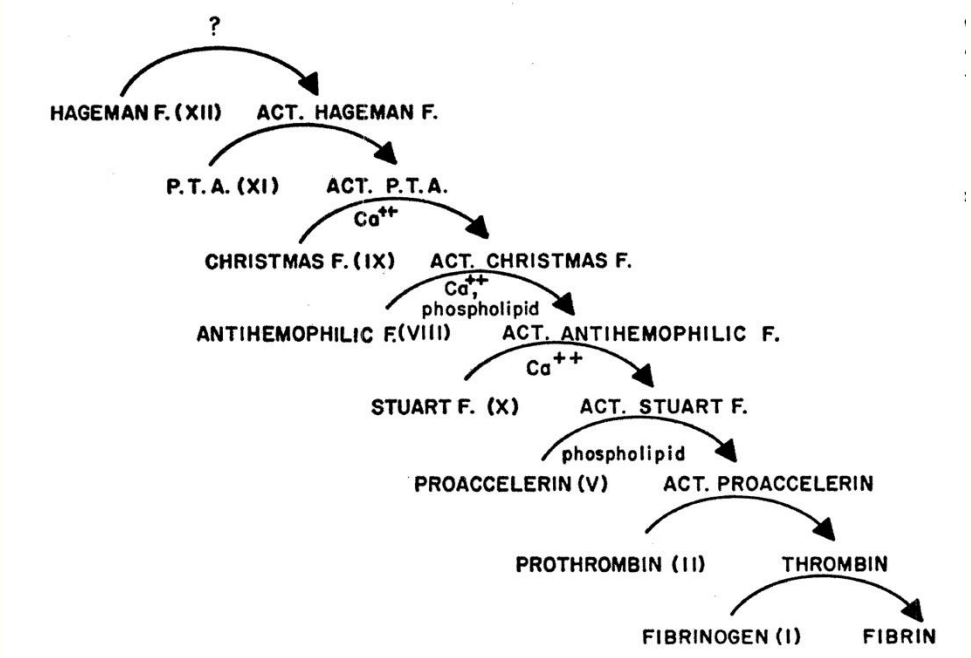
Mixing Studies Identified New Factors (New Deficiencies)

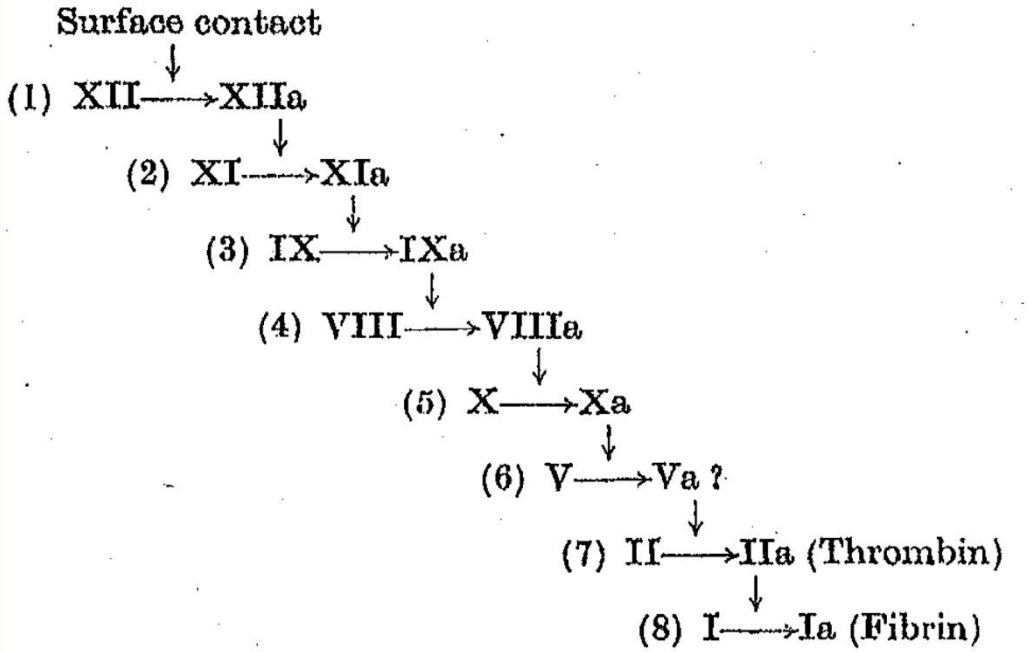
Plasma from patient with unknown bleeding disorder

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

- Perform Clotting Time on 1:1 mix of patient with known deficiency with an unrelated individual.
- If the 1:1 mix remains prolonged, then the unknown sample has the same deficiency as the known deficiency.
- If the 1:1 mix "corrects," then the unknown sample has a different deficiency than the known deficiency.
- From the 1930s through the 1950s, most of the factors were identified in this way.
- Limitations:
  - Hematologists and labs needed to have access to many patient-derived aliquots of plasma.
  - Inhibitors will interfere.

Original Publications Of Coagulation Cascade



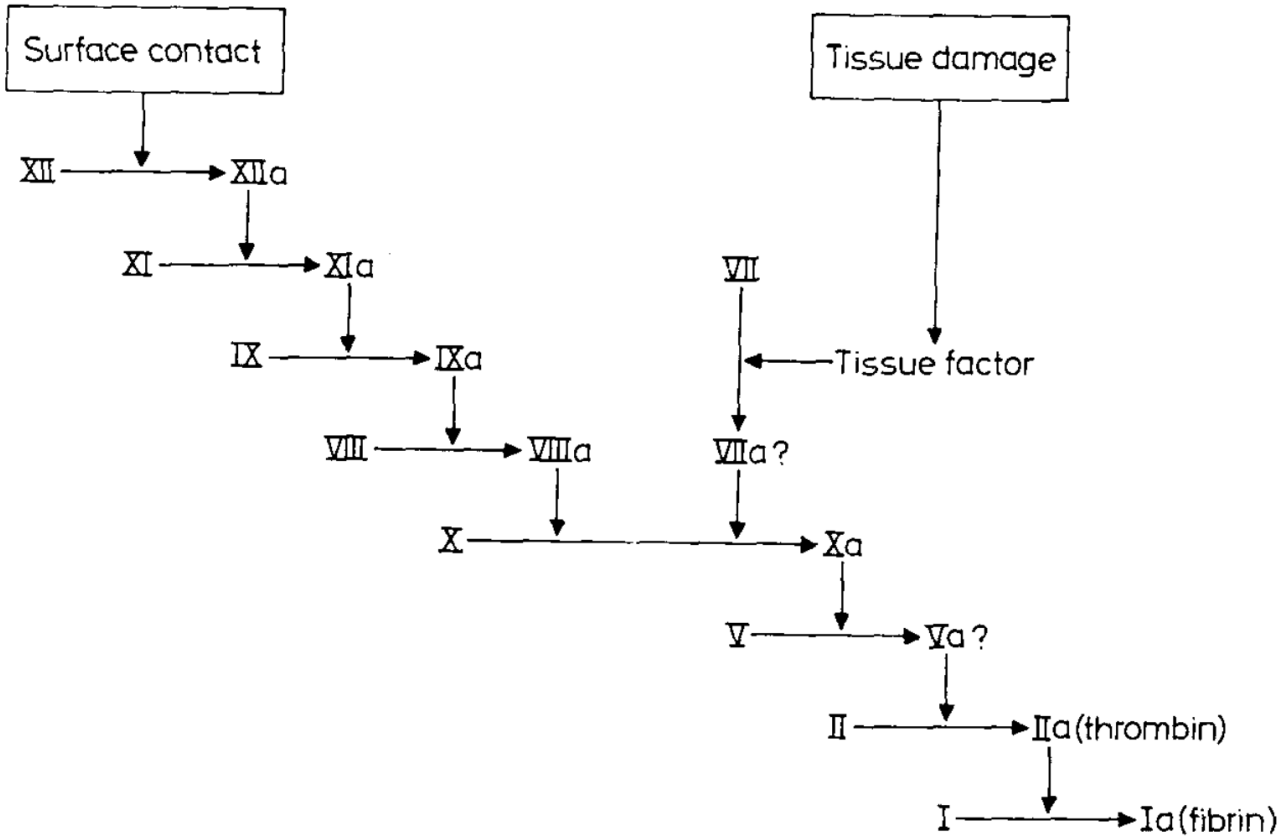


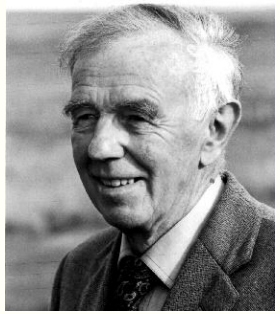
Davie, E. W., and Ratnoff, O. D. "Waterfall sequence for intrinsic blood clotting." Science 1964: 145, 1310–1312

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

Note: Neither representation included Factor VII, of the Extrinsic Pathway!

"A clotting scheme for 1964"
The First Representation of the Current Cascade.



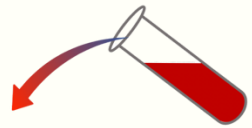


- Robert Gwyn Macfarlane,

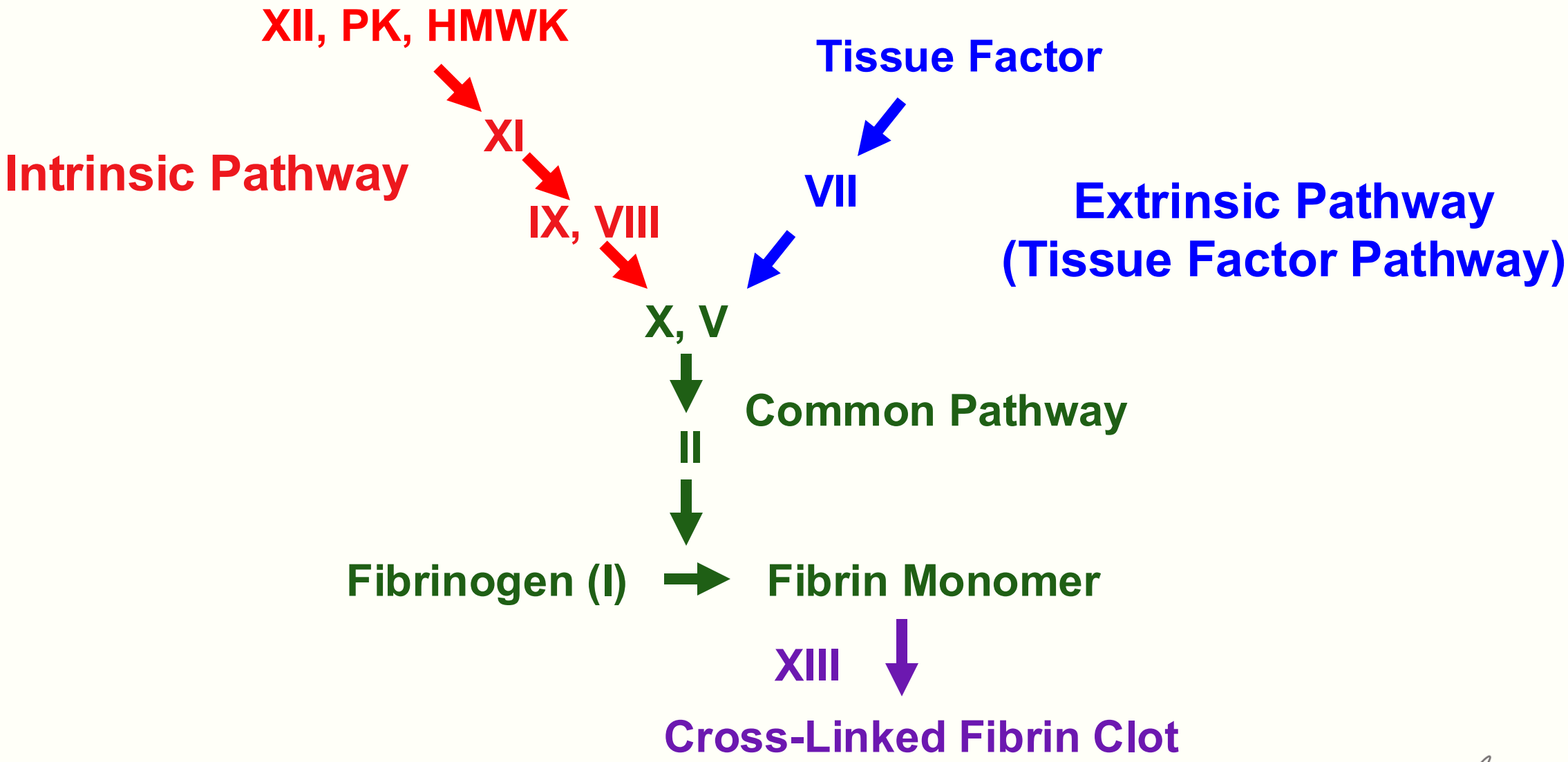
Macfarlane, RG. "A clotting scheme for 1964". Thrombosis et Diathesis Haemorrhagica, supplement. 17: 45-52, 1965.

Key Concepts From Original Publications Of The Coagulation Cascade

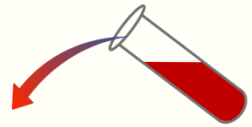
1. Coagulation involves a sequence of reactions.
2. Convention has shifted from names (of the first probands) to Roman numerals.
3. Factors circulate in non-activated forms.
   a. Zymogens or pro-enzymes
   b. Pro-cofactors
4. Factors are activated by proteolytic cleavage by an "upstream" factor and in turn activate a "downstream" factor.
5. Terminology:
   a. Subscript "a" designates activated factor. (VIII → VIIIa)
   b. "i" refers to inactivated. (VIII → VIIIa →VIIIi)
6. A number of gaps, corrections, and open questions remained. (To be discussed below).



