



The Coagulation System

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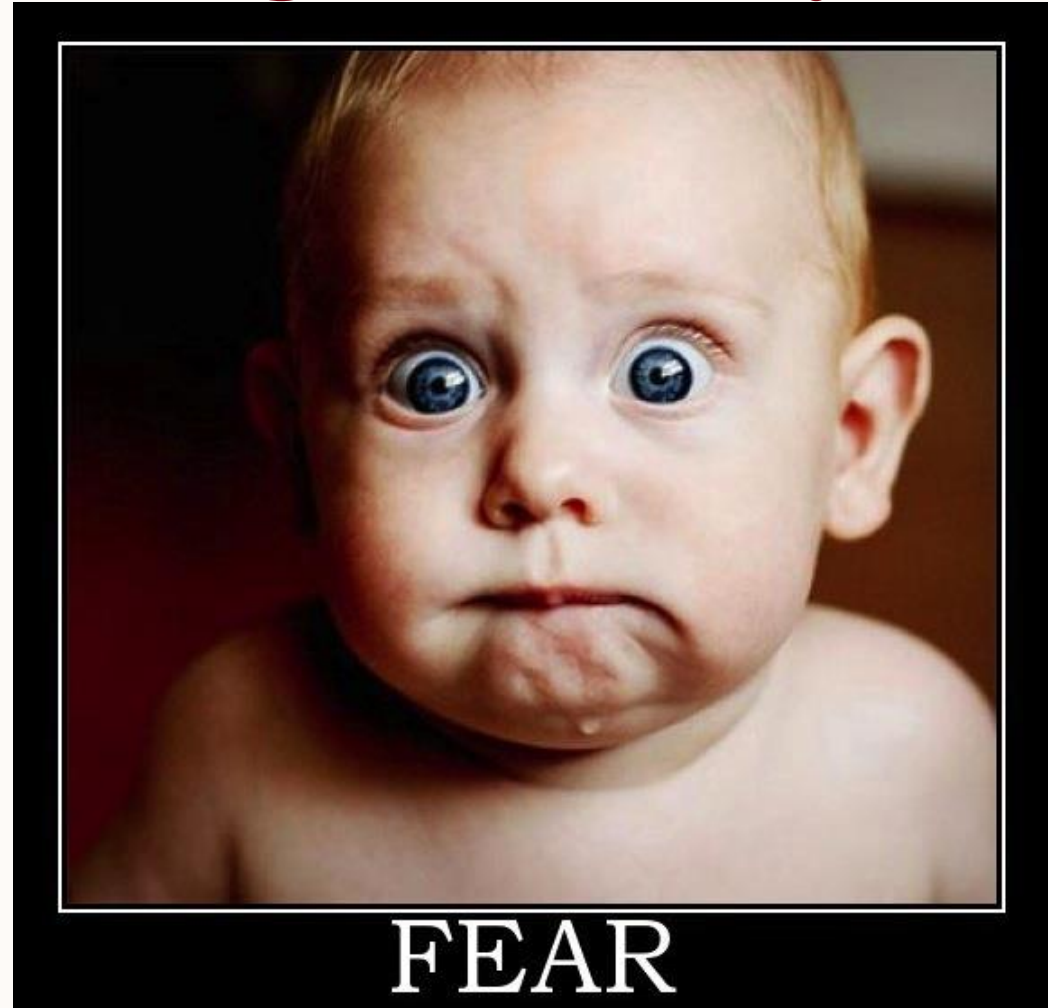


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 - Amgen
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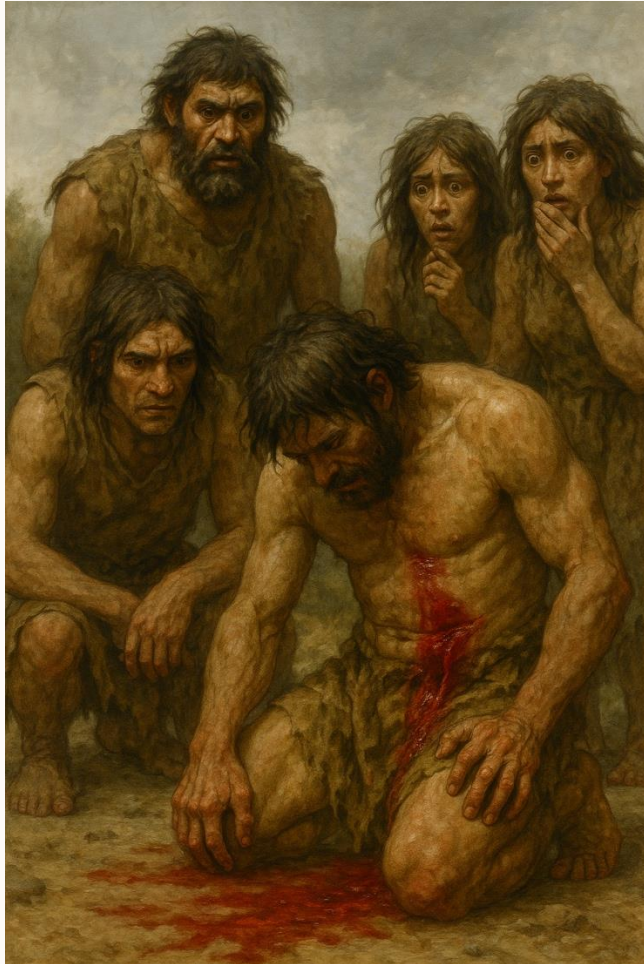
- Data Safety Monitoring Committee
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Coagulation System

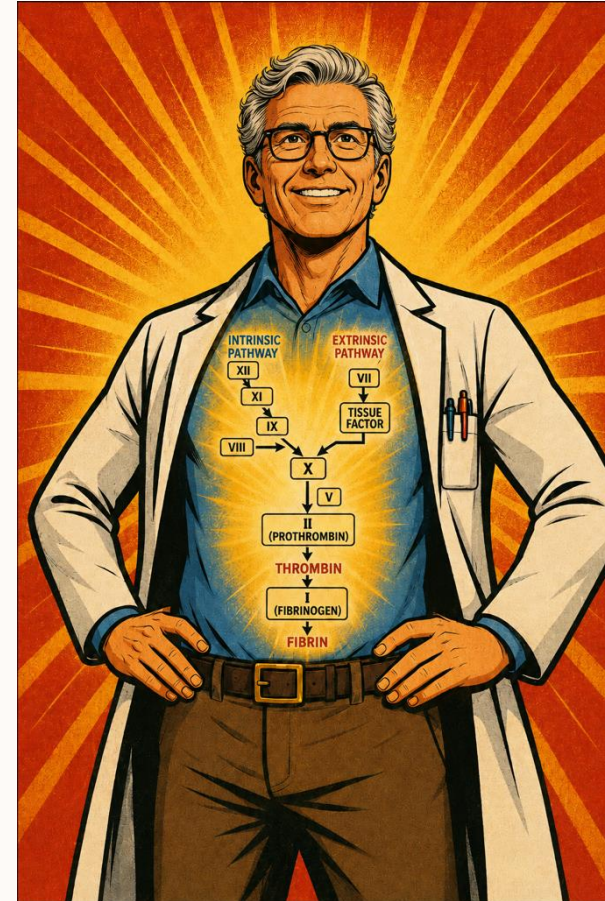


<https://kidsfirstpediatrics.com/babies-separation-anxiety/>

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?



AI-Generated
Figures

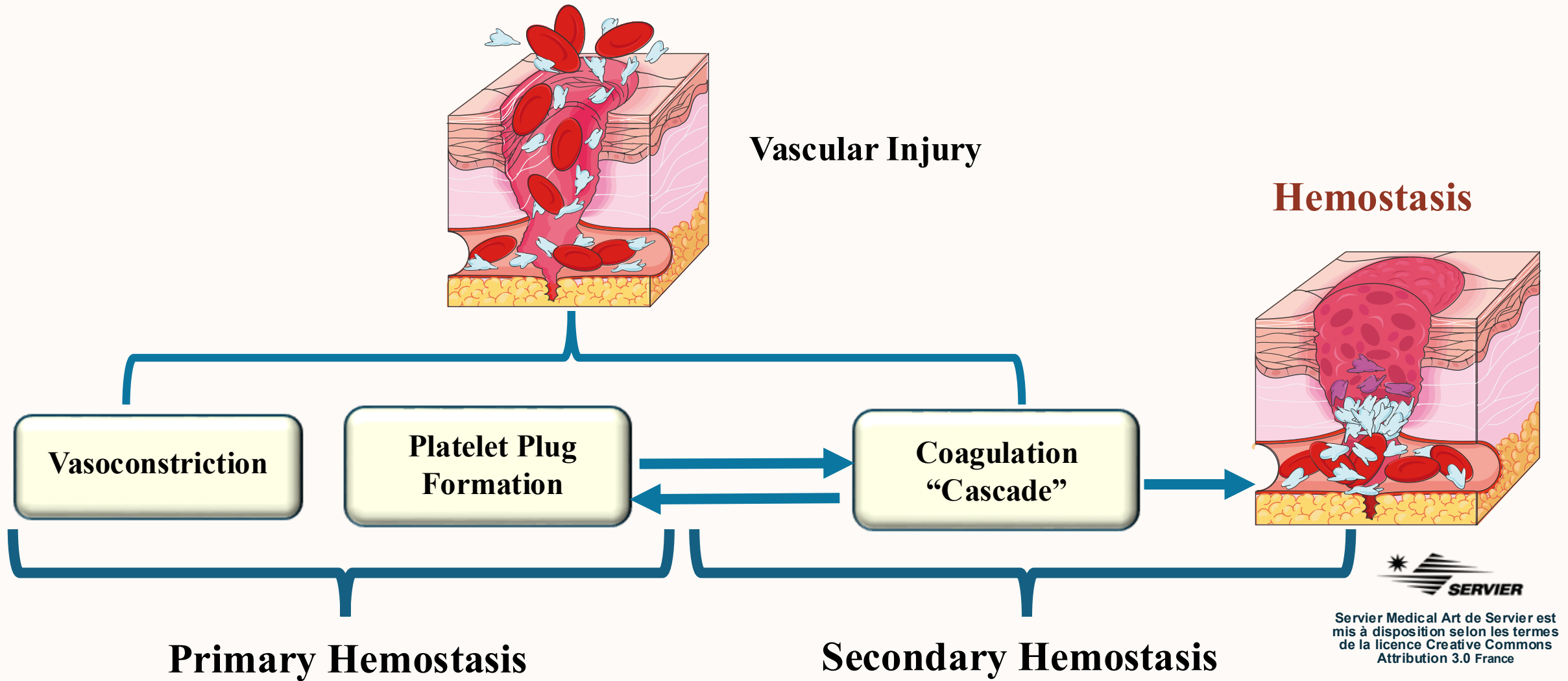
What We'll Cover

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

1. 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)
 - 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



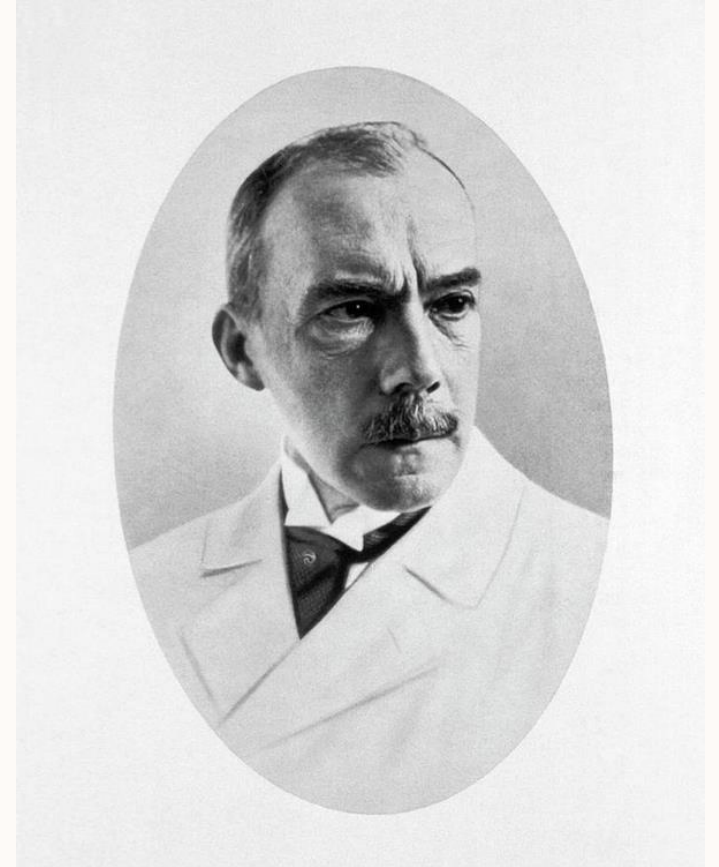
The Dawn of *In Vitro* Studies

- Contact Activation:
 - Described by Joseph Lister in 1863
 - Blood would clot when brought in contact with a surface other than vascular endothelium.
- Calcium Chelation could prevent blood clotting, and recalcification could restore the ability to clot:
 - Maurice Arthus and Calixte Pagès, in 1890
- Tissue Thromboplastin:
 - Tissue extract accelerated blood clotting.
 - Described by Nicolas Maurice Arthus in 1902.