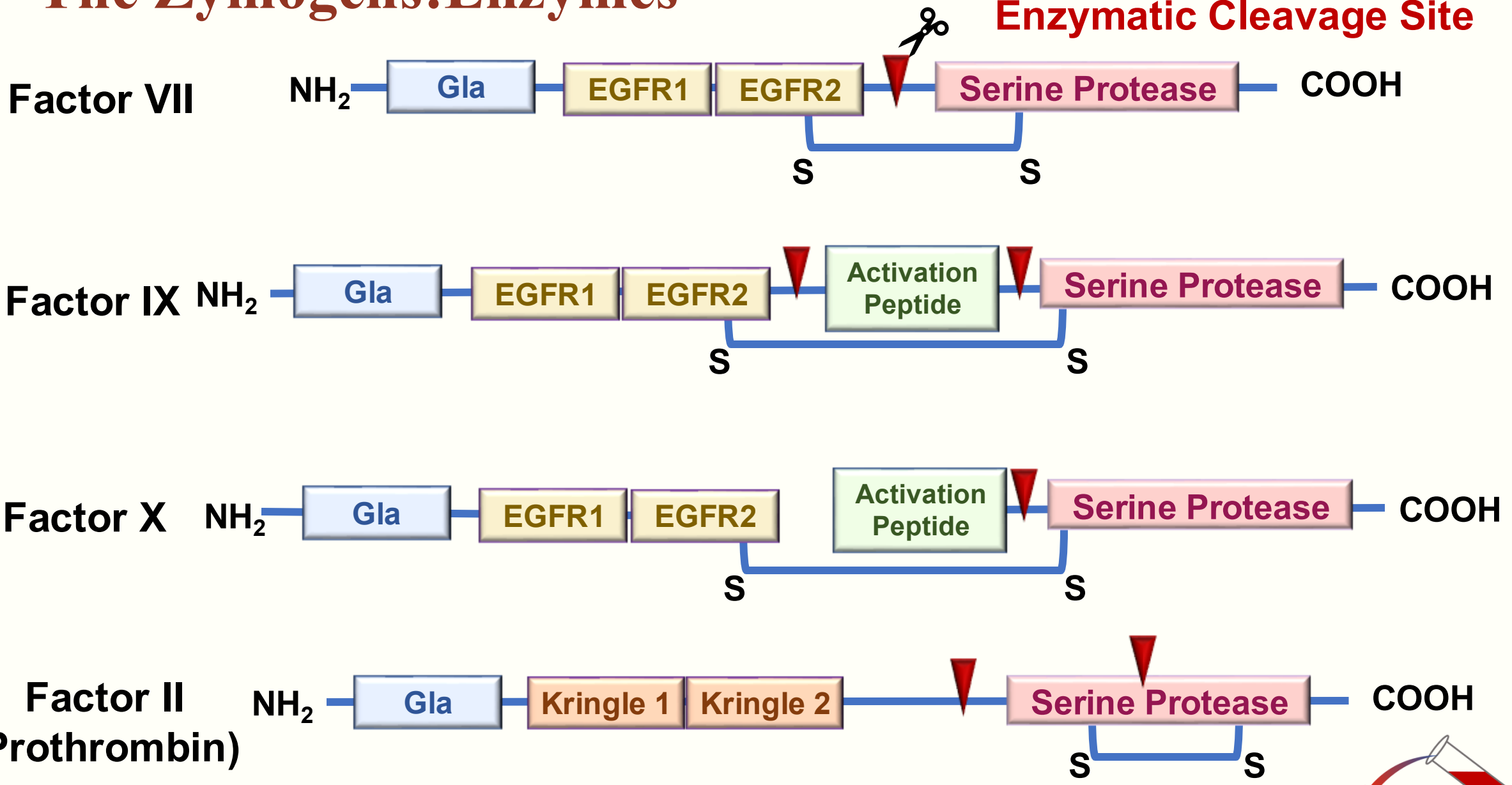
Contemporary Representation of the Coagulation Cascade



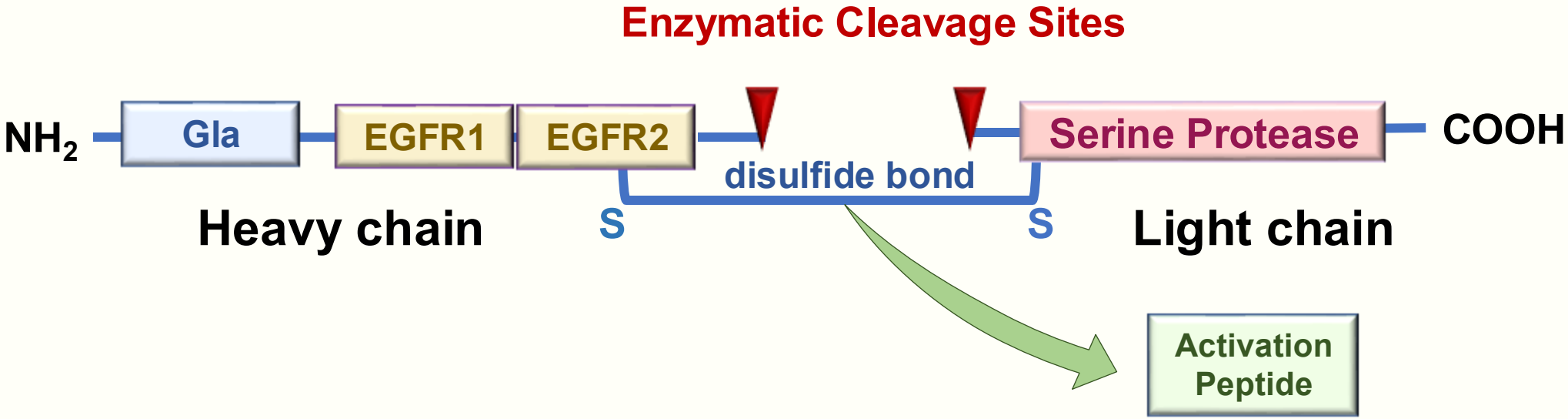
The Structure of the Coagulation Factors



The Zymogens:Enzymes

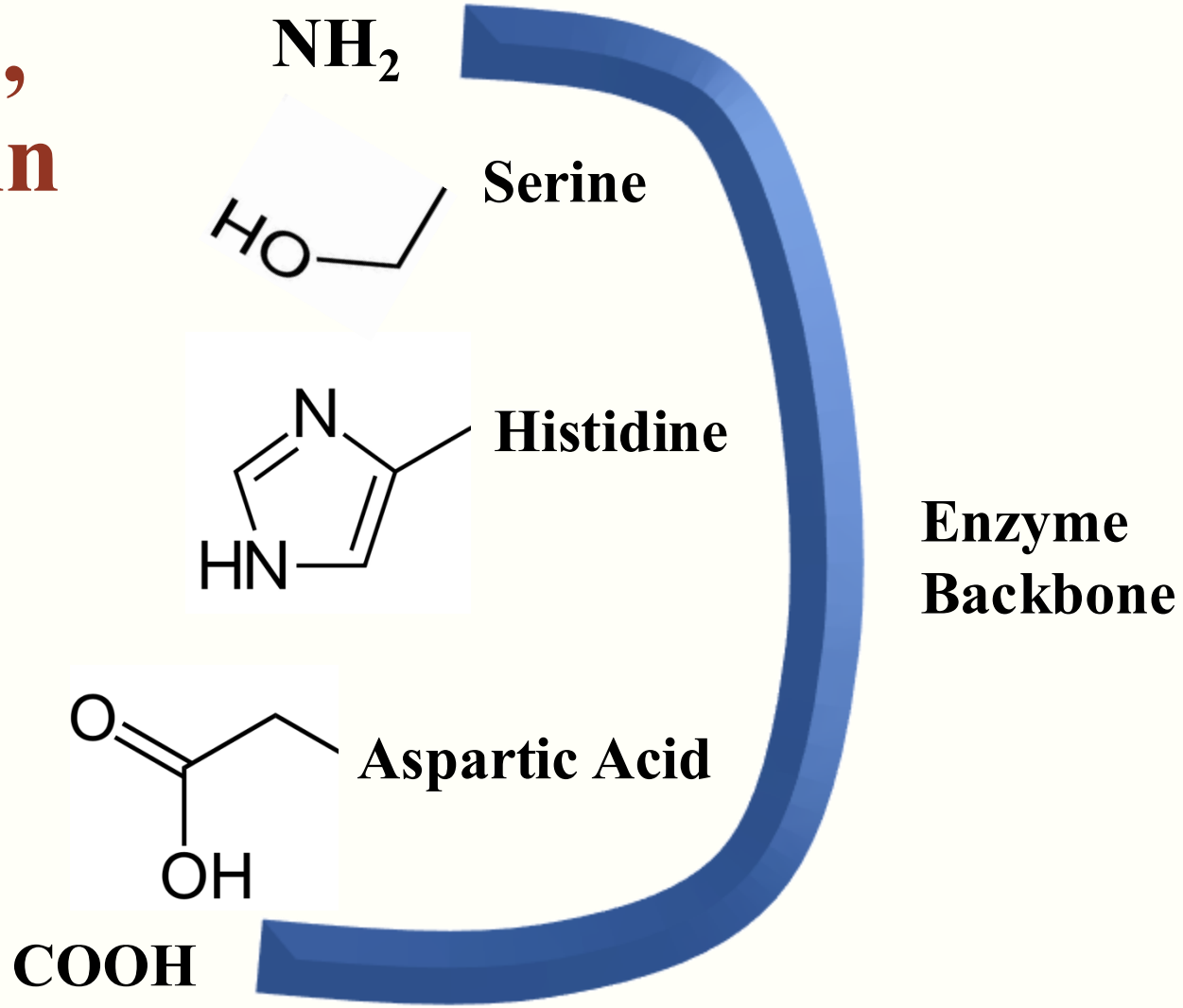


Factor IX Activation: Two Step Enzymatic Cleavage Site



- 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

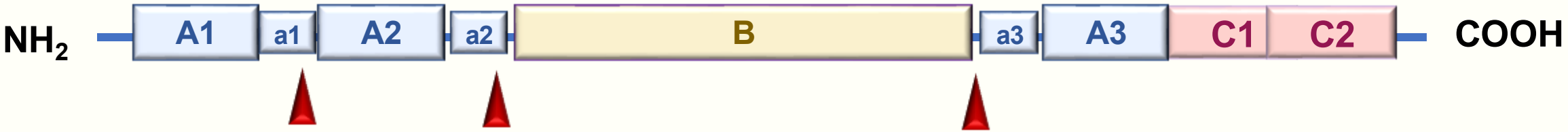
Serine Protease, Catalytic Domain



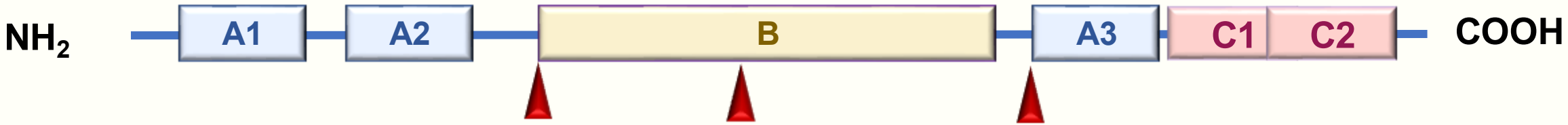
Serine, Histidine and Aspartic acid; amino acids in catalytic domain.

Cofactors

Factor VIII

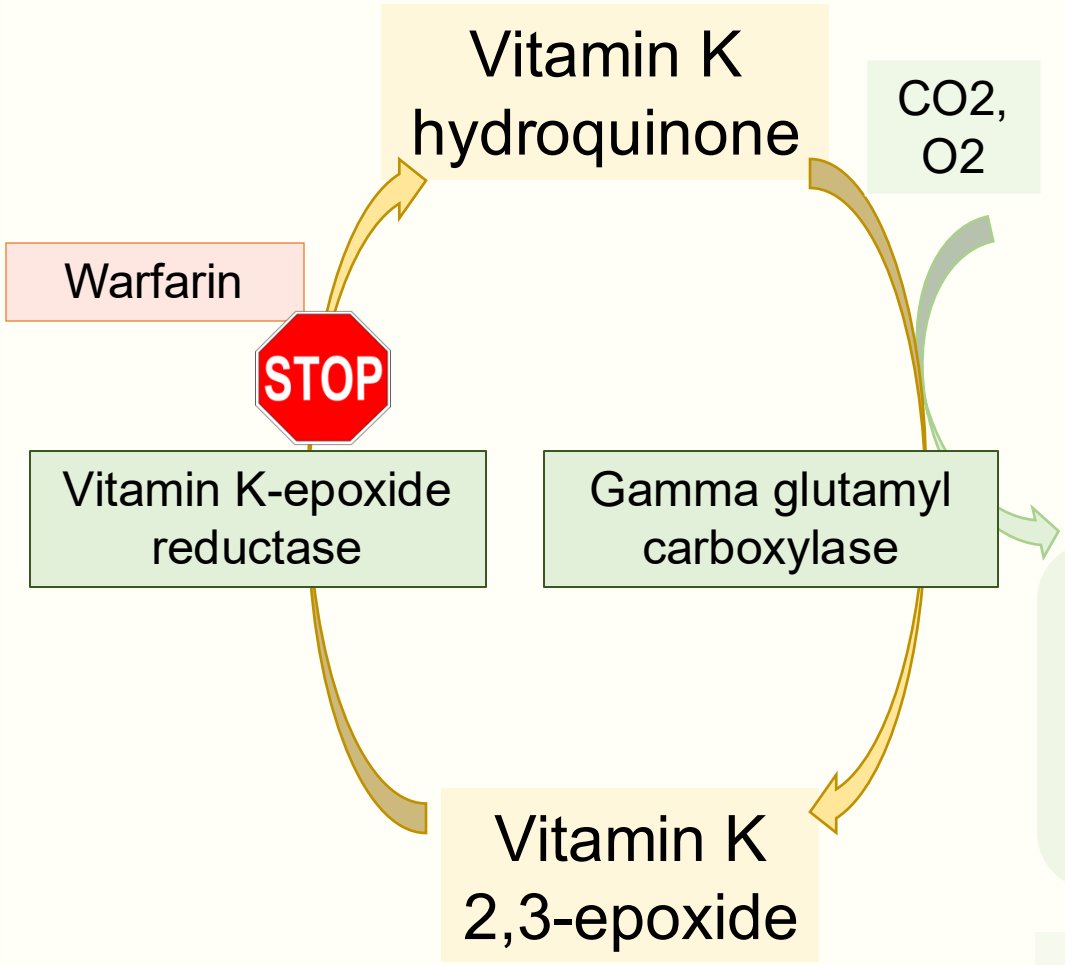


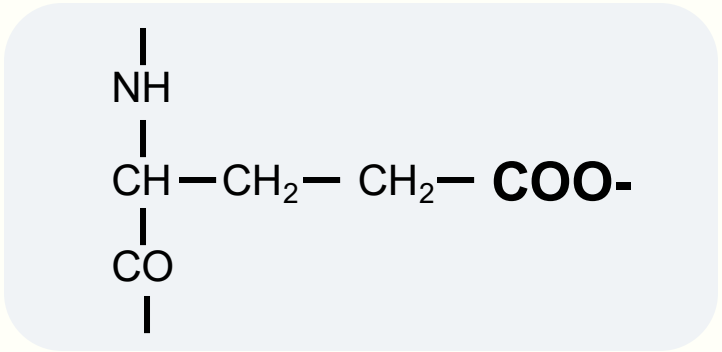
Factor V



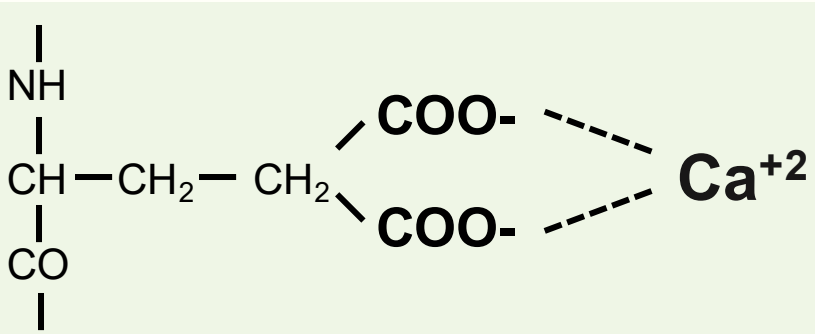
Dahlback B. JTH 15: 1241-1250, 2017
Camire & Bos. JTH, 7: 1951–1961, 2009

Vitamin K Mediated γ-Carboxylation of Glutamic Acid





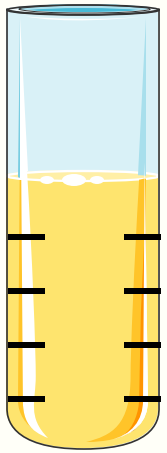
Glutamic Acid:
Single negative charge



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

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

Intrinsic Pathway/Contact Pathway:
Contact with a Negatively Charged Surface



XII, PK, HMWK

↓

XIIa

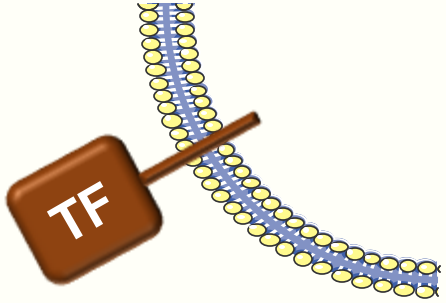
Extrinsic Pathway:
Addition of Tissue Thromboplastin (Tissue Factor and Phospholipid)

TF:VII

Auto-Activation

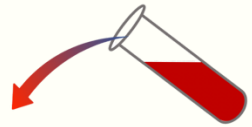
↓

VIIa



TF bound to cell surface

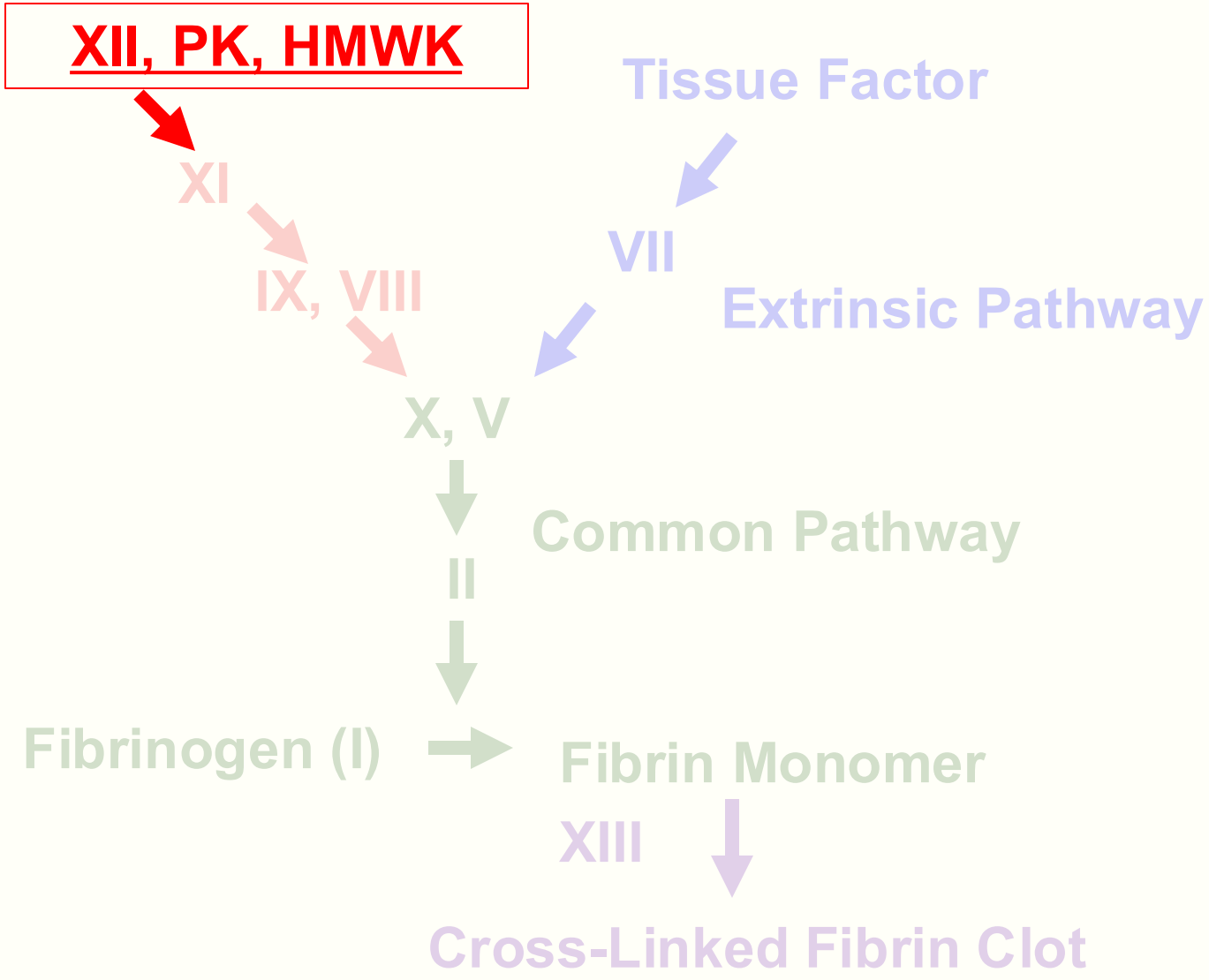
Overview of the Contact Phase: Initiation of Intrinsic Pathway



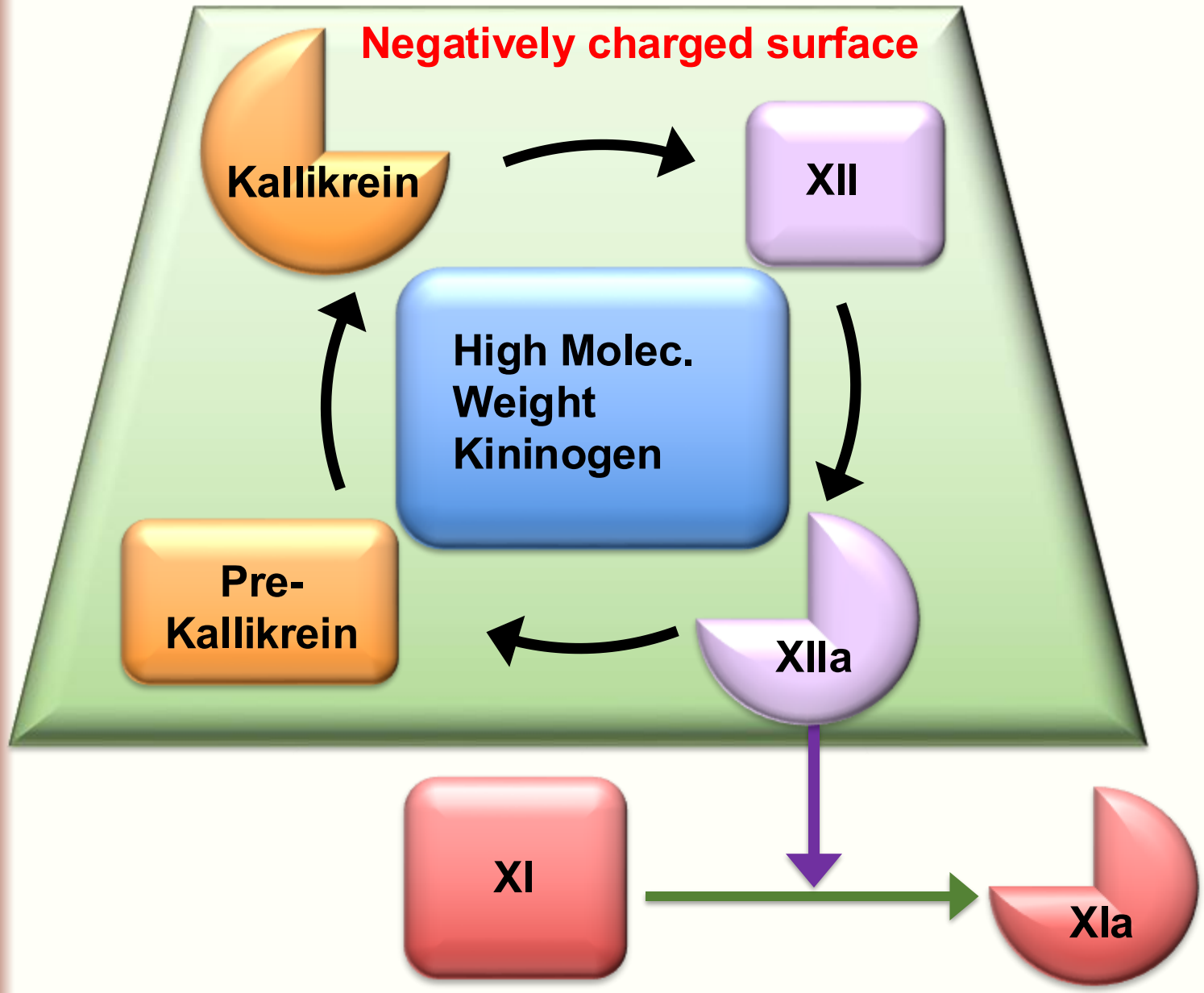
Contact System

- Activated by binding to a negatively charged surface or substance.
- Initiates the Intrinsic Pathway

- Factor XII
- Prekallikrein
- High Molecular Weight Kininogen

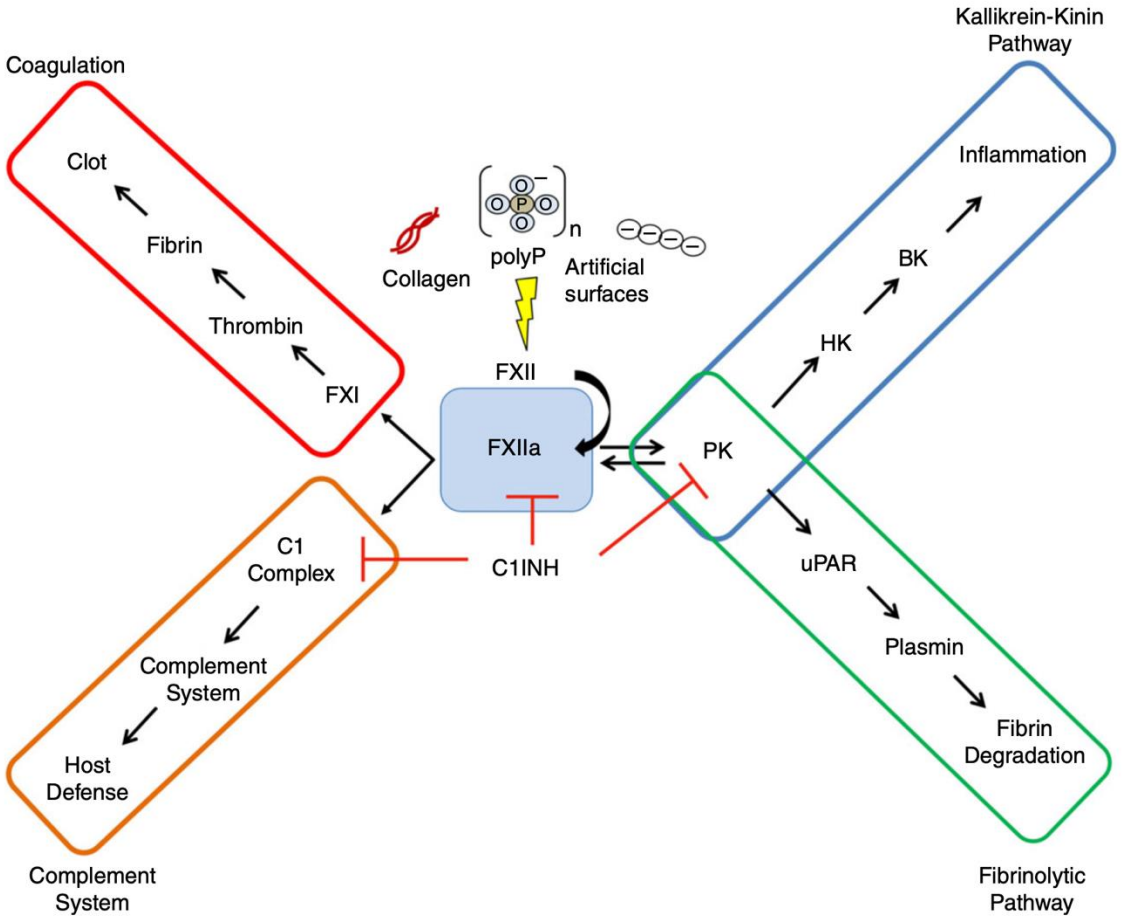


Contact System



- **<u>Minimal contribution to hemostasis in physiologic situations.</u>**
- Deficiencies of the Contact Factors are not associated with bleeding tendency.
- Bradykinin (Derived from HMWK)
  - Role in inflammation, vascular tone.
- Increasing evidence that the Contact System has a role in pathological activation of coagulation and thrombosis.

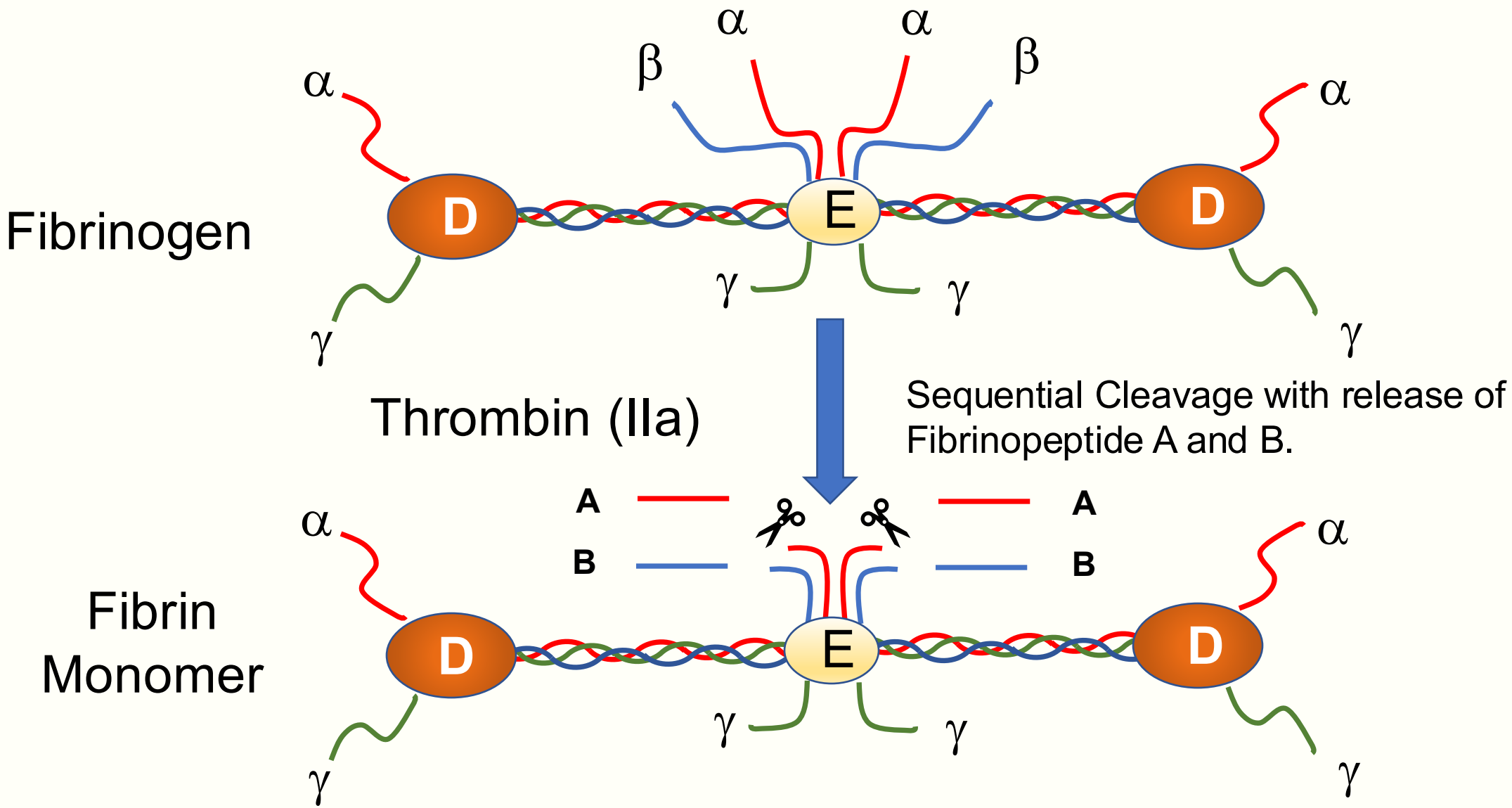
“Contact system revisited: an interface between inflammation, coagulation, and innate immunity”