2. The “Classic Coagulation Cascade”

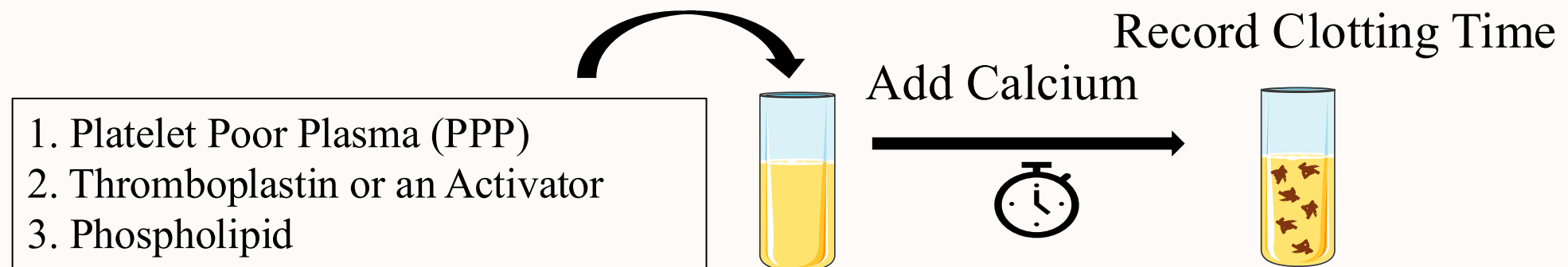
Early Understanding of Coagulation

- 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)

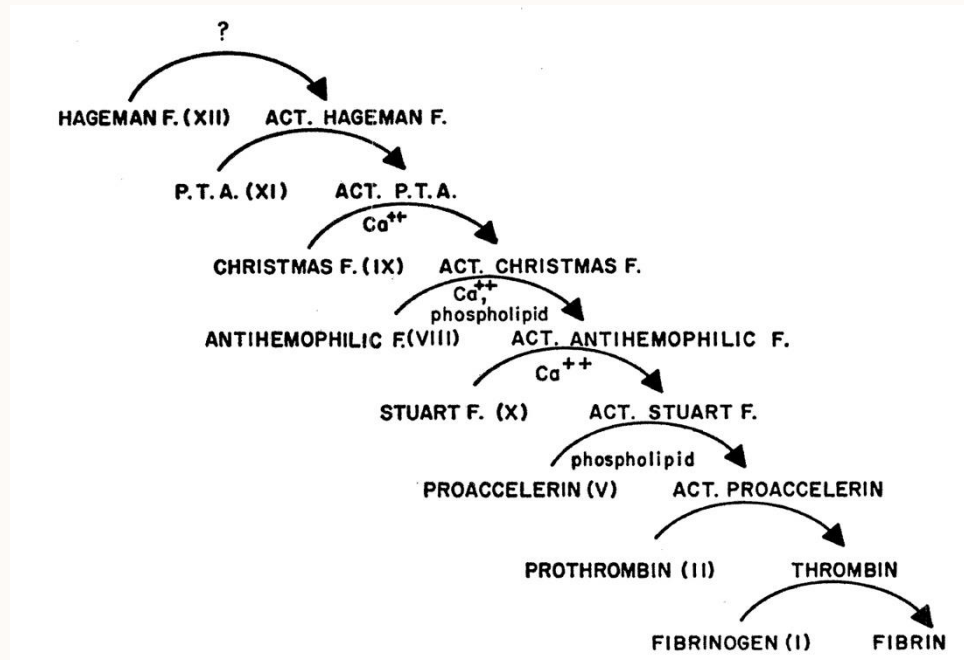
Plasma from patient with
unknown bleeding disorder



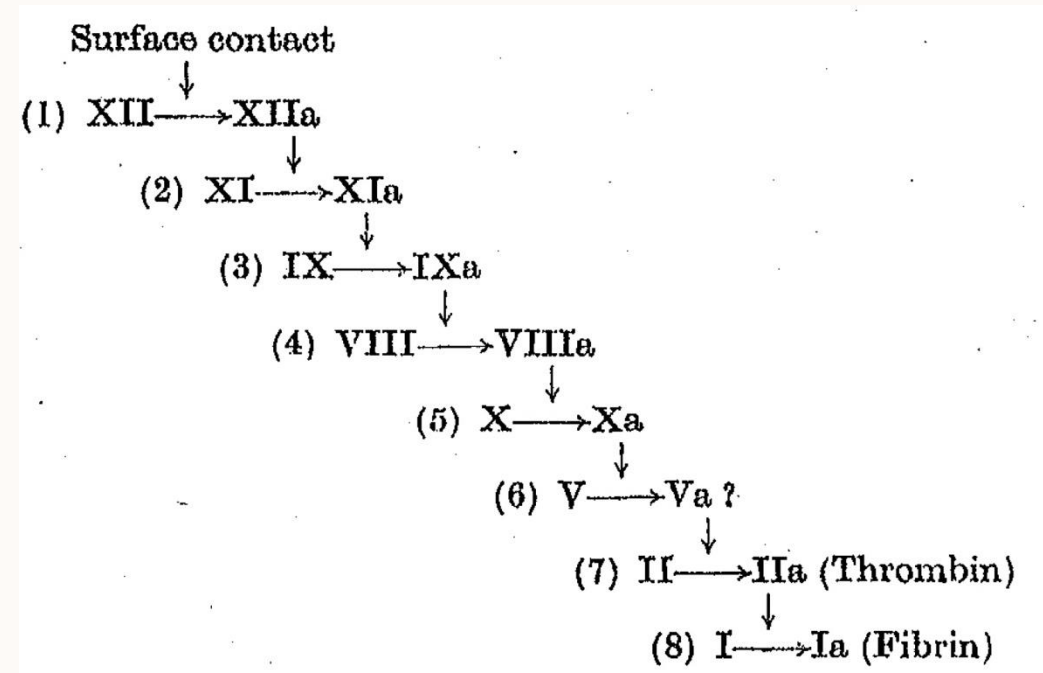
Plasma from patient with
known bleeding disorder
(i.e. Hemophilia A)

- 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

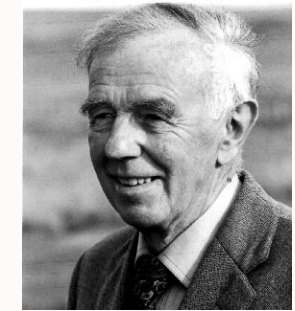
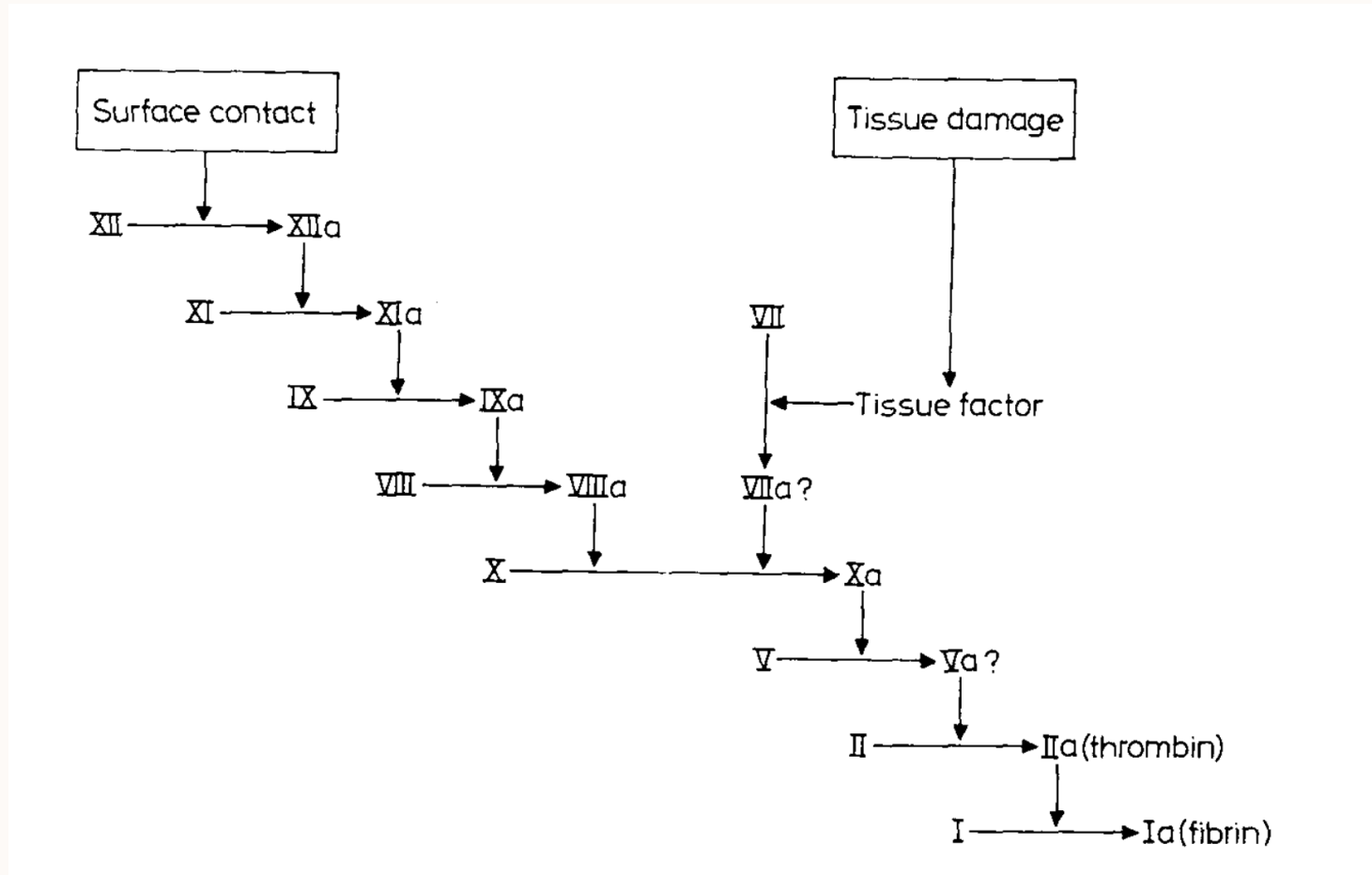


MacFarlane R.G. “An enzyme **cascade** in the blood clotting mechanism, and its function as a biological amplifier.” Nature 1964; 202: 498-9

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. Numerous gaps, corrections, and open questions remained. (To be discussed below).