- **FXII deficiency is not physiologically associated with an increased bleeding risk.**
- **Contact system has a role in inflammation, complement system, fibrinolysis, and pathologic thrombosis.**

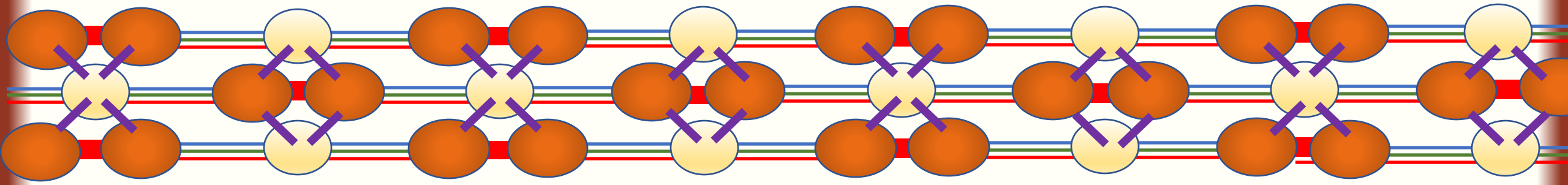
- **Polyphosphate (polyP) from activated platelets and bacteria can activate Factor XII.**
- **Neutrophil extracellular traps (NETs), chromatin extruded from activate.d neutrophils can activate the Contact System.**
- **F XIIa may increase vascular leak in allergic conditions.**
- Long AT, et al. J Thromb Haemost 2016; 14: 427–37.

Fibrinogen: Fibrin

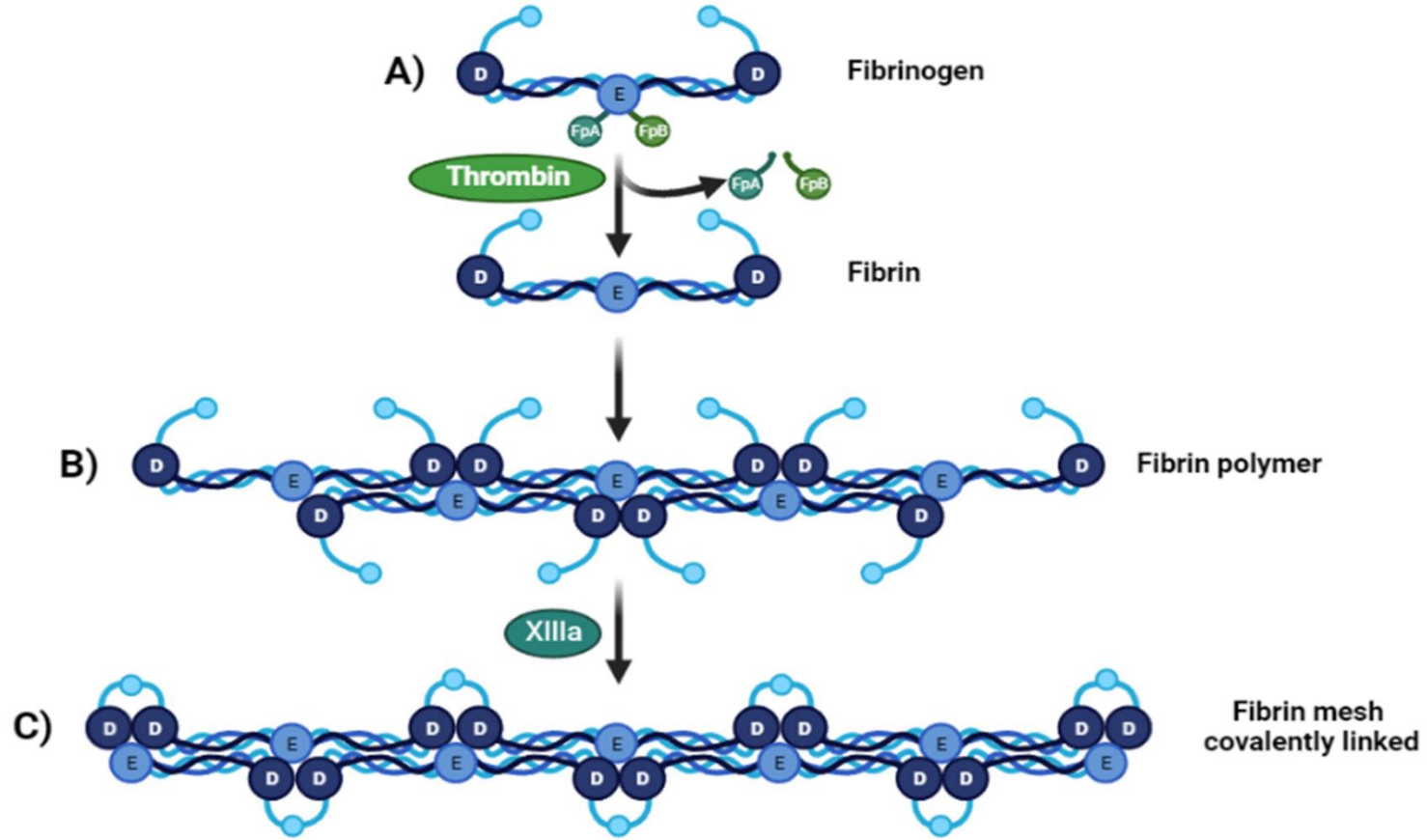


Factor XIIIa (Transglutaminase): Cross-Link Fibrin

XIIIa: Cross-Links Fibrin Clot



From fibrinogen to fibrin Mesh



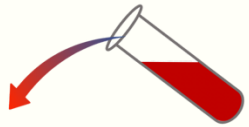
- (**A**) Fibrinogen D:E:D regions interact with thrombin-realizing fibrinopeptides (FpA and FpB)
- (**B**). Soluble fibrin is then activated by Factor XIIIa, permitting sulfide bonding to crosslink among fibrin, converting it to a
- (**C**) crosslinked fibrin polymer.

Rojas-Murillo, J.A. et al, Physical, Mechanical, and Biological Properties of Fibrin Scaffolds for Cartilage Repair. Int. J. Mol. Sci. 2022, 23, 9879. https://doi.org/10.3390/ijms23179879

Limitations of The Classic Coagulation Cascade

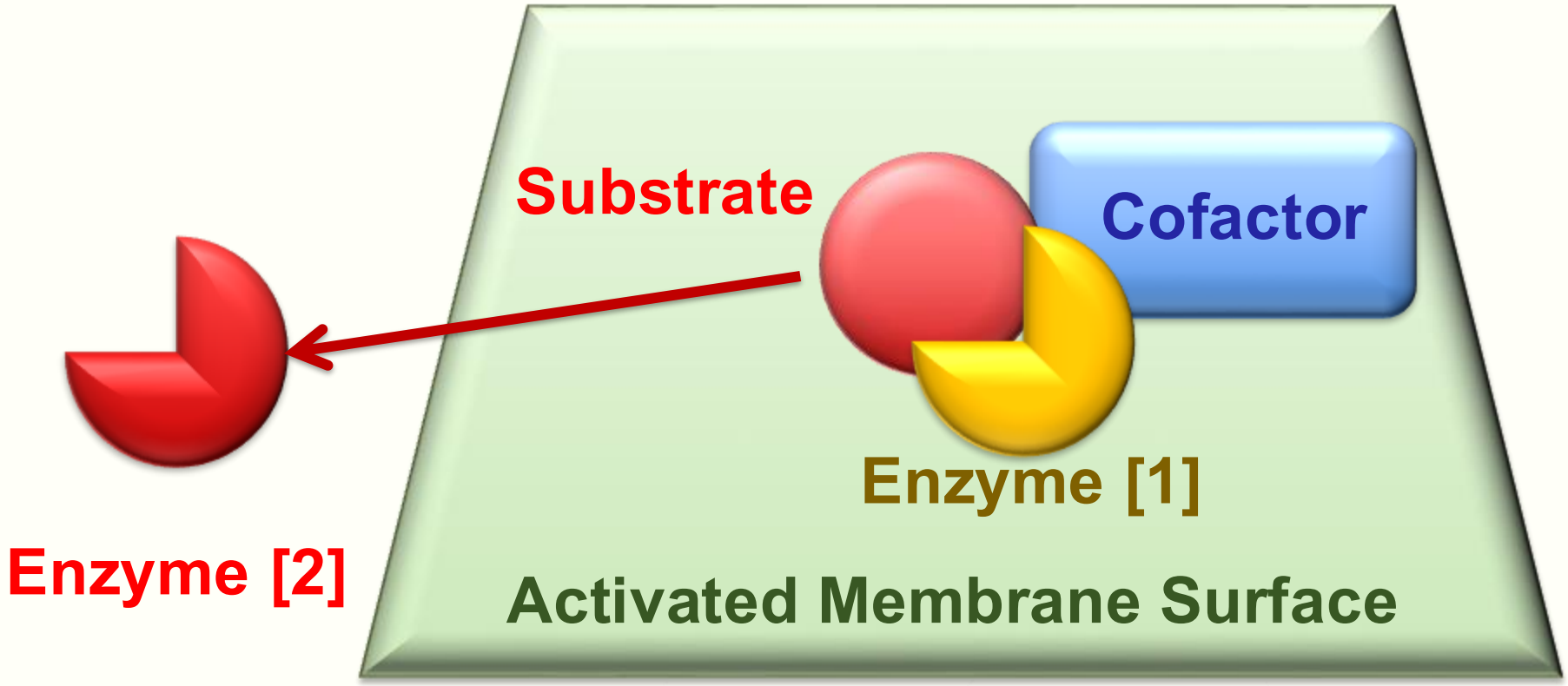
1) For years we have recognized the inconsistencies within these pathways to truly inform us of a patient's hemostatic system.
2) There are markedly different clinical manifestations of deficiencies of different factors, particularly within the Intrinsic Pathway.
   - Why do some deficiencies of the 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.
   a) No "upstream" factor(s) had been shown to activate Factor V or VIII.
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



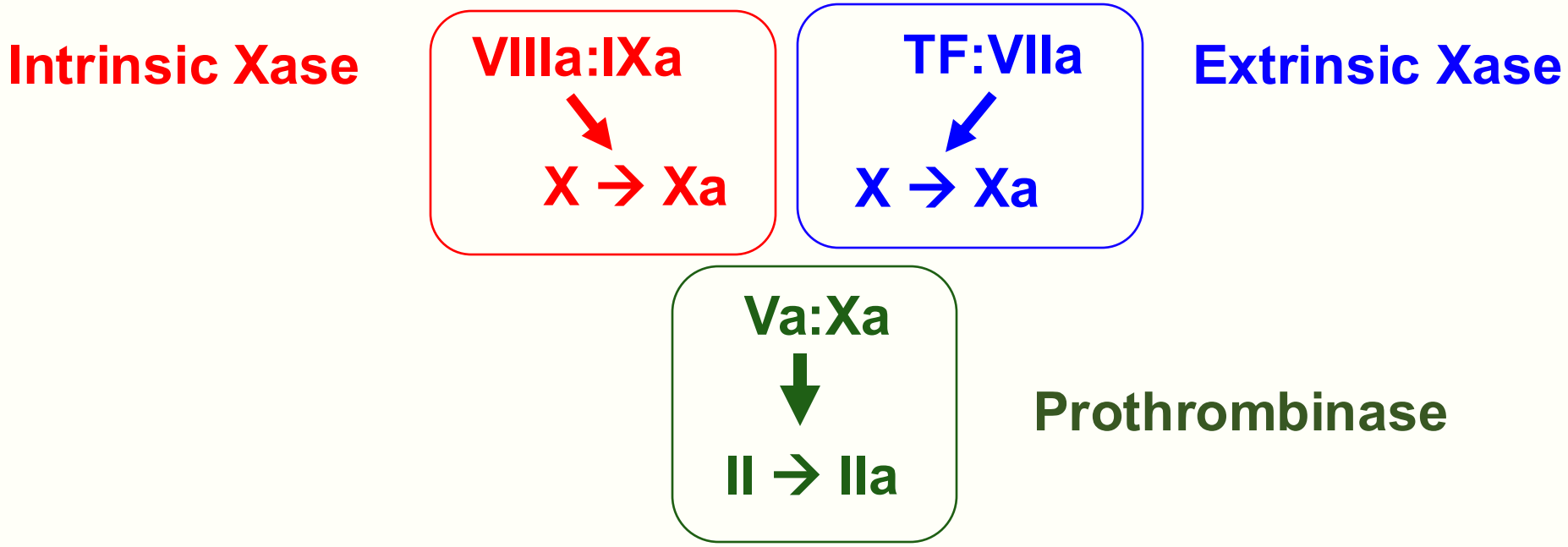
The Cell (Surface) Based Model Of Coagulation

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

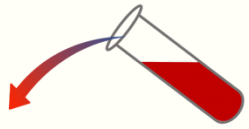


Three Complexes of the "Classic Cascade"

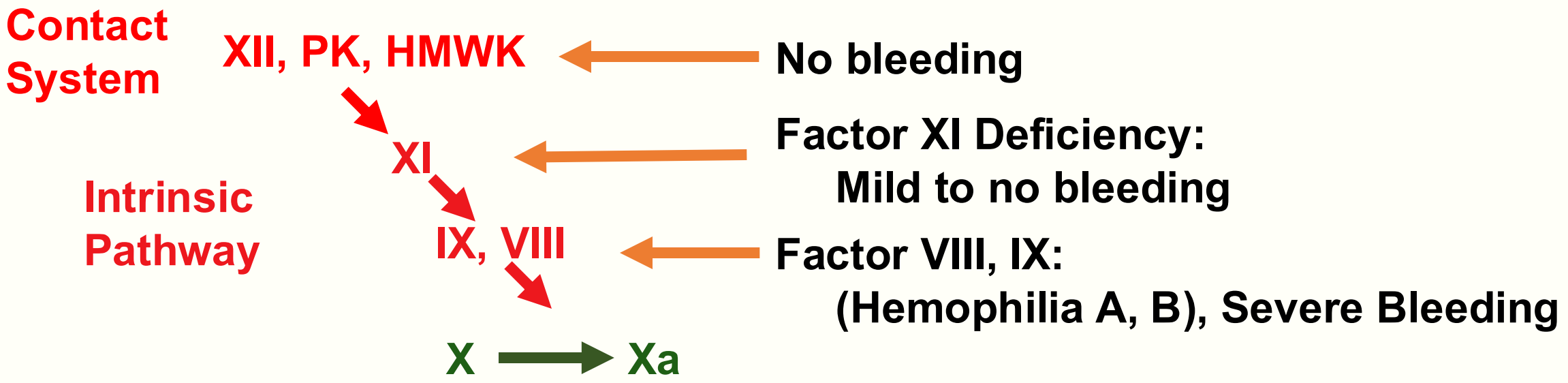
Pathway	Complex	Enzyme	Cofactor	Substrate	Product
Intrinsic Pathway	Intrinsic Xase	IXa	VIIIa	X	Xa
Extrinsic Pathway	Extrinsic Xase	VII/VIIa	TF	X	Xa
Common Pathway	Prothrombinase	Xa	Va	II	IIa



"Cross-Over" of Extrinsic and Intrinsic Pathways

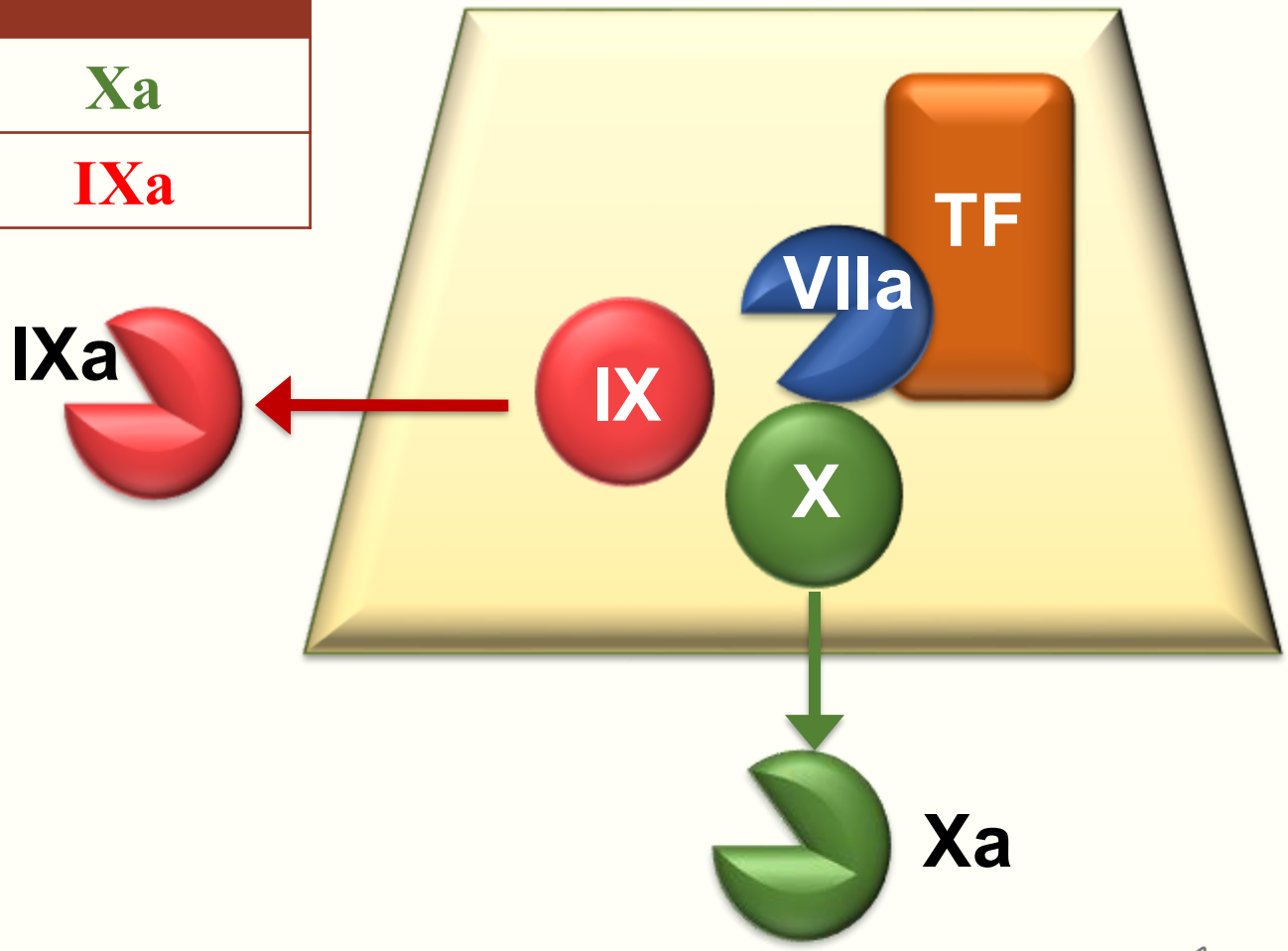


Deficiencies of Different Factors Within the Intrinsic Pathway Lead to Different Clinical Manifestations



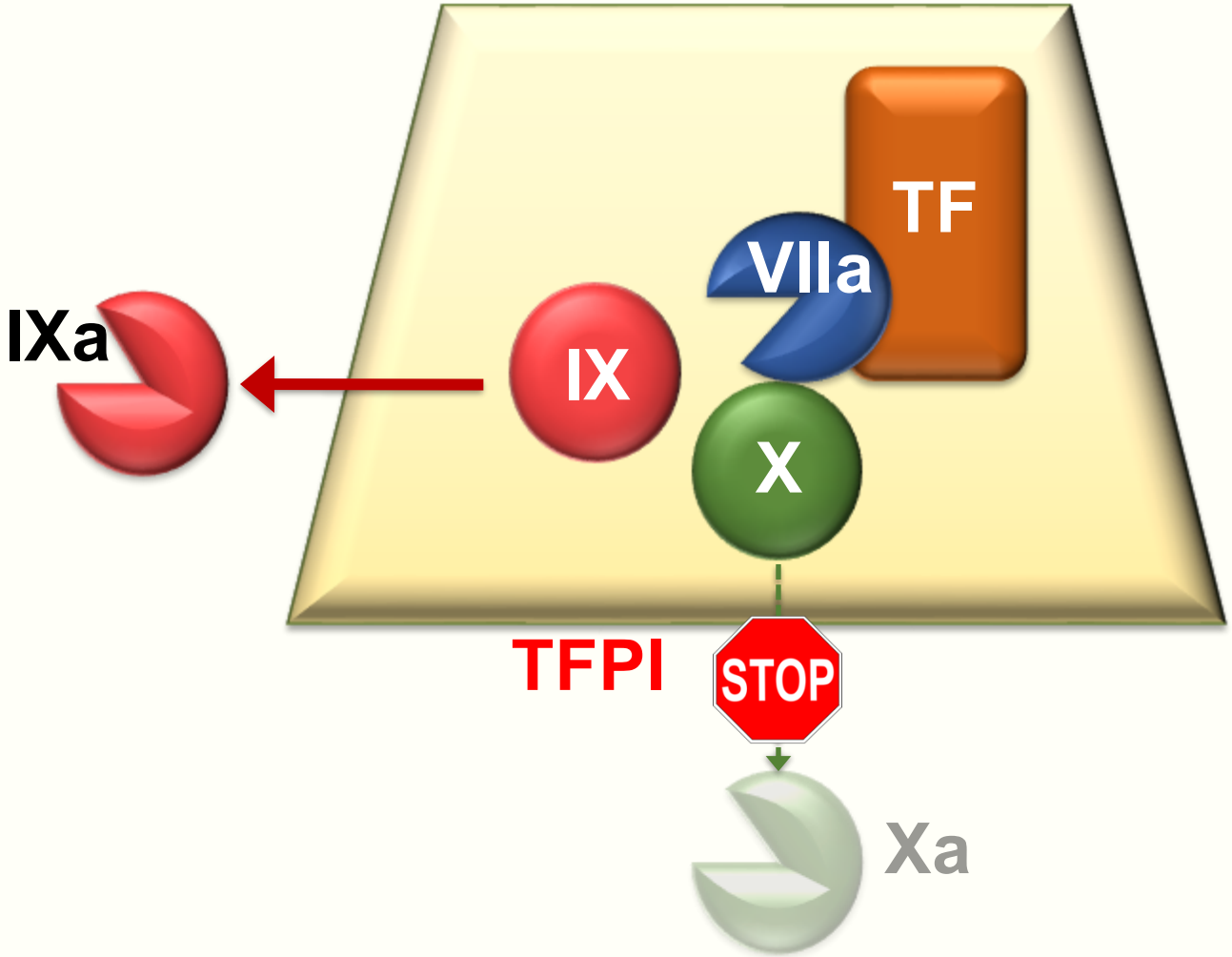
There Are Two Alternative Substrates of TF:VIIa Complex

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

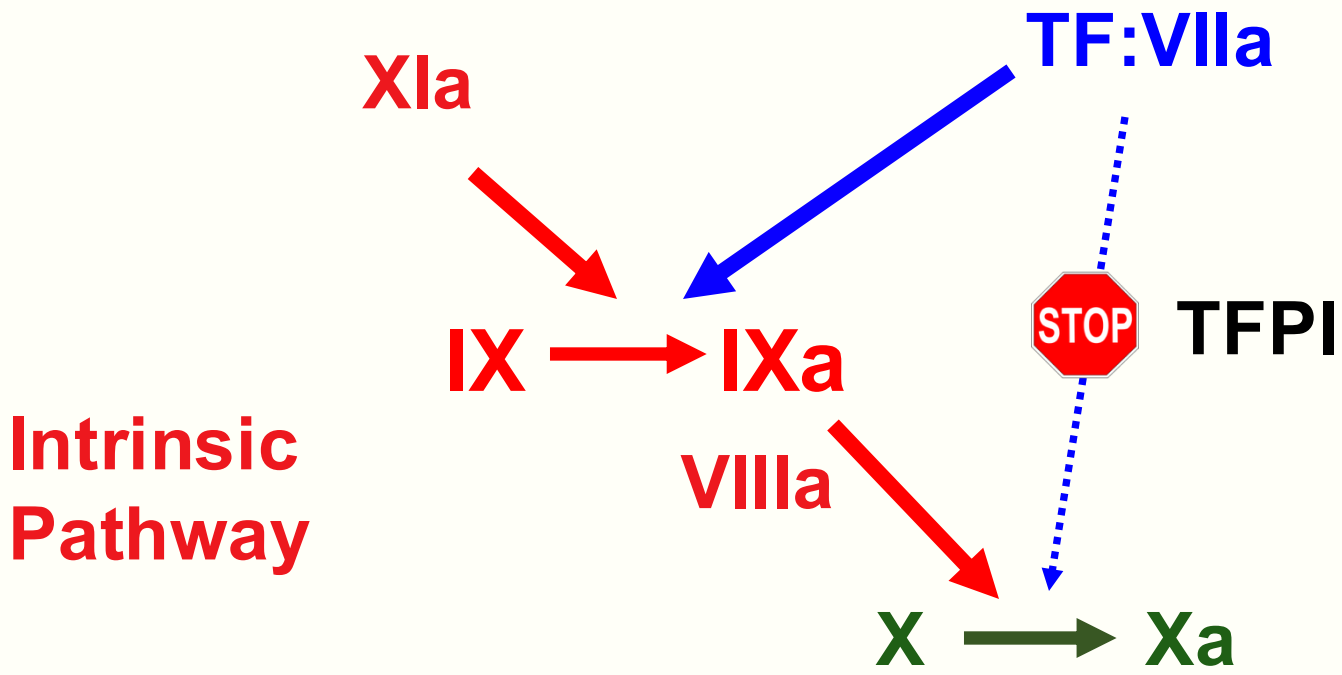


Tissue Factor Pathway Inhibitor (TFPI)

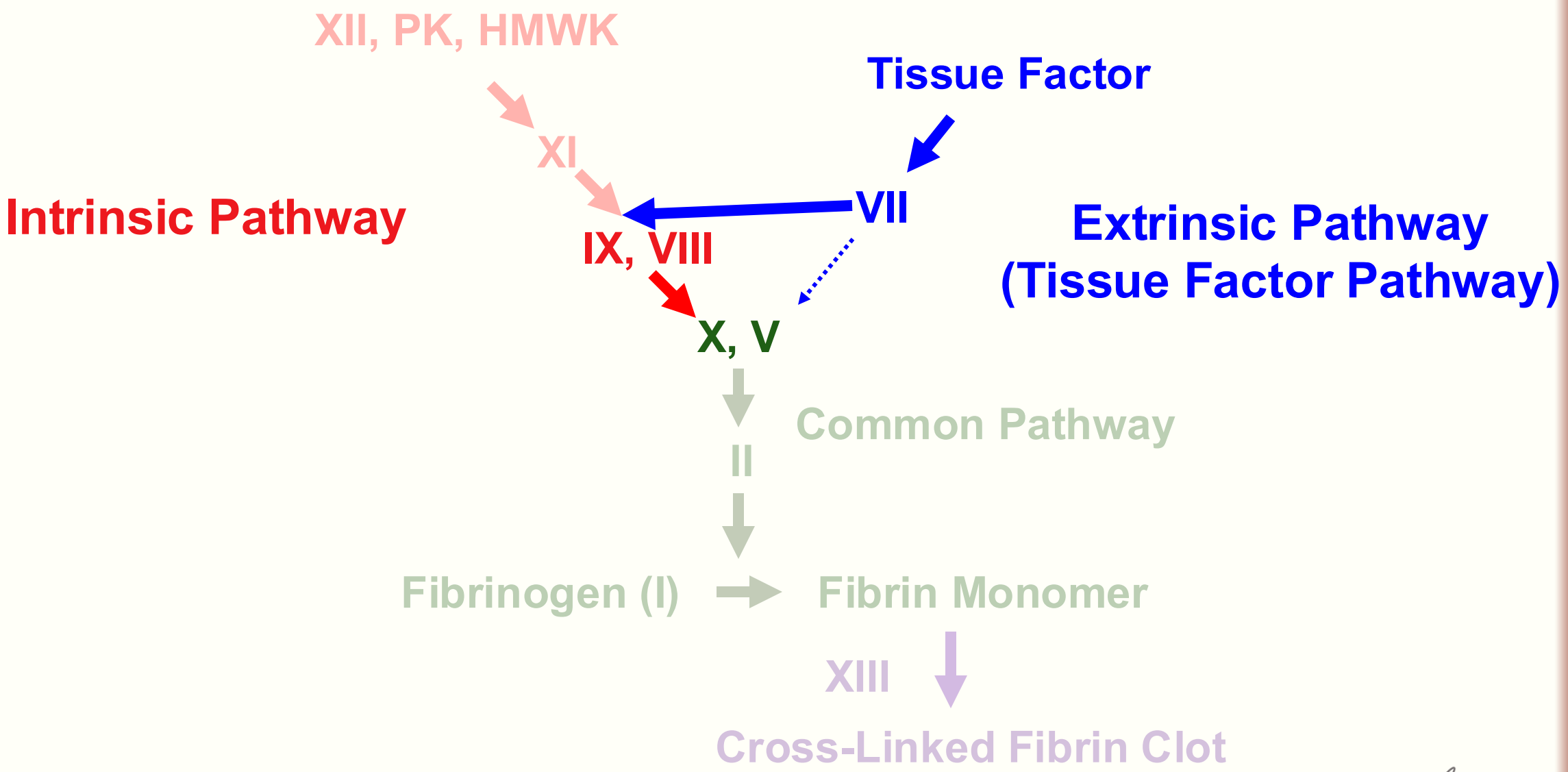
- **TFPI inhibits activation of Factor X by TF:VIIa.**
- **Therefore, *In Vivo, the primary substrate of FVIIa is F IX.***
- ***In addition to activation of Factor X, the Extrinsic Pathway "Crosses Over" into the Intrinsic Pathway.***