Contemporary Representation of the Coagulation Cascade

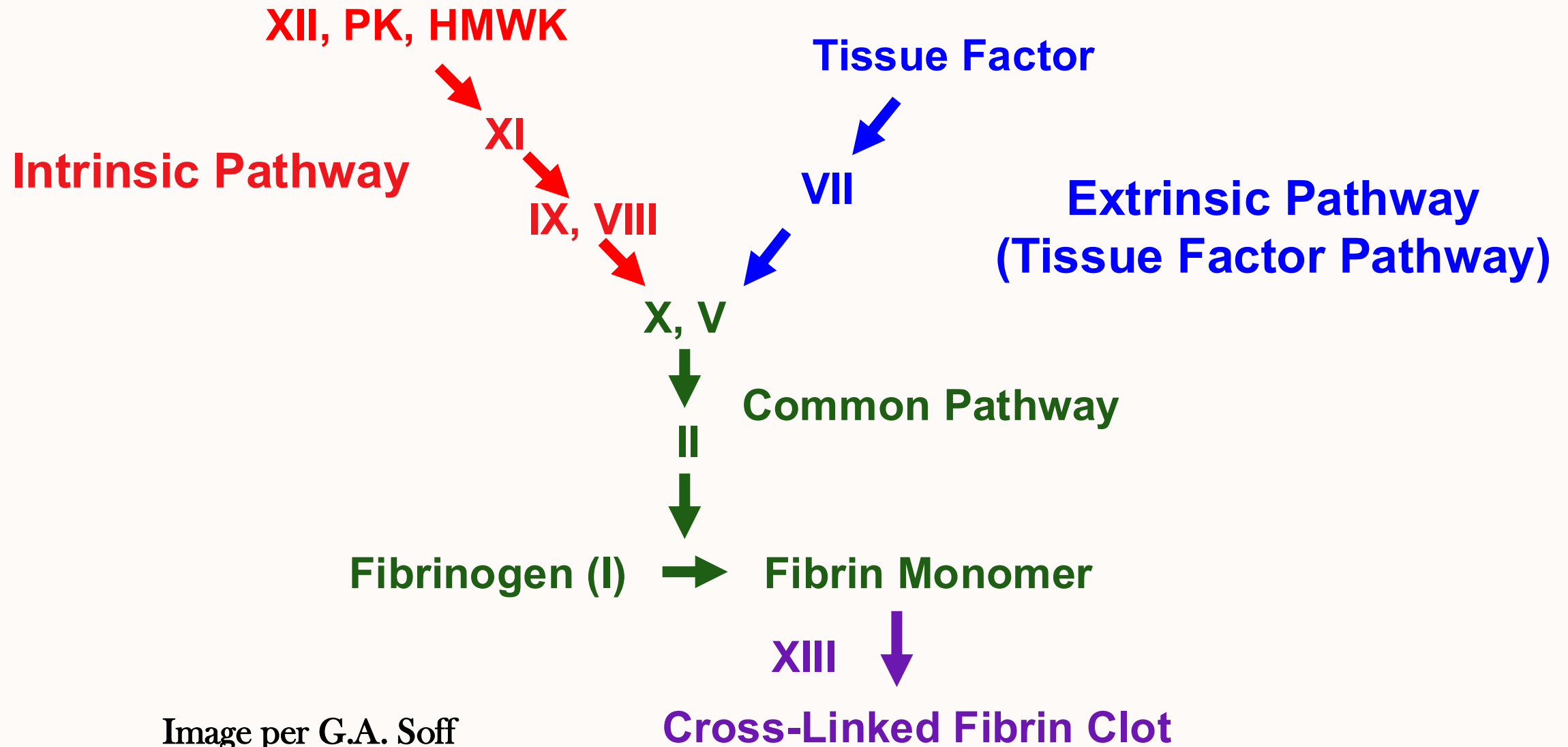
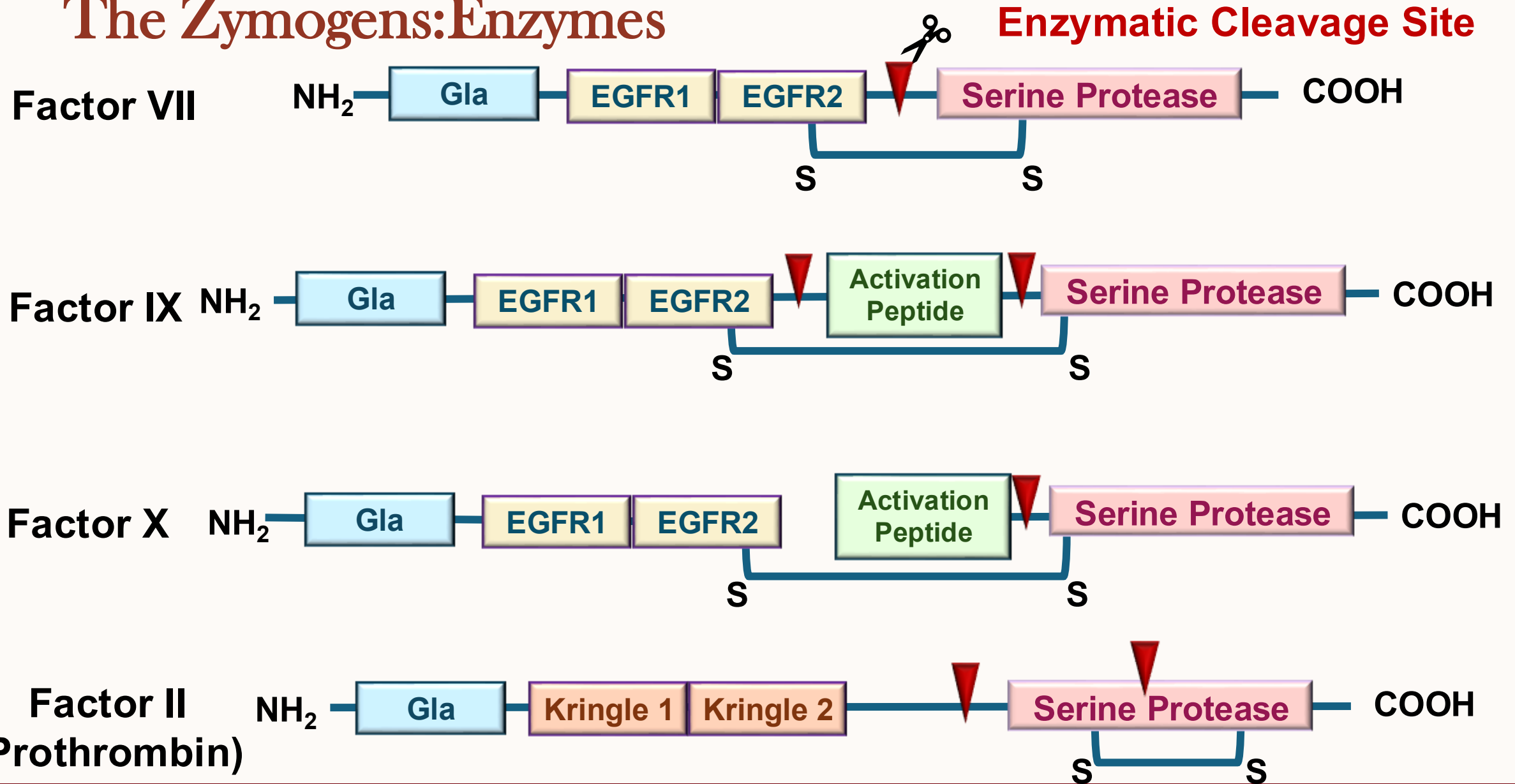