Factor IX Can Be Activated By TF:VIIa or XIa



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

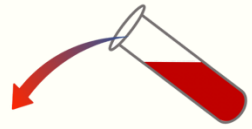

XII, PK, HMWK
XI
Intrinsic Pathway
Tissue Factor
VII
Extrinsic Pathway
(Tissue Factor Pathway)
IX, VIII
X, V
Common Pathway
II
Fibrinogen (I)
Fibrin Monomer
XIII
Cross-Linked Fibrin Clot

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

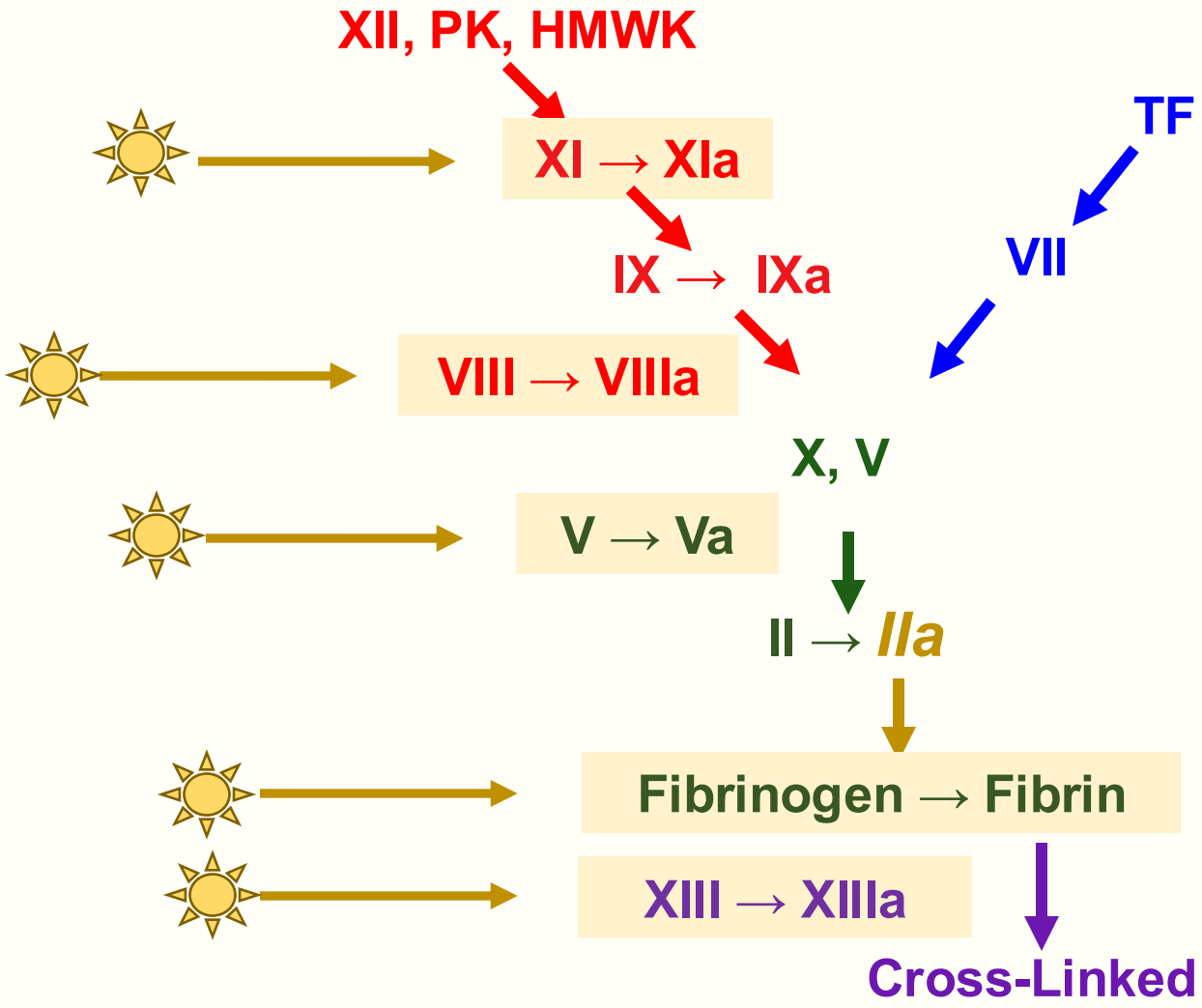
https://commons.wikimedia.org/wiki/File:Most_distant_Gamma-ray_burst.jpg

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.**



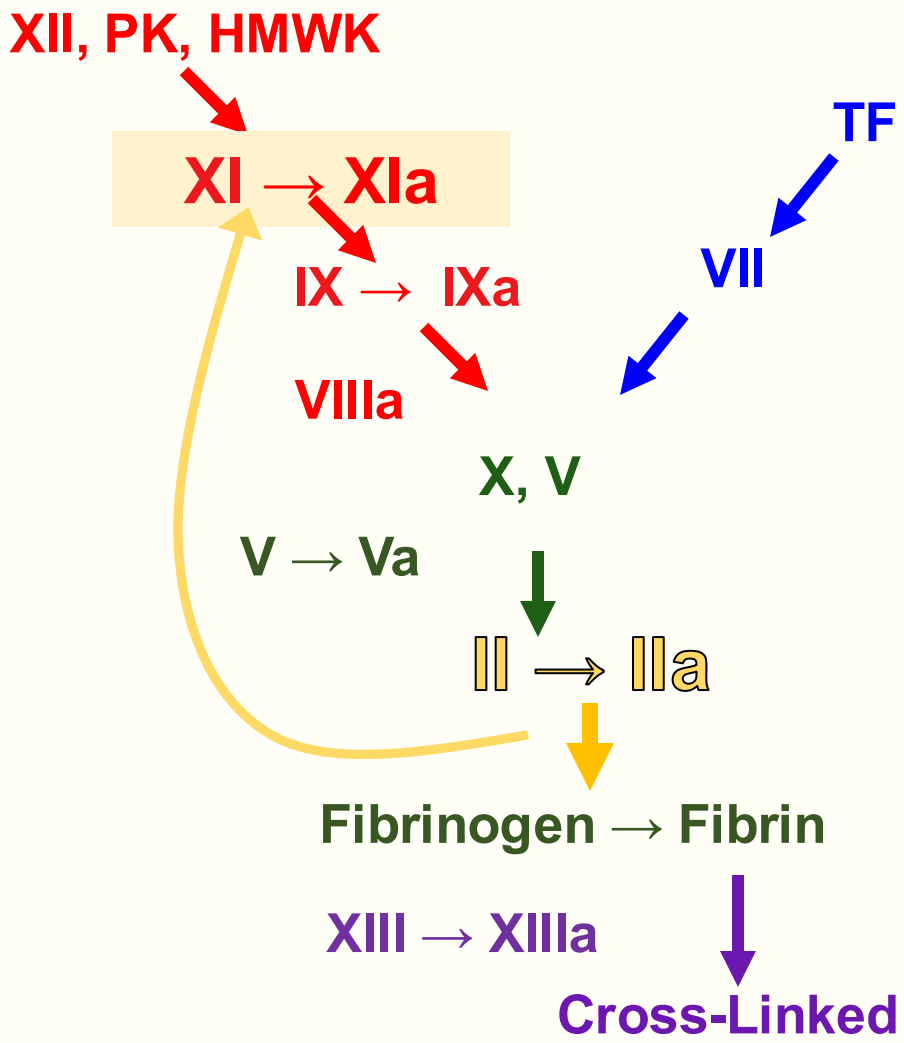
Thrombin Feedback;
Activation of Factors V, VIII, XI, XIII



Procoagulant Activities of Thrombin

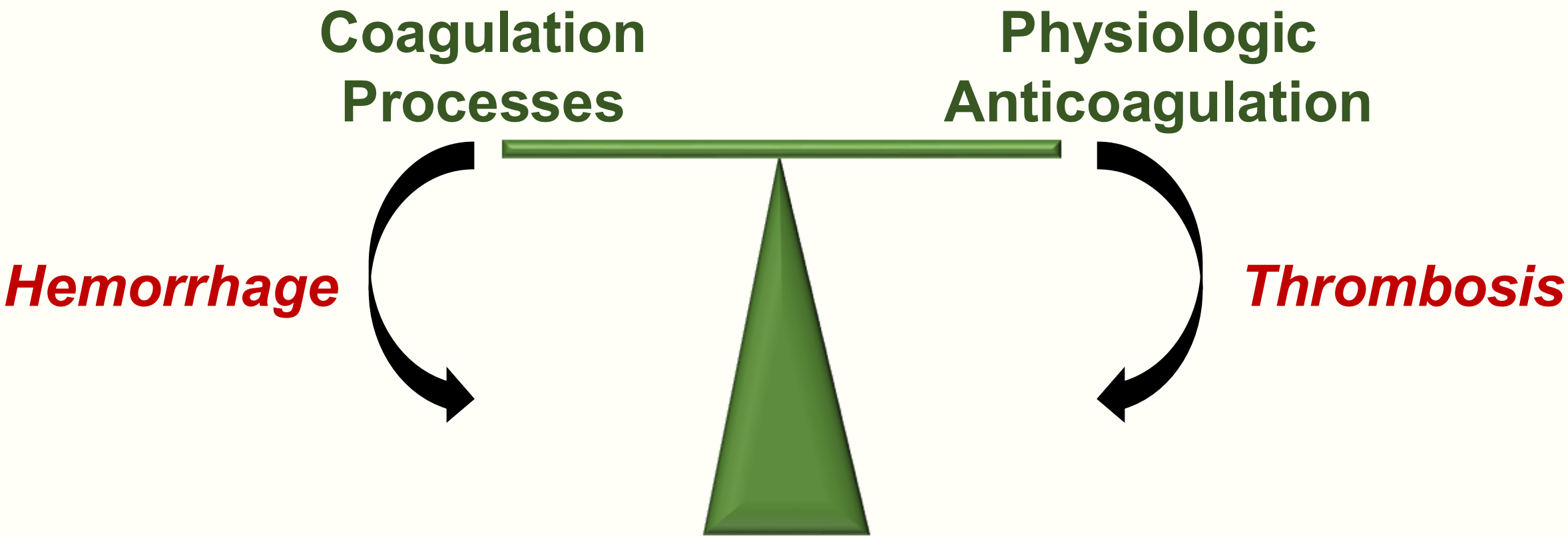
1. Cleavage of Fibrinogen
2. Activation of Factor V
3. Activation of Factor VIII
4. Activation of Factor XI
5. Activation of Factor XIII
6. [Activation of Platelets]

Role of Factor XI



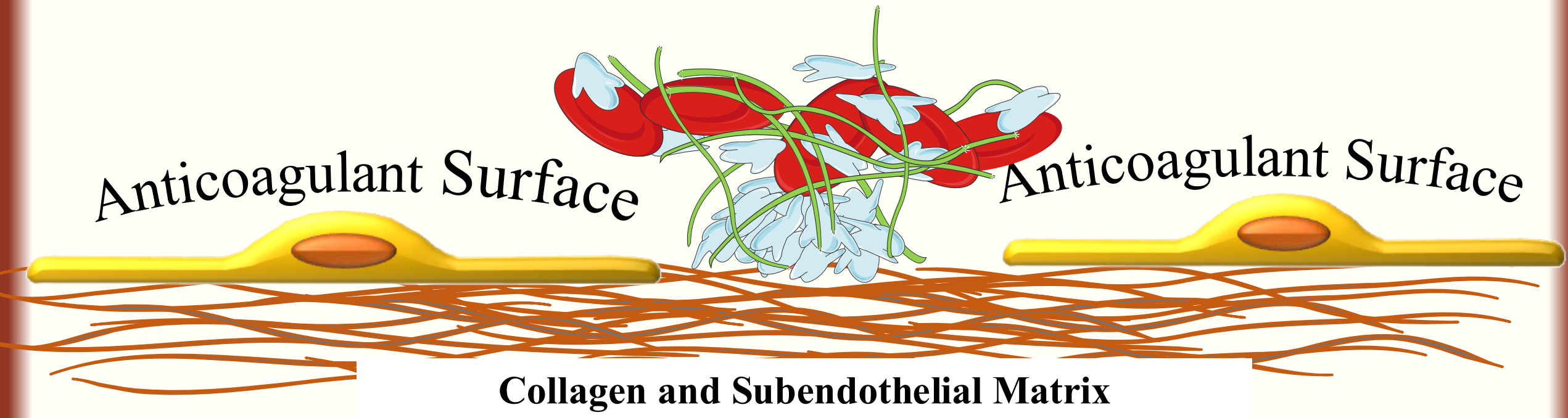
- Factor XI can be activated by XIIa or Thrombin.
- Factor XI is a component of a positive feedback loop.
- Factor XI also links the Contact System with the core coagulation pathway.
- Factor XI deficiency is rarely associated with spontaneous bleeding.
- Inhibition of Factor XI is being explored as new option for therapeutic anticoagulation!

The Hemostatic Balance:
Physiologic Anticoagulation Processes



Vascular Endothelial Cells Present Anticoagulant Surface

- Vascular endothelial cells present anticoagulant surface.
- Disruption of endothelial surface exposes blood to Collagen and Subendothelial Matrix (procoagulants) leading to activation of coagulation.
- Deficiency of physiologic anticoagulants leads to activation of coagulation.