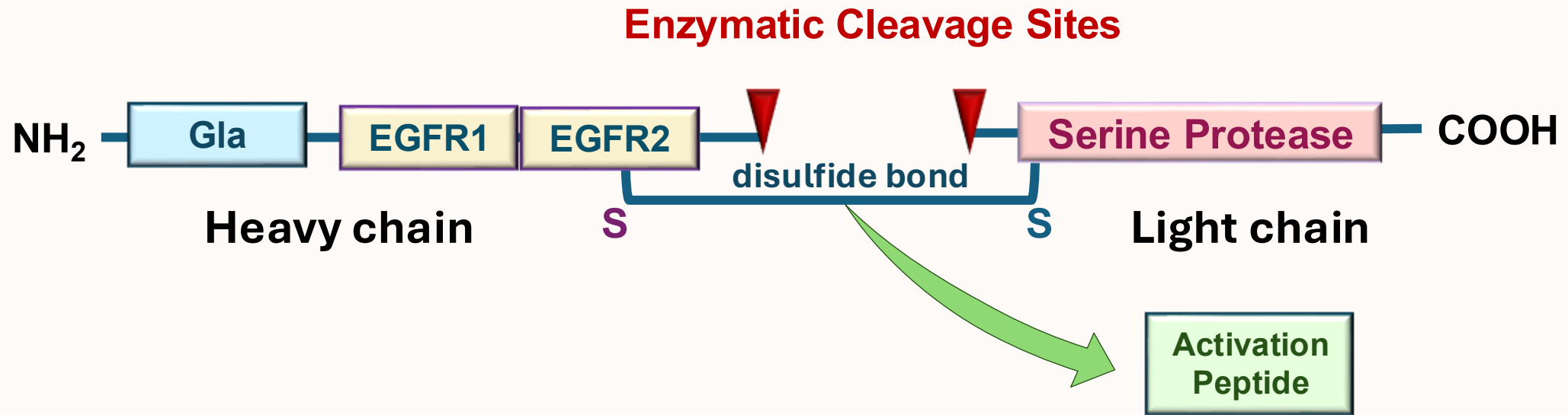
Image per G.A. Soff

3. The Structure of the Coagulation Factors

The Zymogens: Enzymes

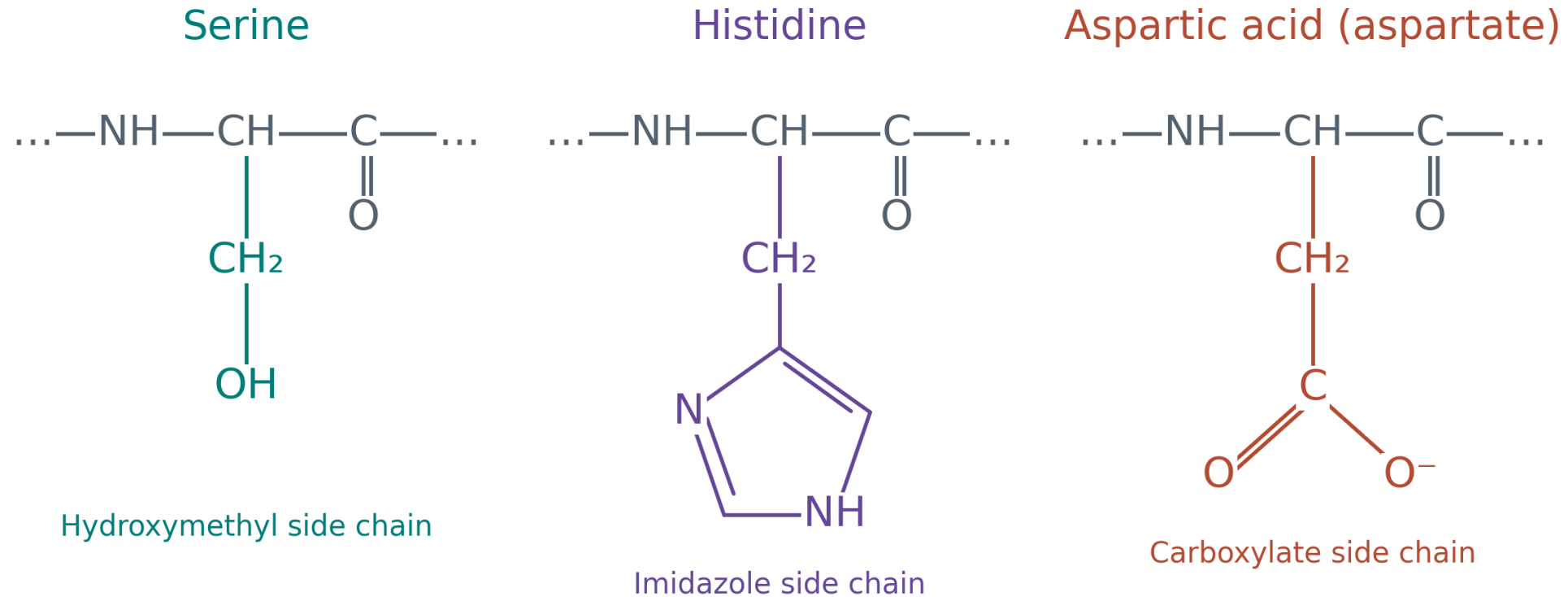


Factor IX Activation: Two Step Enzymatic Cleavage Site



- 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

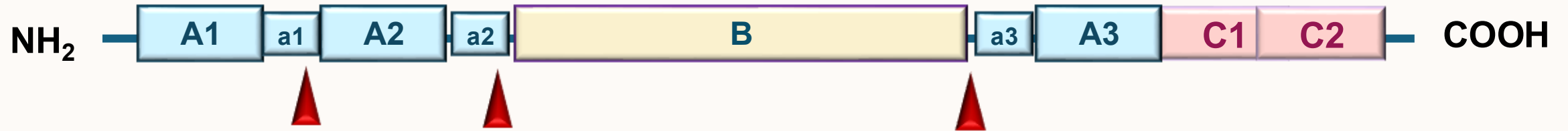


Gray: peptide backbone • Color: side chains • Separate sequence positions; brought together by protein folding

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

Cofactors

Factor VIII



Factor V

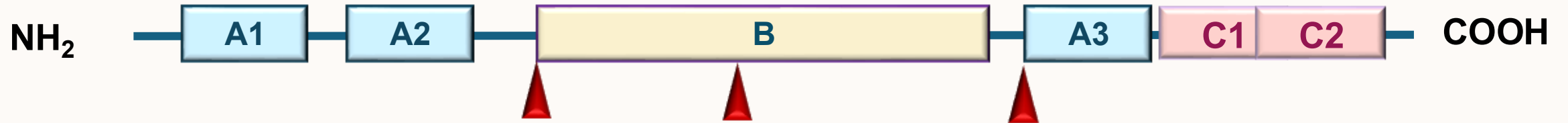


Image per G.A. Soff

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

Vitamin K Mediated γ -Carboxylation of Glutamic Acid

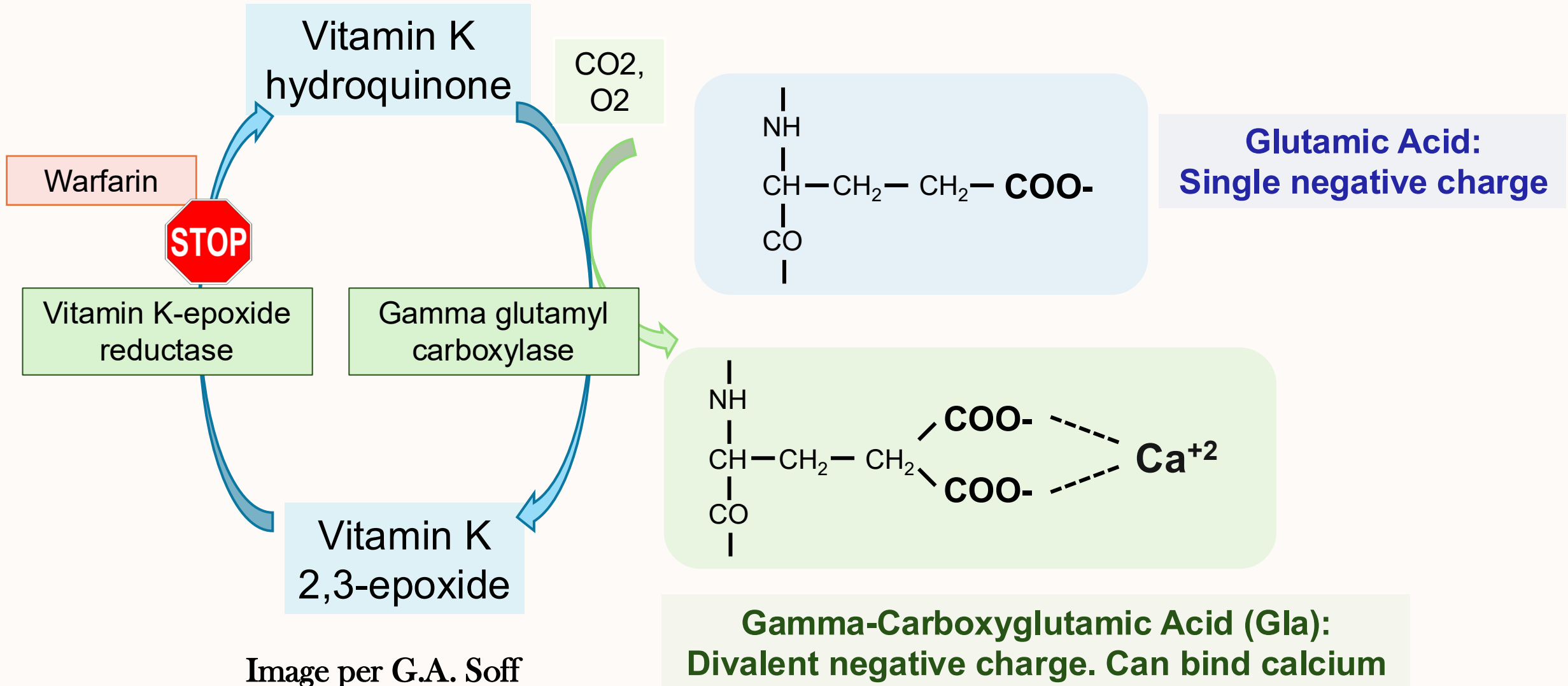
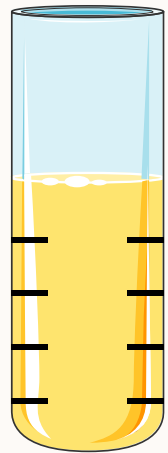


Image per G.A. Soff

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

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



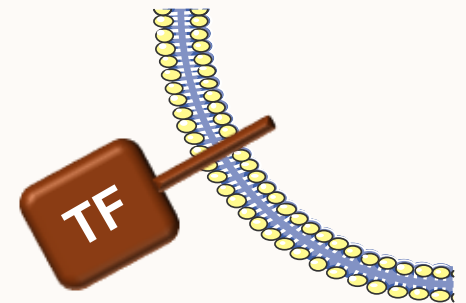
XII, PK, HMWK



XIIa

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

TF:VII
Auto-Activation
↓
VIIa

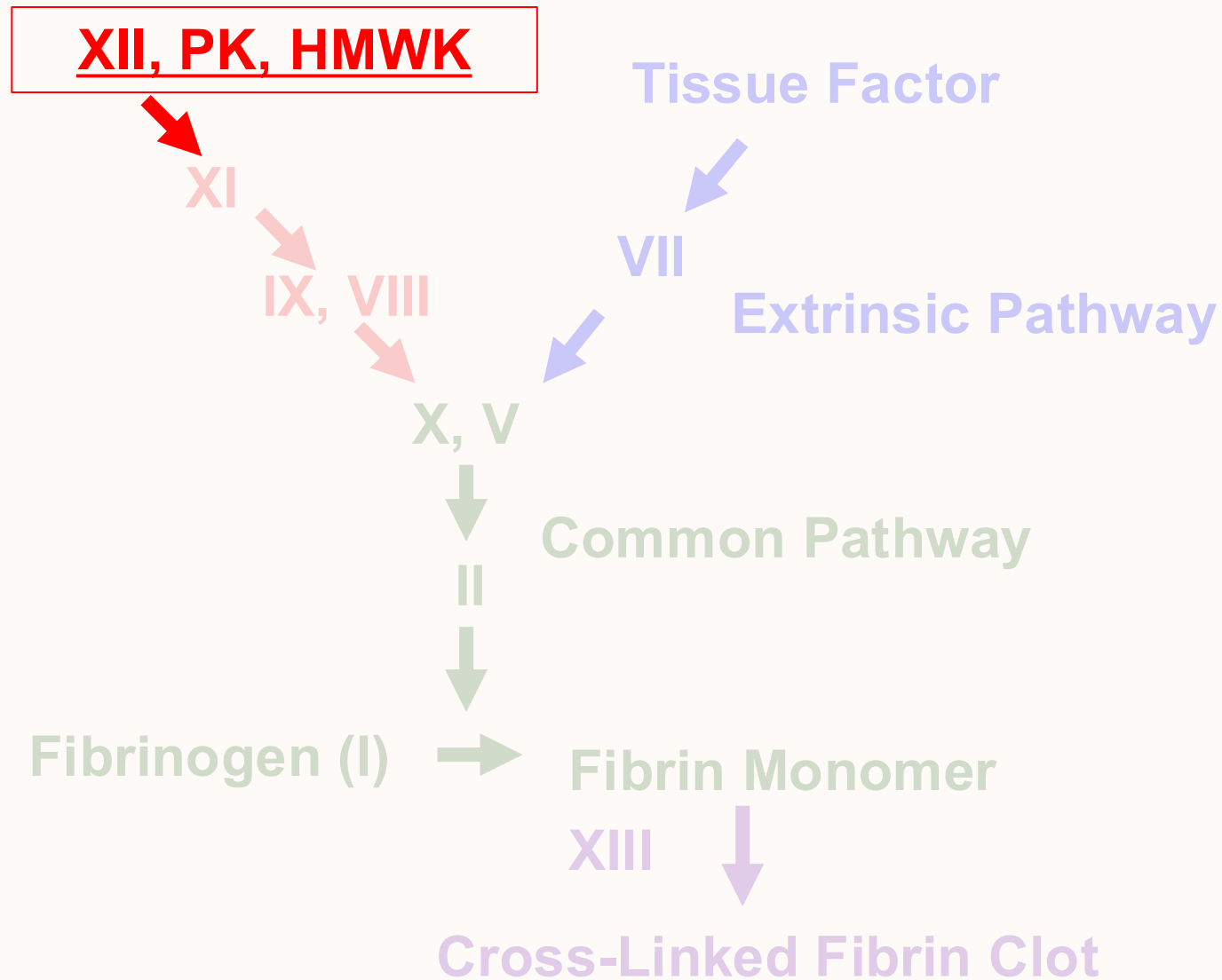


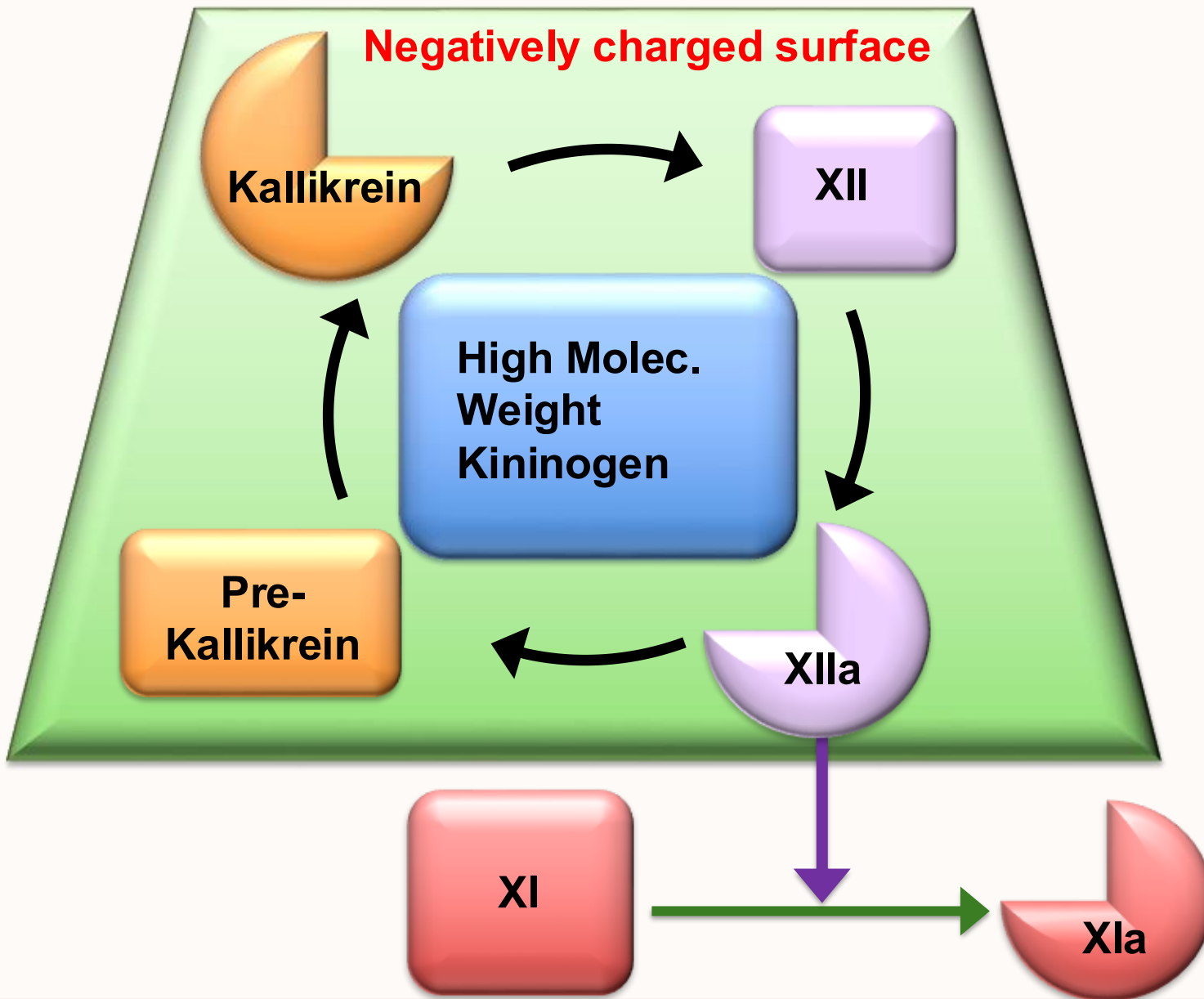
**TF bound to
cell surface**

5. 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

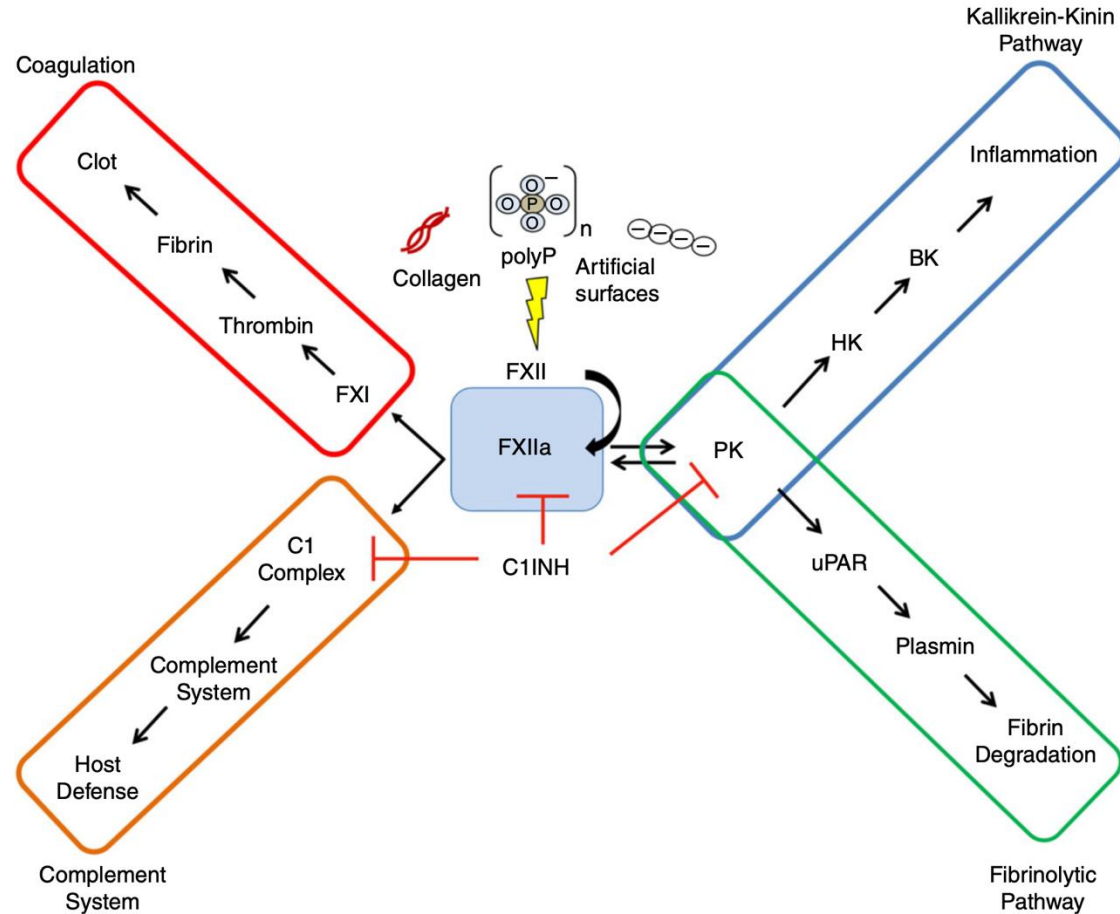




Contact System

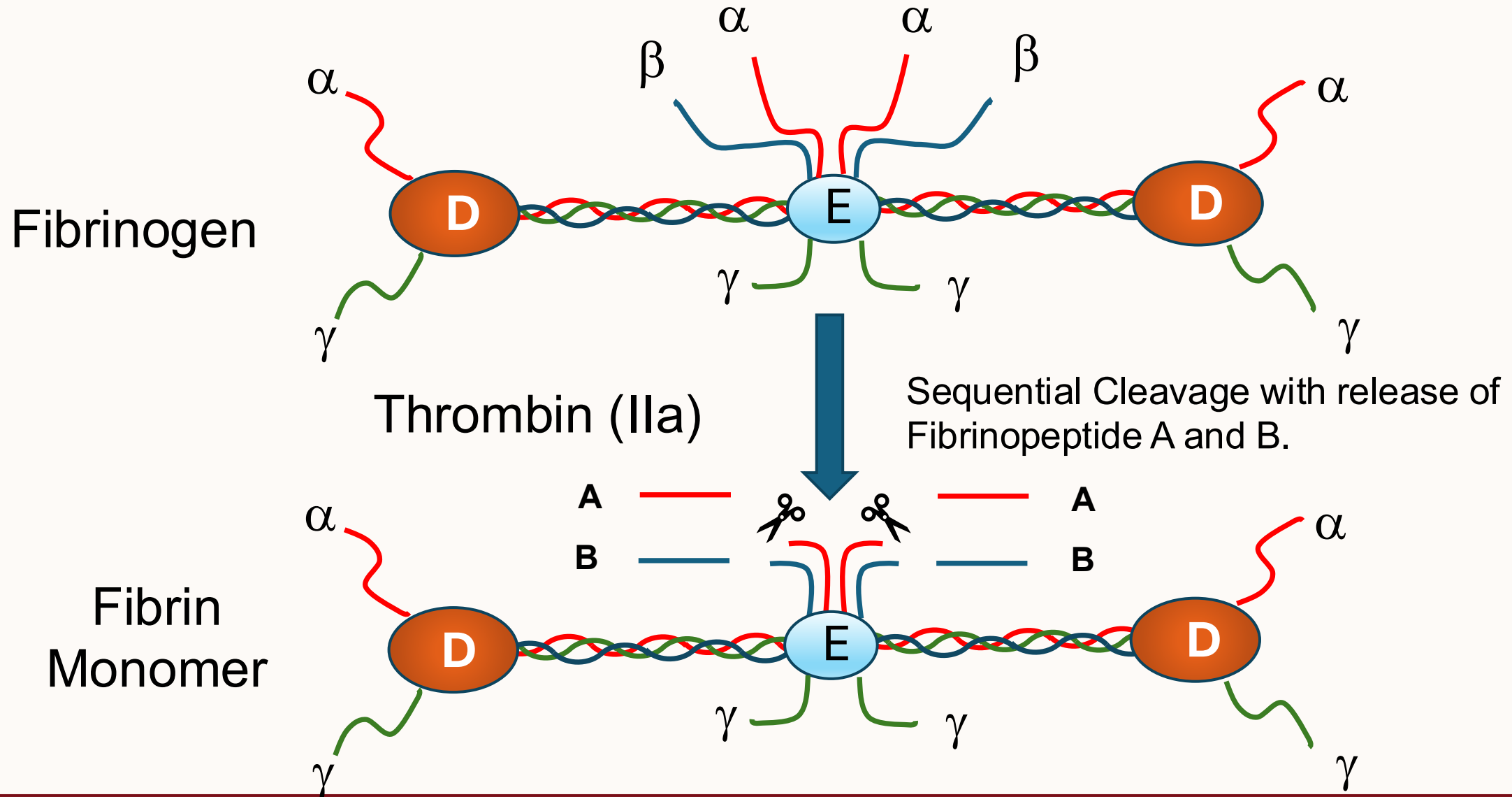
- Minimal contribution to hemostasis in physiologic situations.
- Deficiencies of the Contact Factors are not associated with bleeding tendency.
- Bradykinin (Derived from HMWK)
 - Role in inflammation, vascular tone.
- Increasing evidence that the Contact System has a role in pathological activation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