Physiologic Anticoagulation Processes on Endothelial Cells

Pathway	Activity	Effect
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	Directs TF:VIIa activity towards activation of F IX to IXa.
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

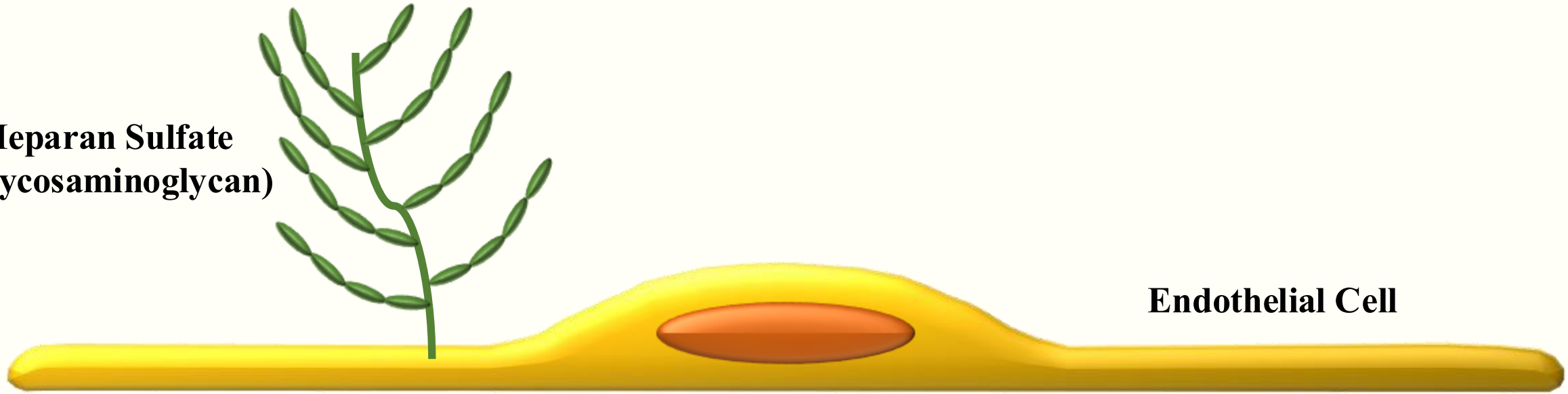
Antithrombin

Antithrombin:
Inactive Conformation

Thrombin

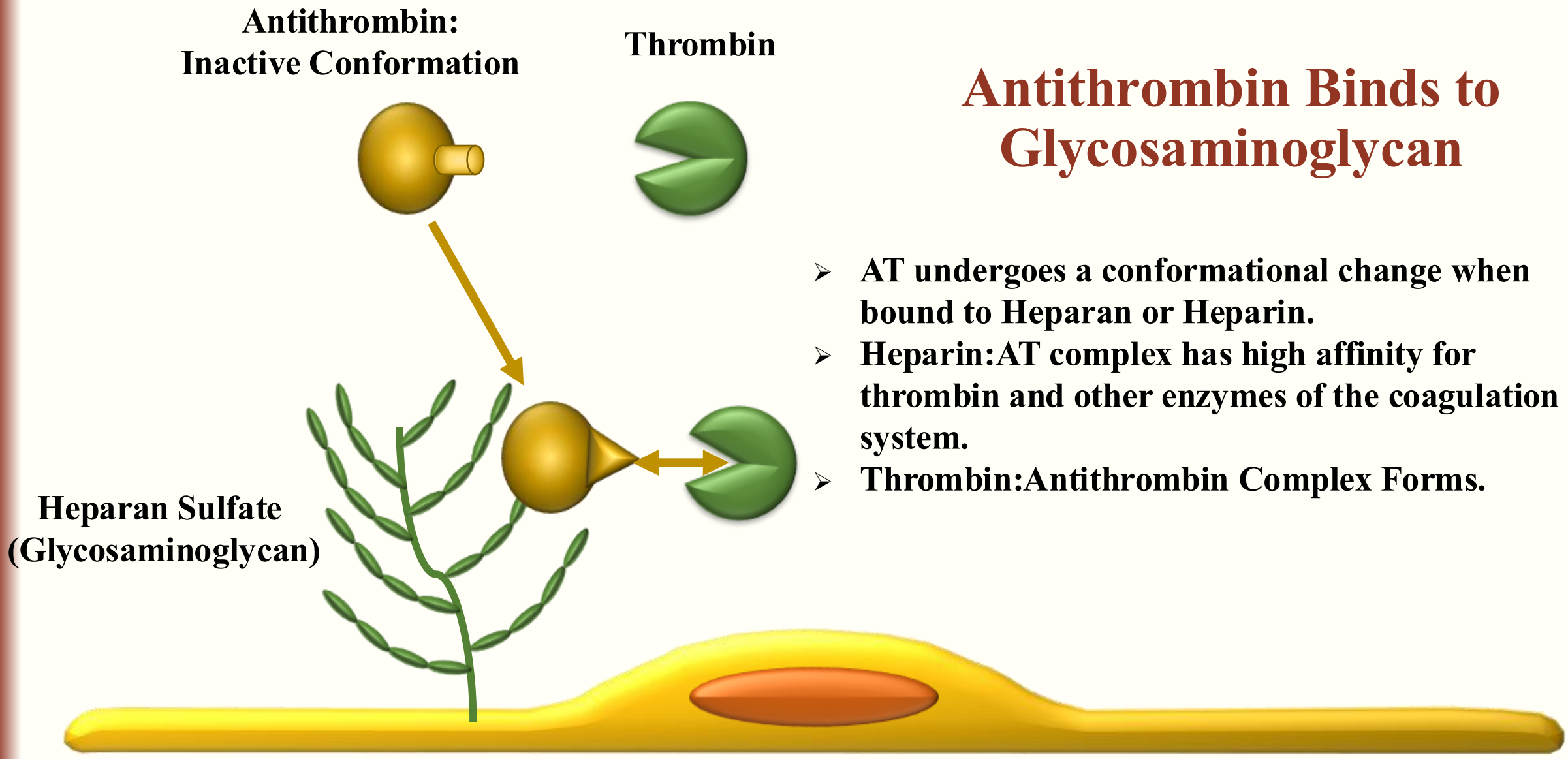
Antithrombin, in fluid phase is unable to bind thrombin or other enzymes.

Heparan Sulfate
(Glycosaminoglycan)



Endothelial Cell

Antithrombin Binds to Glycosaminoglycan

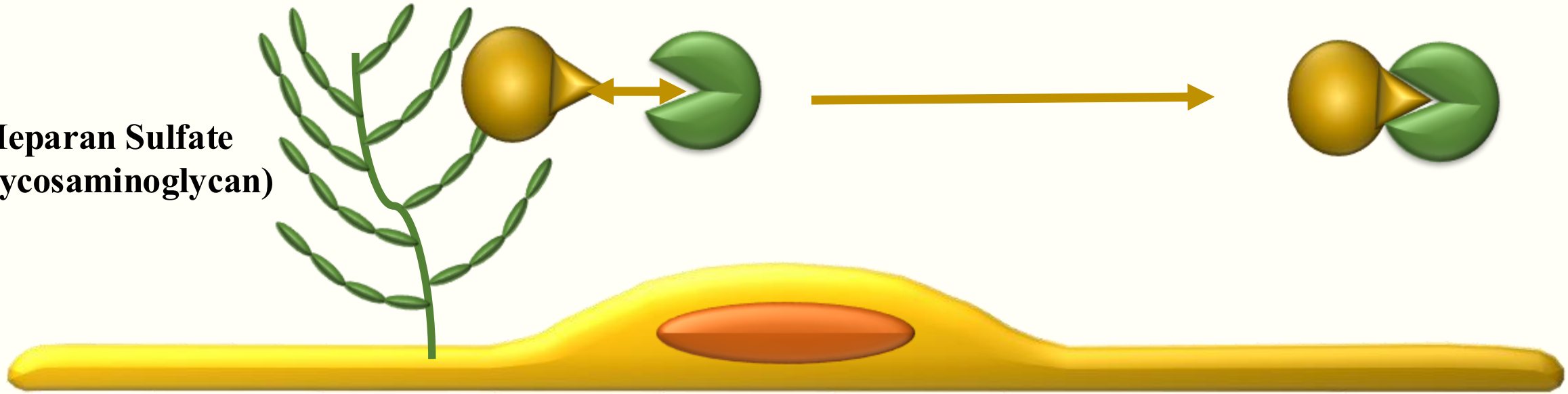


- AT undergoes a conformational change when bound to Heparan or Heparin.
- Heparin:AT complex has high affinity for thrombin and other enzymes of the coagulation system.
- Thrombin:Antithrombin Complex Forms.

Antithrombin: Glycosaminoglycan

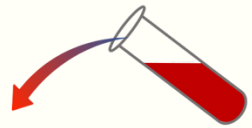
Thrombin:Antithrombin Complex Dissociates from Glycosaminoglycan and is cleared in the liver.

Heparan Sulfate (Glycosaminoglycan)

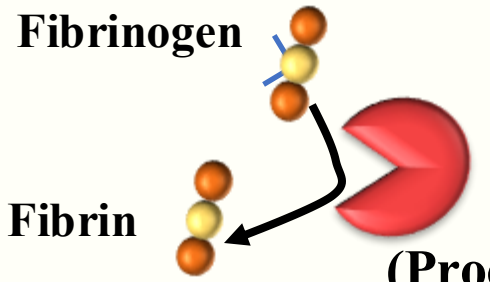


Protein C/Protein S/Thrombomodulin System

- 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.

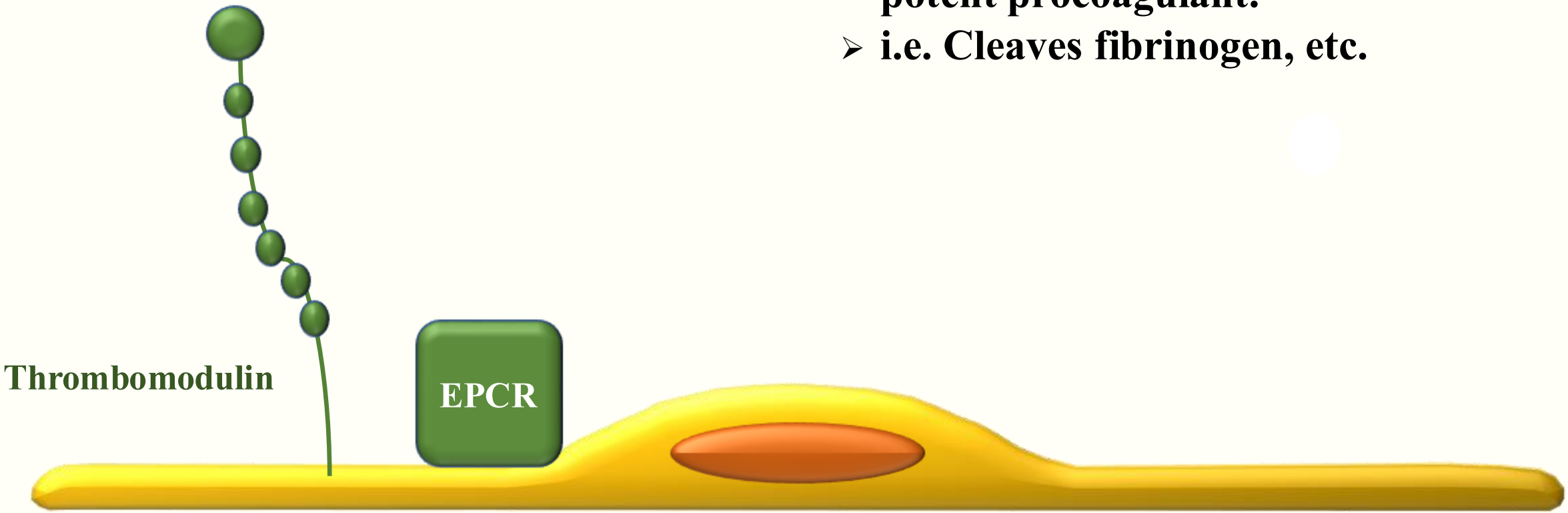


Protein C/Protein S/Thrombomodulin



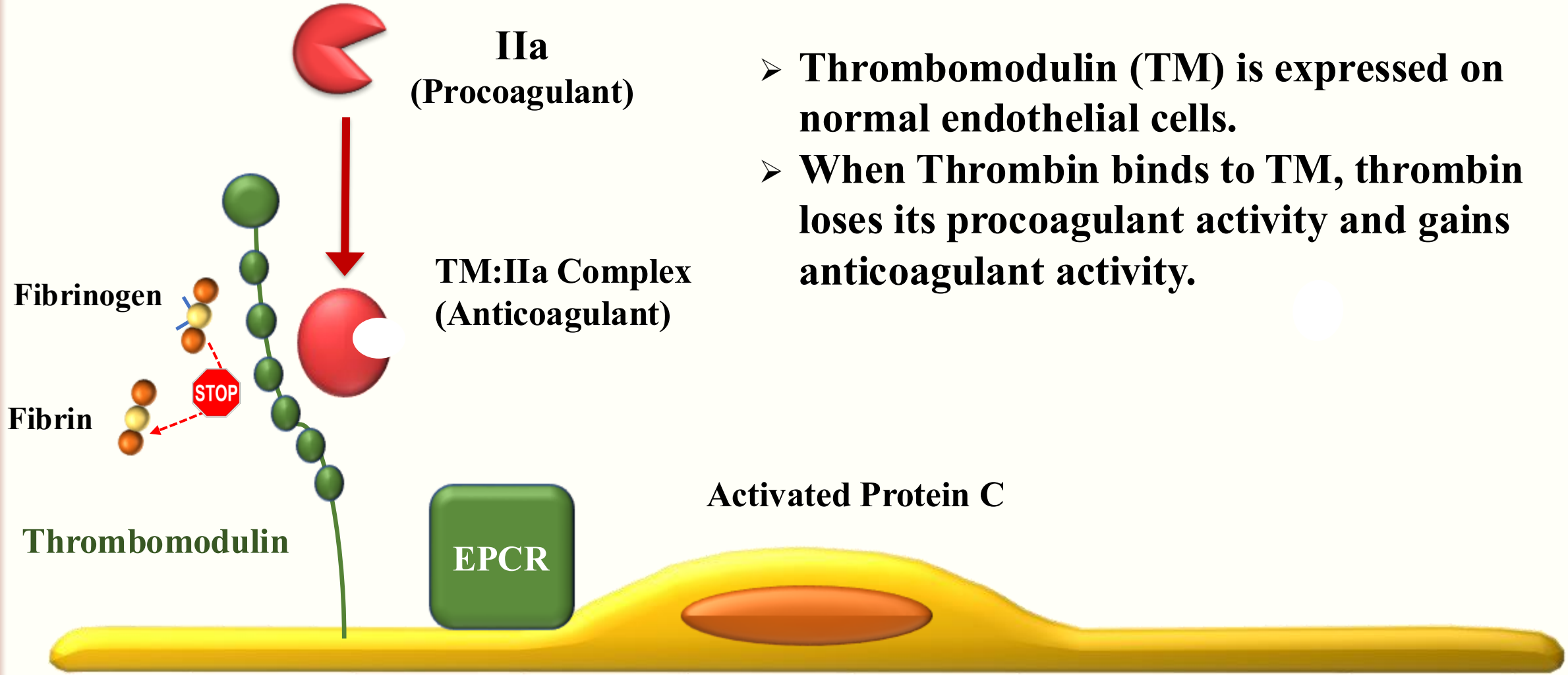
IIa
(Procoagulant)

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



EPCR: Endothelial Cell Protein C Receptor

Protein C/Protein S/Thrombomodulin

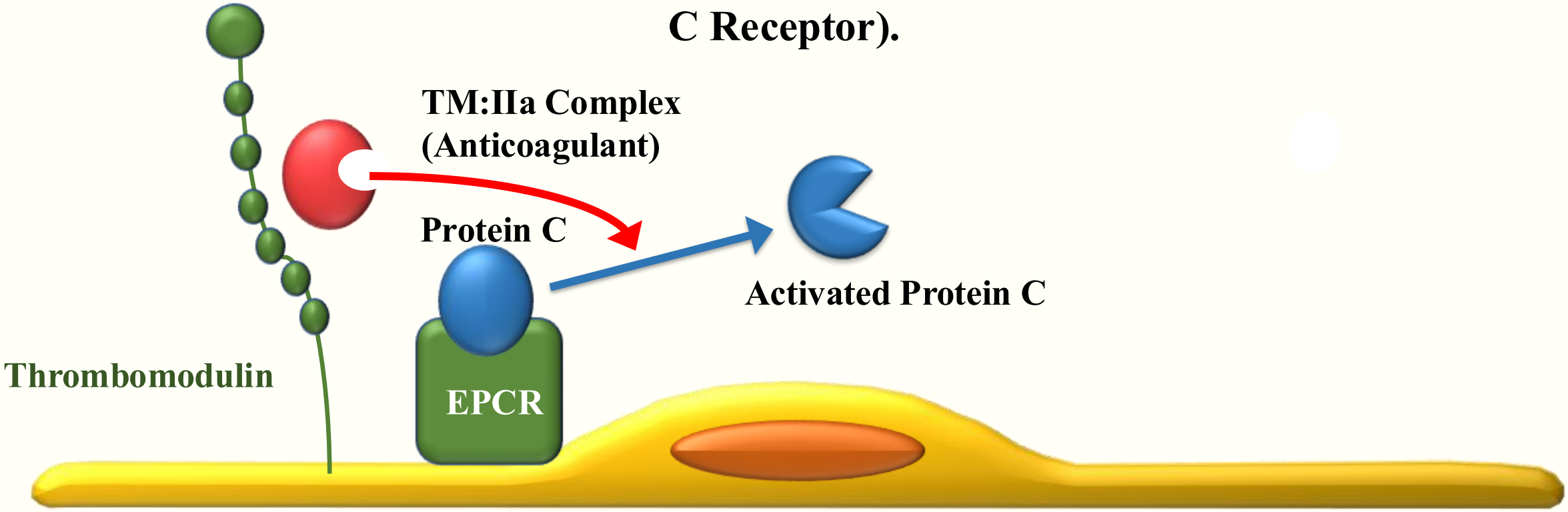


- Thrombomodulin (TM) is expressed on normal endothelial cells.
- When Thrombin binds to TM, thrombin loses its procoagulant activity and gains anticoagulant activity.