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

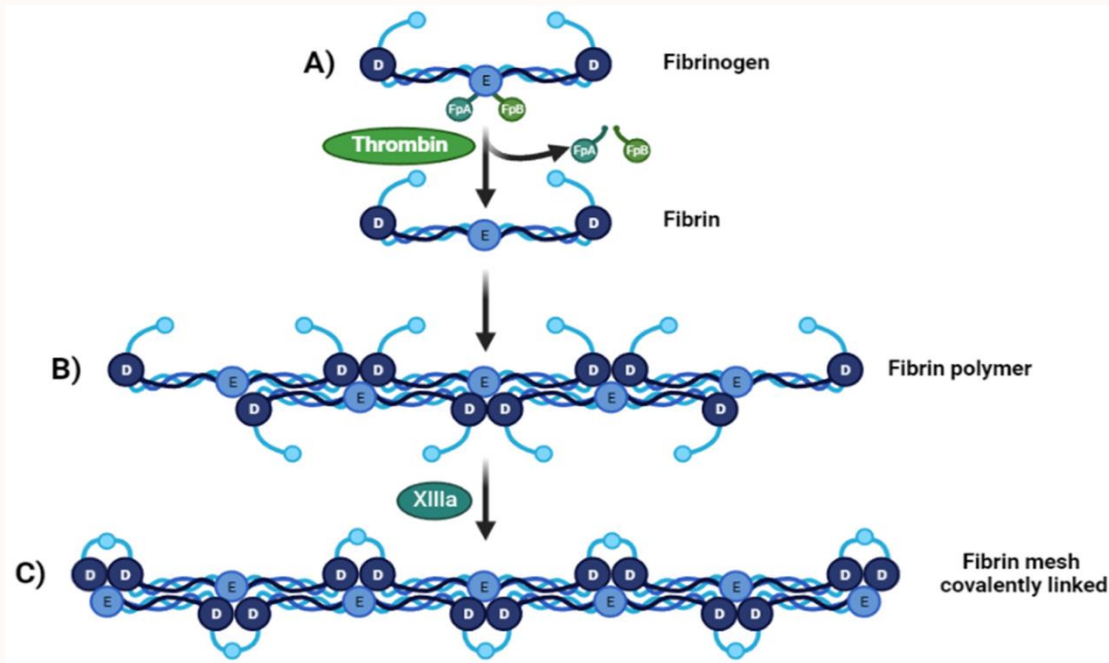


- FXII deficiency is not physiologically associated with an increased bleeding risk.
- Contact system has a role in inflammation, complement system, fibrinolysis, and pathologic thrombosis.
- Polyphosphate (polyP) from activated platelets and bacteria can activate Factor XII.
- Neutrophil extracellular traps (NETs), chromatin extruded from activated neutrophils can activate the Contact System.
- FXIIa may increase vascular leak in allergic conditions.
 - Long AT, et al. J Thromb Haemost 2016; 14: 427-37.

6. Fibrinogen: Fibrin



Factor XIIIa (Transglutaminase): Cross-Link Fibrin

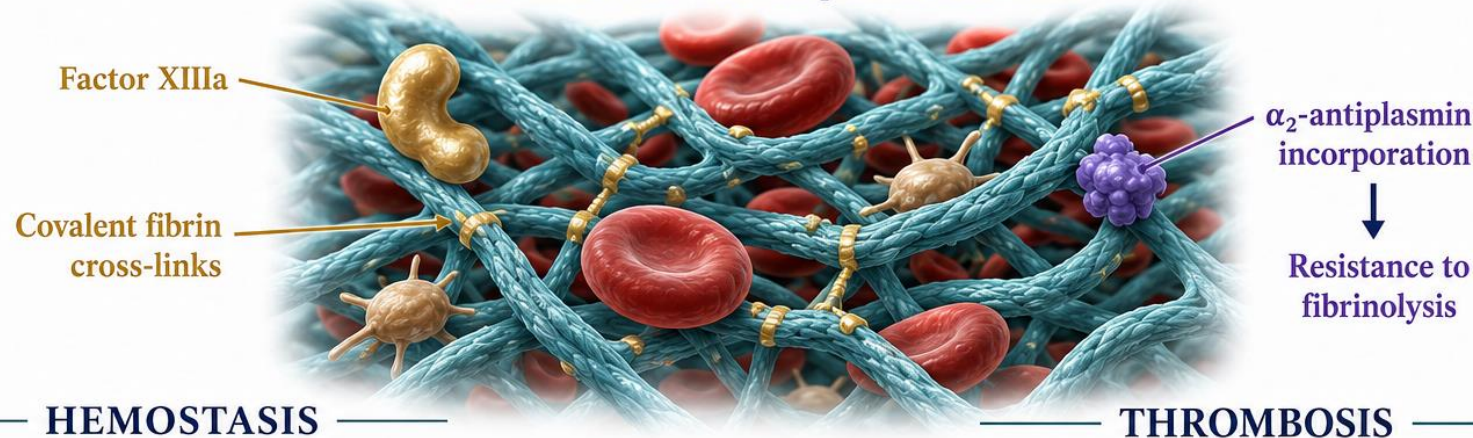


- Ca^{2+} -dependent transglutaminase
- Covalently cross-links adjacent fibrin monomers by forming ϵ -(γ -glutamyl)lysine isopeptide bonds between glutamine and lysine residues, converting a weak soluble clot into a stable, insoluble one.
- These cross-links occur in three forms:
 - 1) Major γ - γ links between the C-terminal γ -chains (D domains),
 - 2) Major α - α links among the flexible α C regions,
 - 3) Minor mixed γ - α links—collectively increasing clot stiffness and resistance to both mechanical disruption and fibrinolysis.

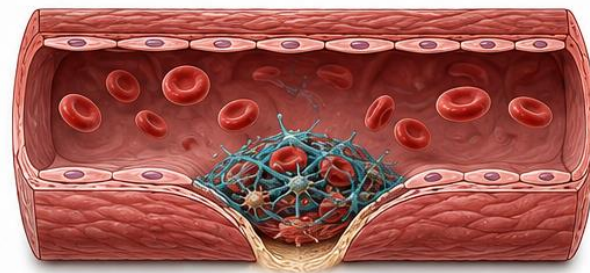
Luyendyk JP, Flick MJ, Wolberg AS. Factor XIII: driving (cross-)links in hemostasis, thrombosis, and disease. *Blood*. 2025 Sep 18;146(12):1412-1421. doi: 10.1182/blood.2024025321. PMID: 39869827; PMCID: PMC12535669.

Factor XIII: driving (cross-)links in hemostasis and thrombosis

Thrombin + Ca^{2+} activate plasma Factor XIII

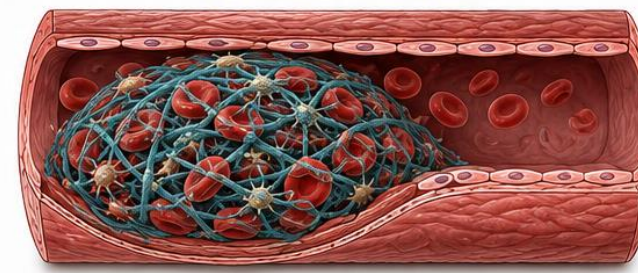


“FXIIIa-mediated cross-linking of α_2 -antiplasmin to fibrin increases resistance to fibrinolysis.”



Stabilizes the hemostatic plug

**Stronger fibrin •
Greater resistance
to breakdown**



Promotes thrombus stability

Figure generated with AI assistance, based on content of Luyenyk et al 2025.

Reference: Luyendyk JP, Flick MJ, Wolberg AS. *Blood*. 2025;146(12):1412–1421.
doi:10.1182/blood.2024025321 | PMID:39869827
Original conceptual illustration based on the cited review.

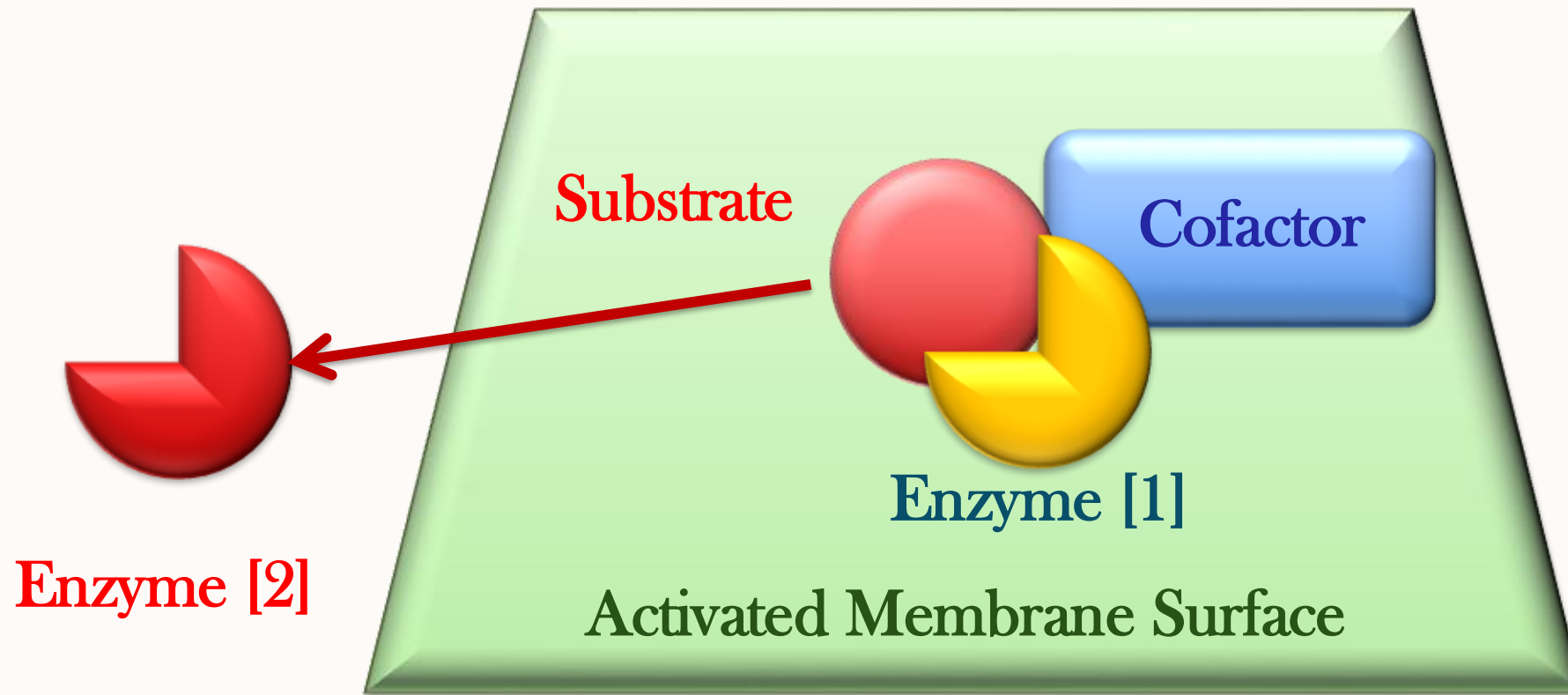
7. Limitations of The Classic Coagulation Cascade

- 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.

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

The Cell (Surface) Based Model Of Coagulation

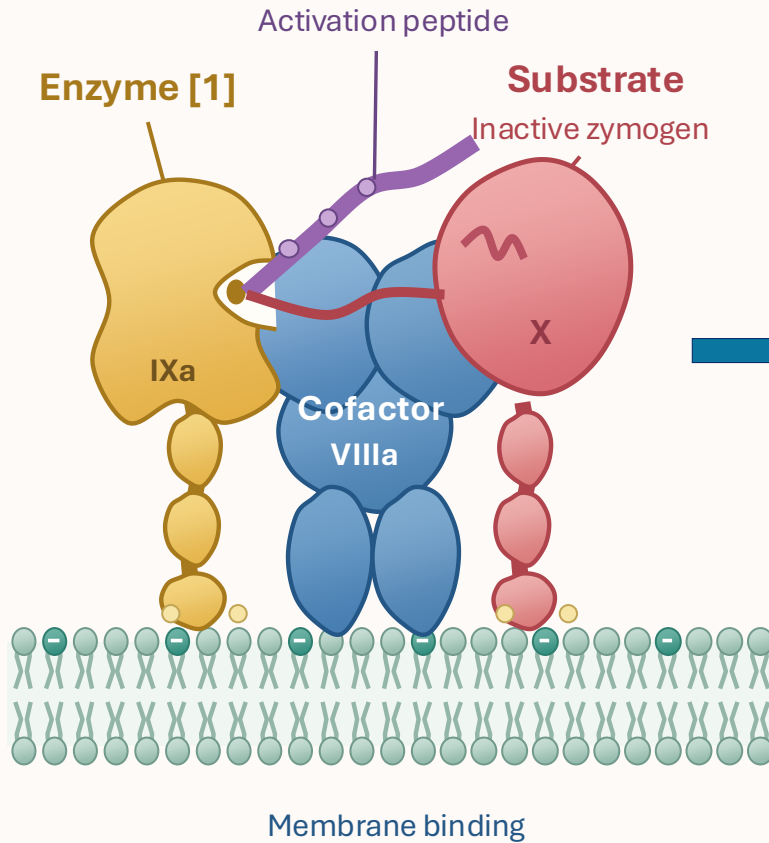
Coagulation is “Best” understood as a series of membrane-bound complexes: enzyme/cofactor/substrate.