EPCR: Endothelial Cell Protein C Receptor

Protein C/Protein S/Thrombomodulin

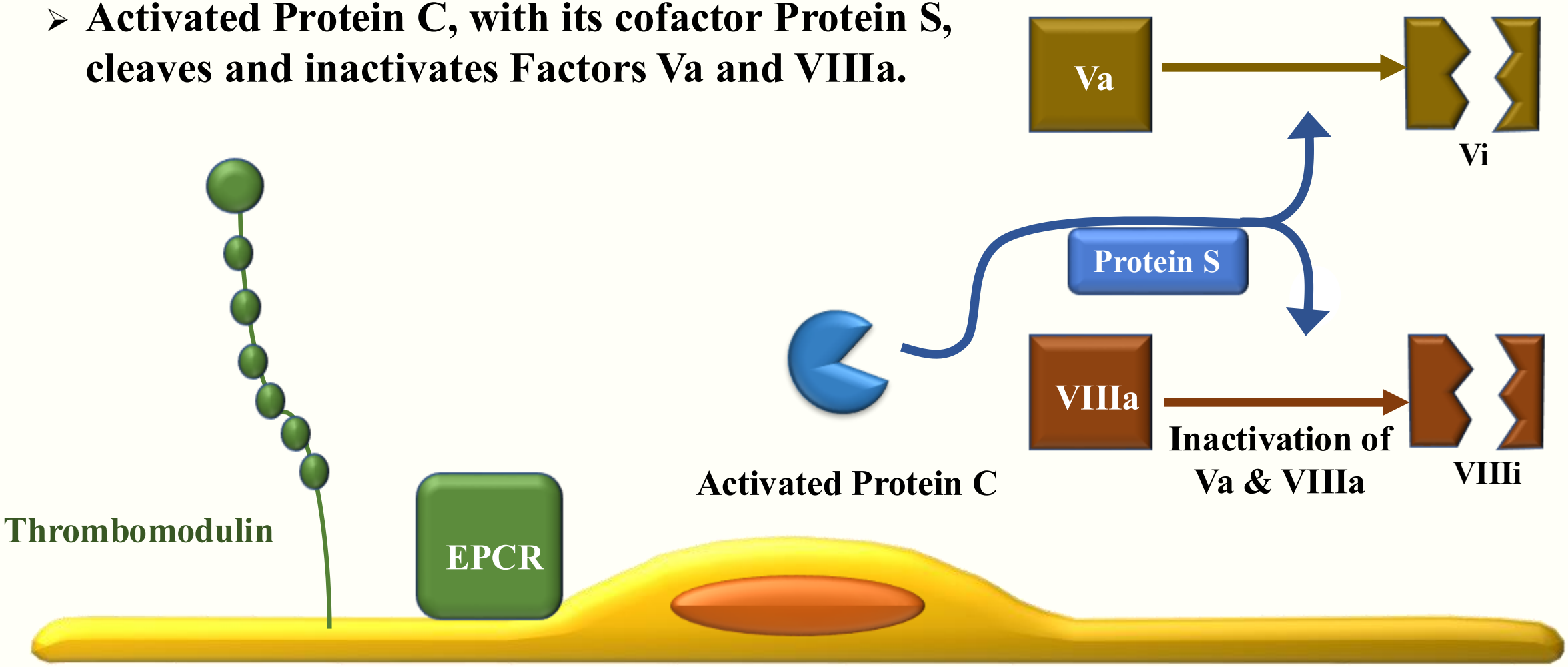
- Thrombin:Thrombomodulin complex cleaves and activates Protein C.
- (Protein C localizes to Endothelial Cell Protein C Receptor).



EPCR: Endothelial Cell Protein C Receptor

Protein C/Protein S/Thrombomodulin

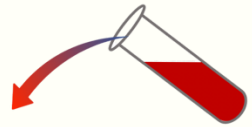
- **Activated Protein C, with its cofactor Protein S, cleaves and inactivates Factors Va and VIIIa.**



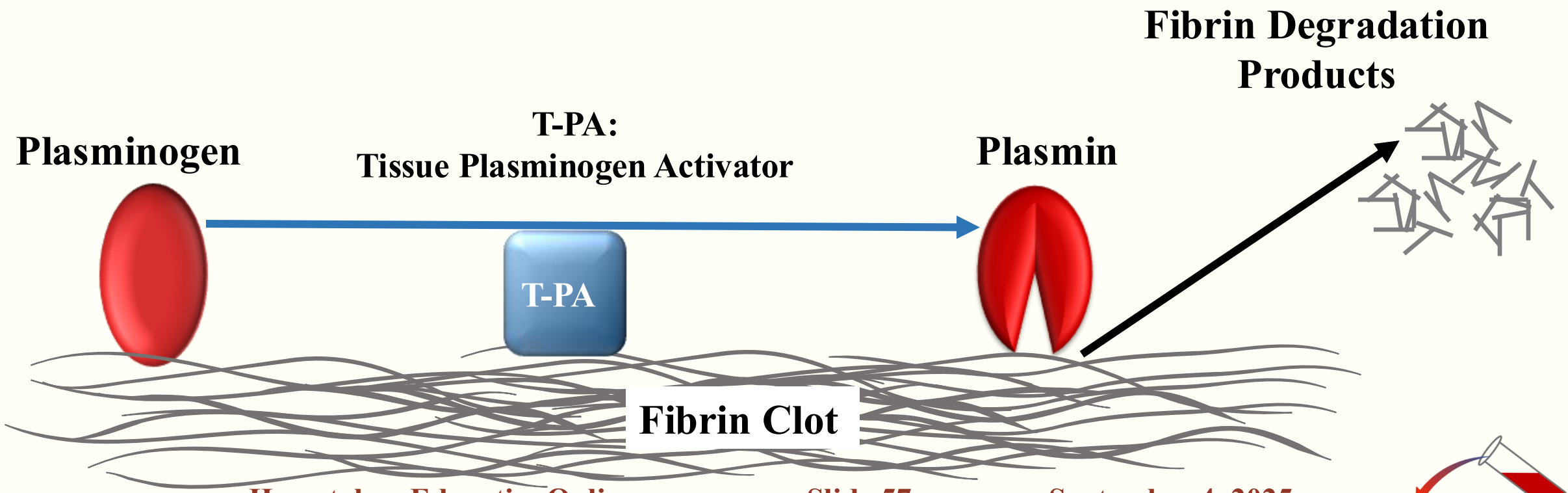
EPCR: Endothelial Cell Protein C Receptor

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.

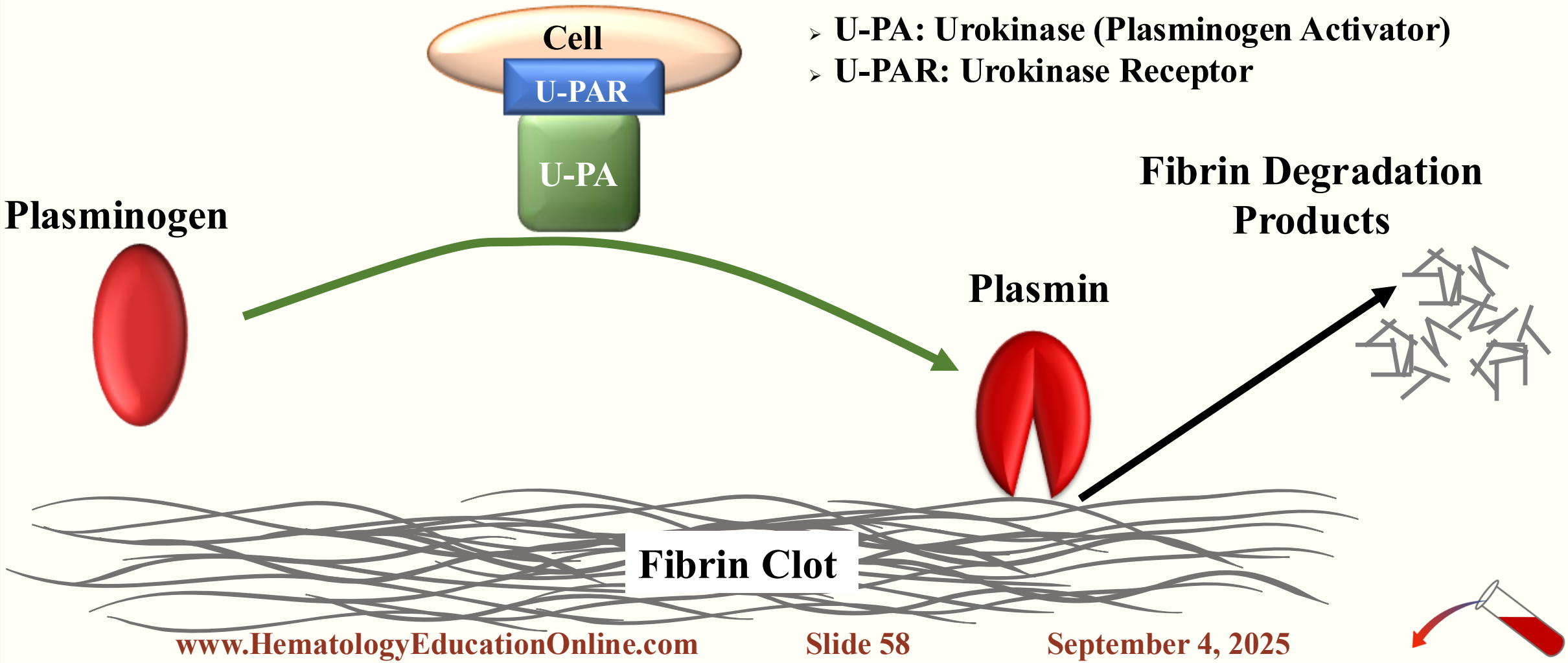


Fibrinolytic Pathway:
T-PA, Fibrin Clot Based Activation

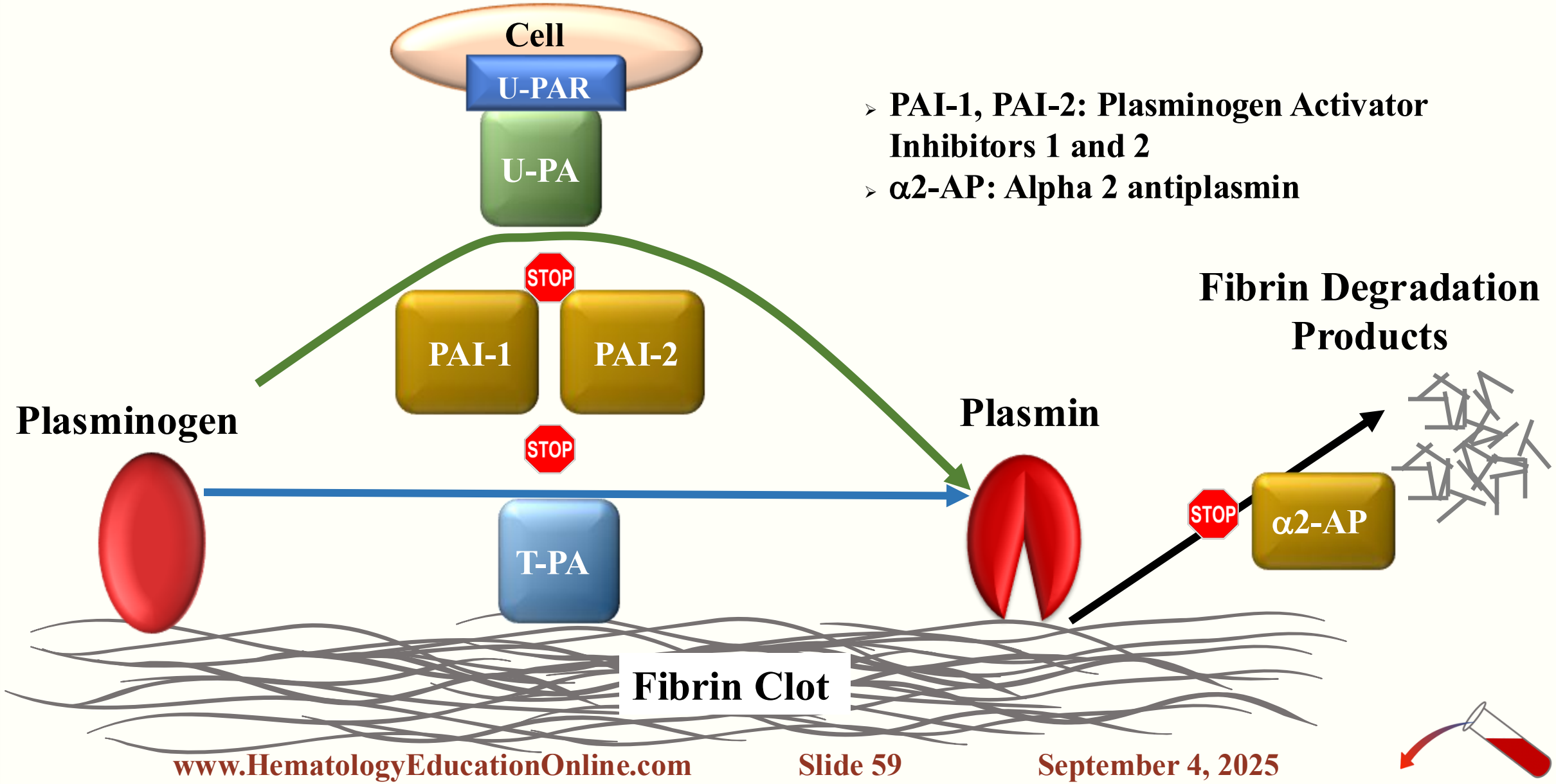


Fibrinolytic Pathway: U-PA/U-PAR, Cell Based Activation

- 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