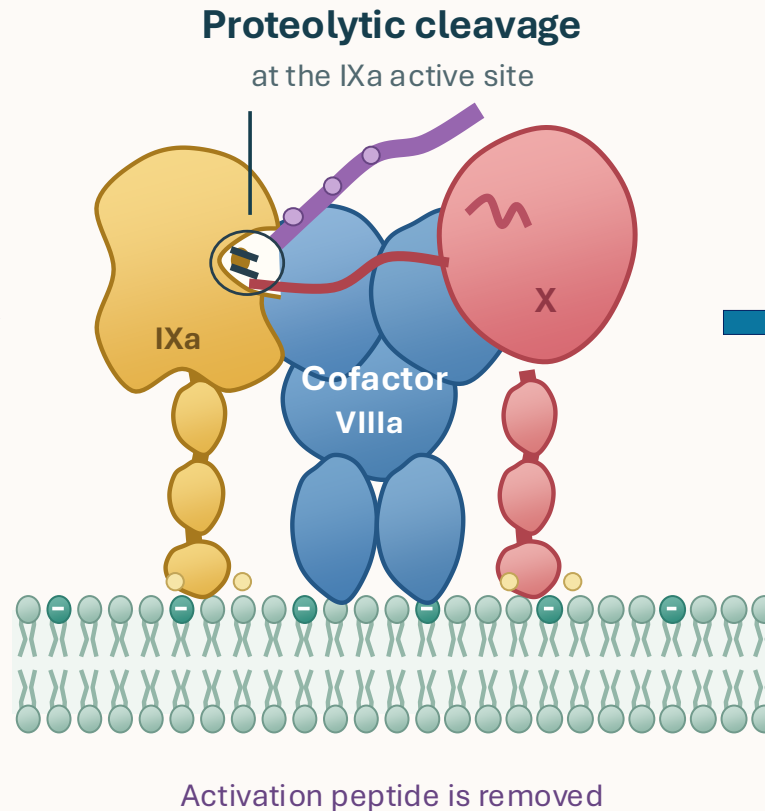
The Cell (Surface) Based Model Of Coagulation

Intrinsic tenase example: cofactor VIIIa positions enzyme IXa and substrate X on a phospholipid membrane

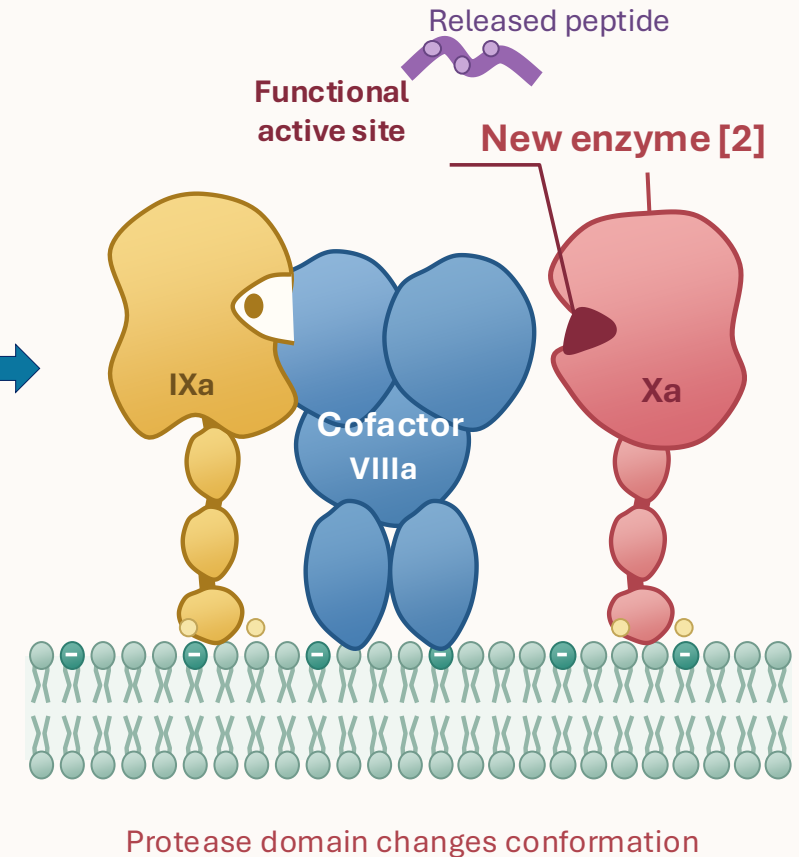
1 Assemble



2 Cleave



3 Activate

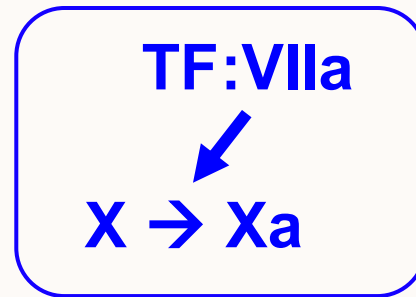
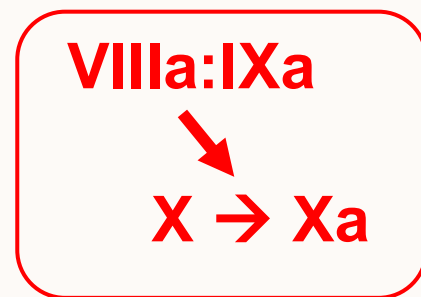


Phospholipid bilayer with exposed phosphatidylserine (-) and Ca^{2+} -dependent protein binding

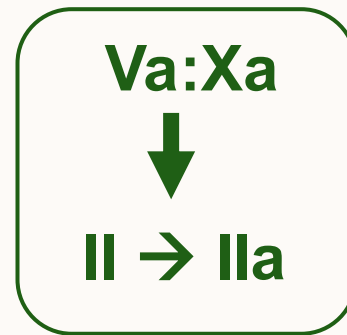
Three Complexes of the “Classic Cascade”

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

Intrinsic Xase



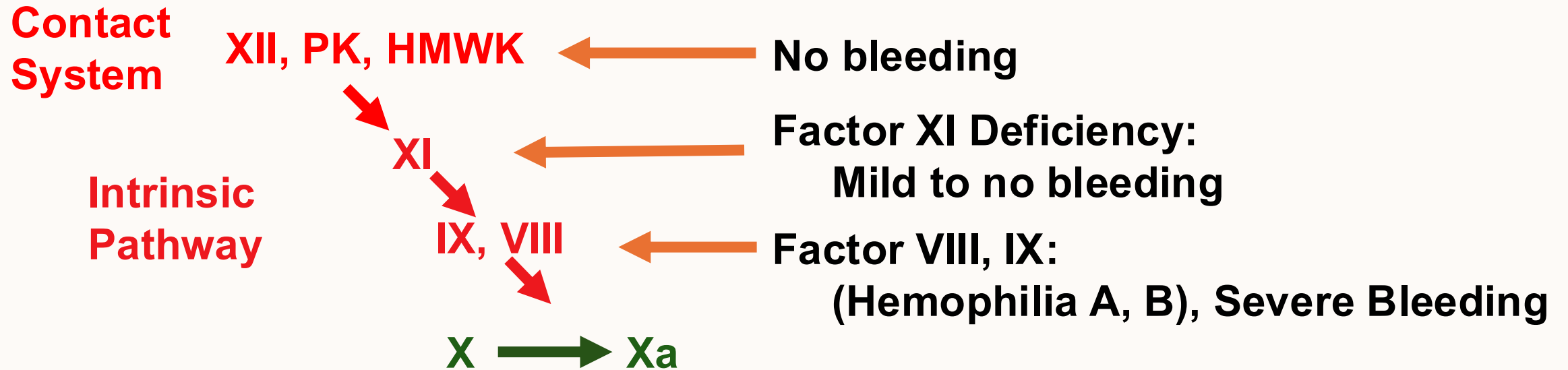
Extrinsic Xase



Prothrombinase

9. “Cross-Over” of Extrinsic and Intrinsic Pathways

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



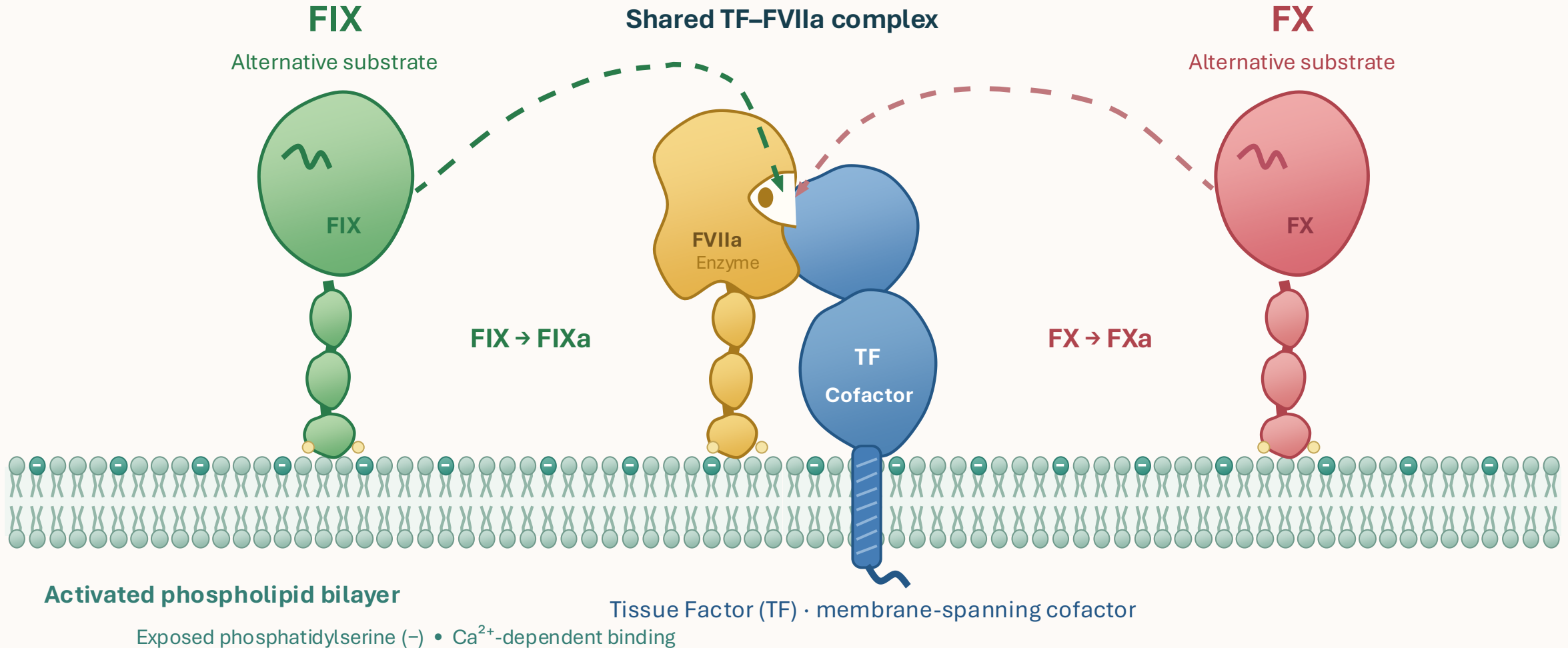
There Are Two Alternative Substrates of TF:VIIa Complex

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

- "TFPI throttles the catalyst (limiting both FIXa and FXa), while antithrombin preferentially eliminates FXa—together yielding a net output in which FIXaβ predominates and can feed the intrinsic tenase to sustain FXa generation."
- Lu G, Broze GJ Jr, Krishnaswamy S. Formation of factors IXa and Xa by the extrinsic pathway: differential regulation by tissue factor pathway inhibitor and antithrombin III. J Biol Chem. 2004 Apr 23;279(17):17241-9. doi: 10.1074/jbc.M312827200. Epub 2004 Feb 12. PMID: 14963035.

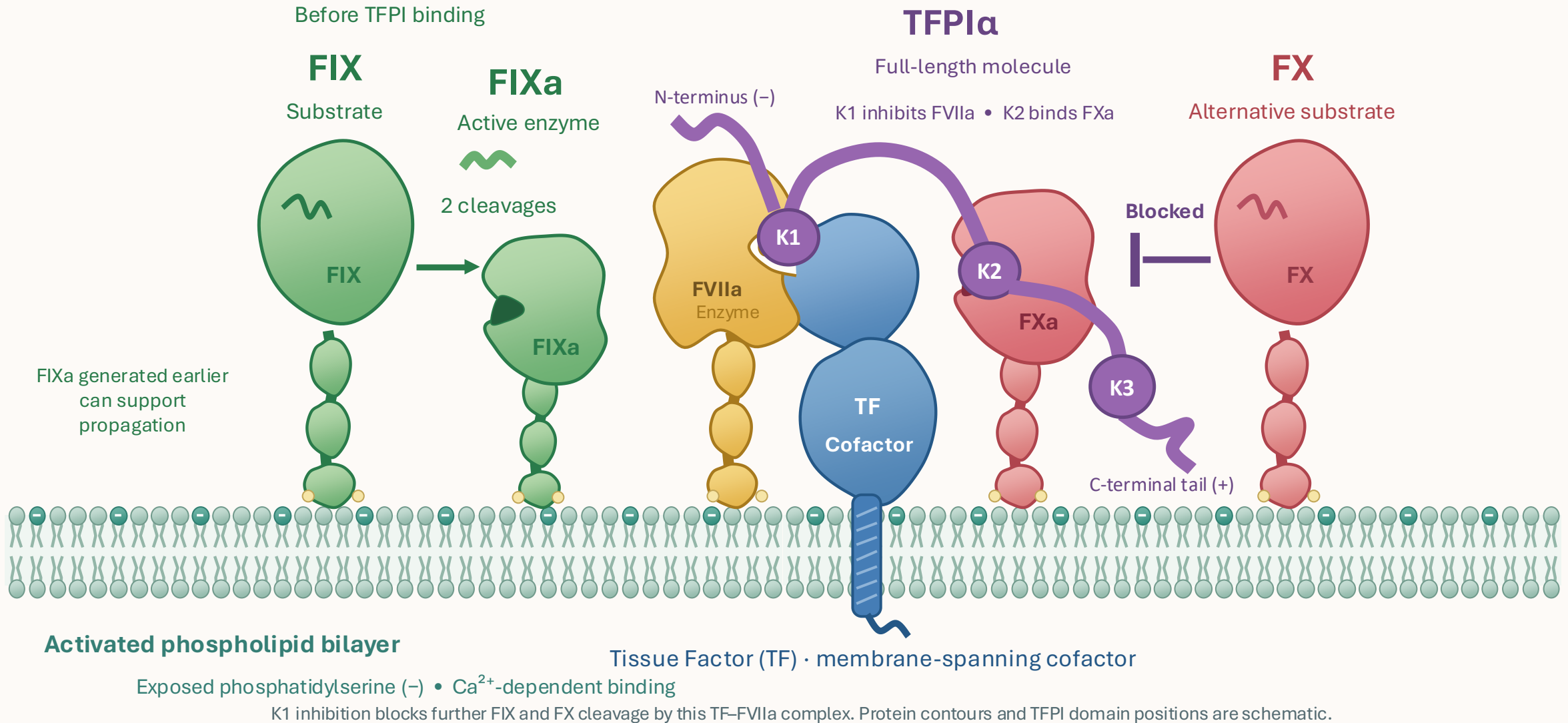
TF-FVIIa activates FIX or FX

Two alternative substrates engage one membrane-bound enzyme-cofactor complex

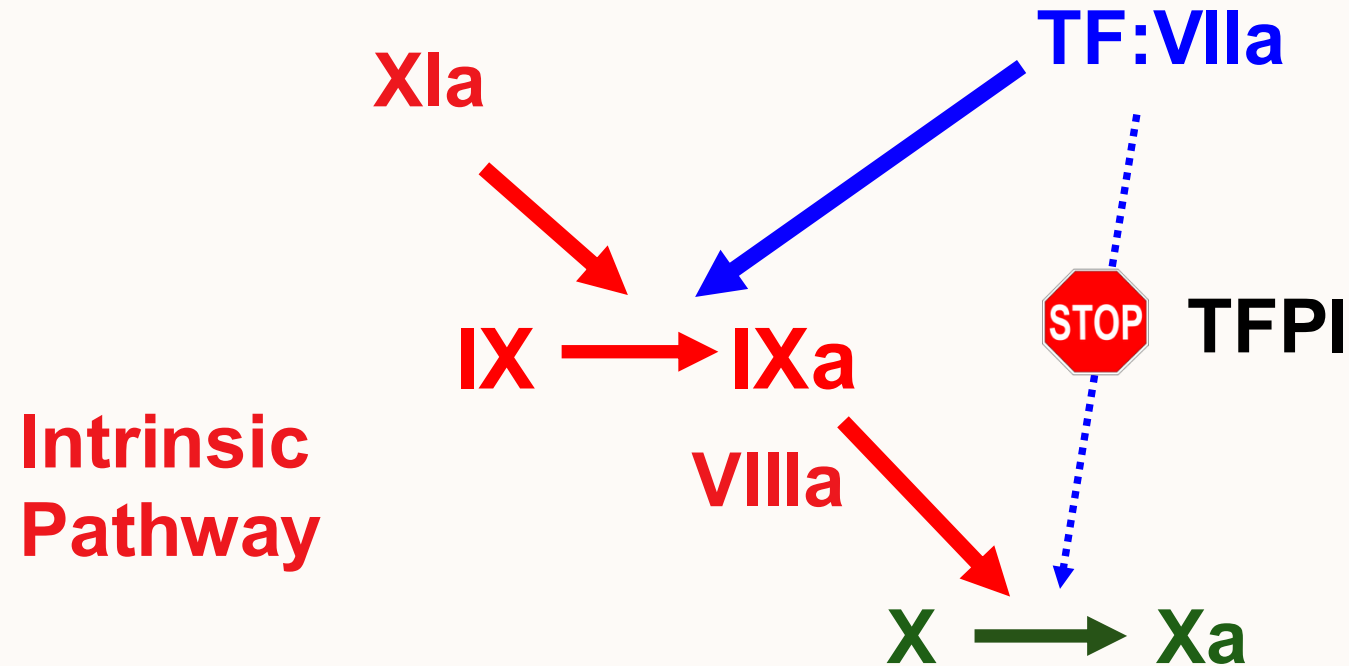


FIX and FX are shown as alternative binding events, not simultaneous occupancy. Molecular shapes are schematic.

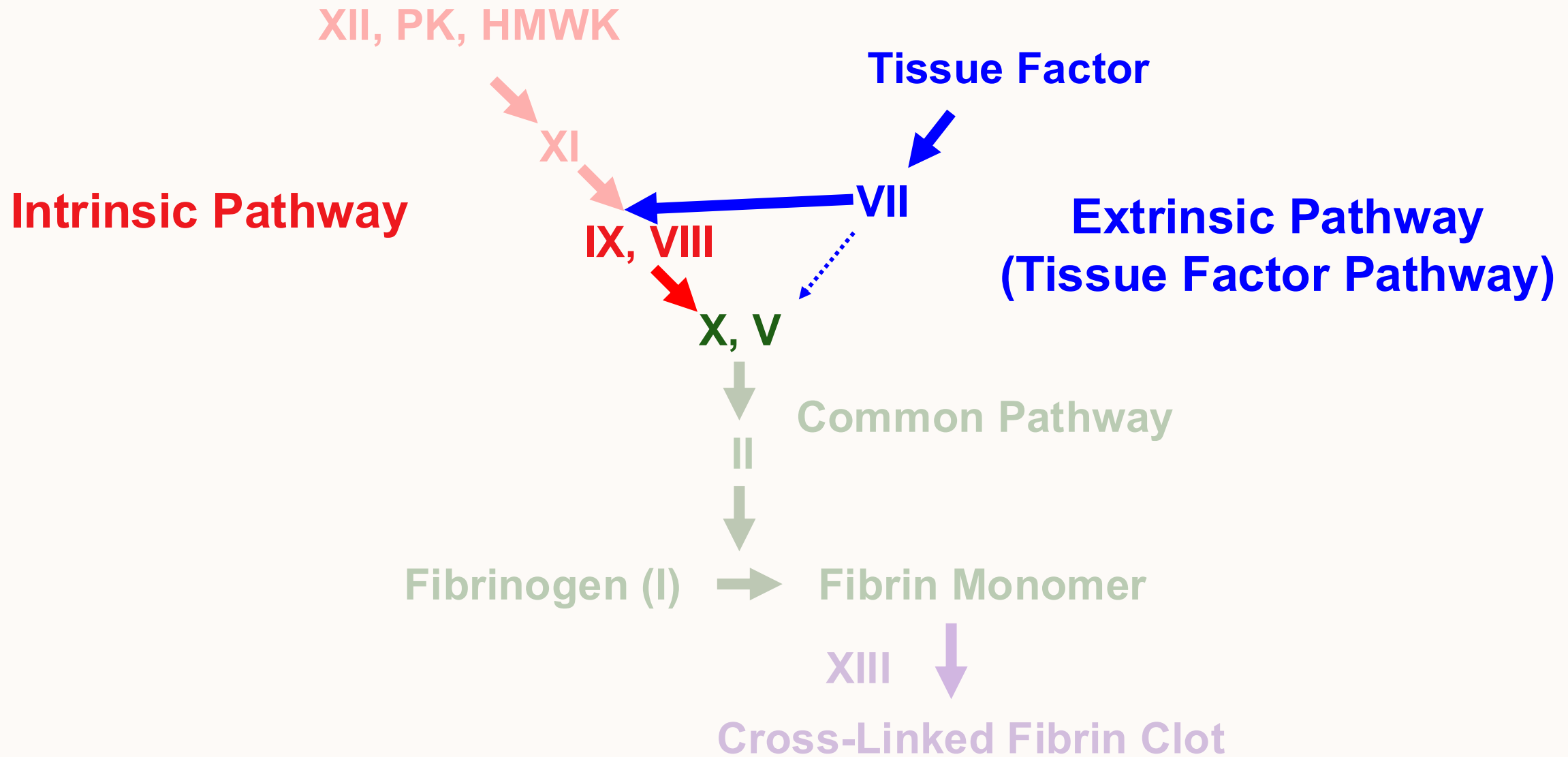
TFPI blocks the TF-FVIIa-FX reaction



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.



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

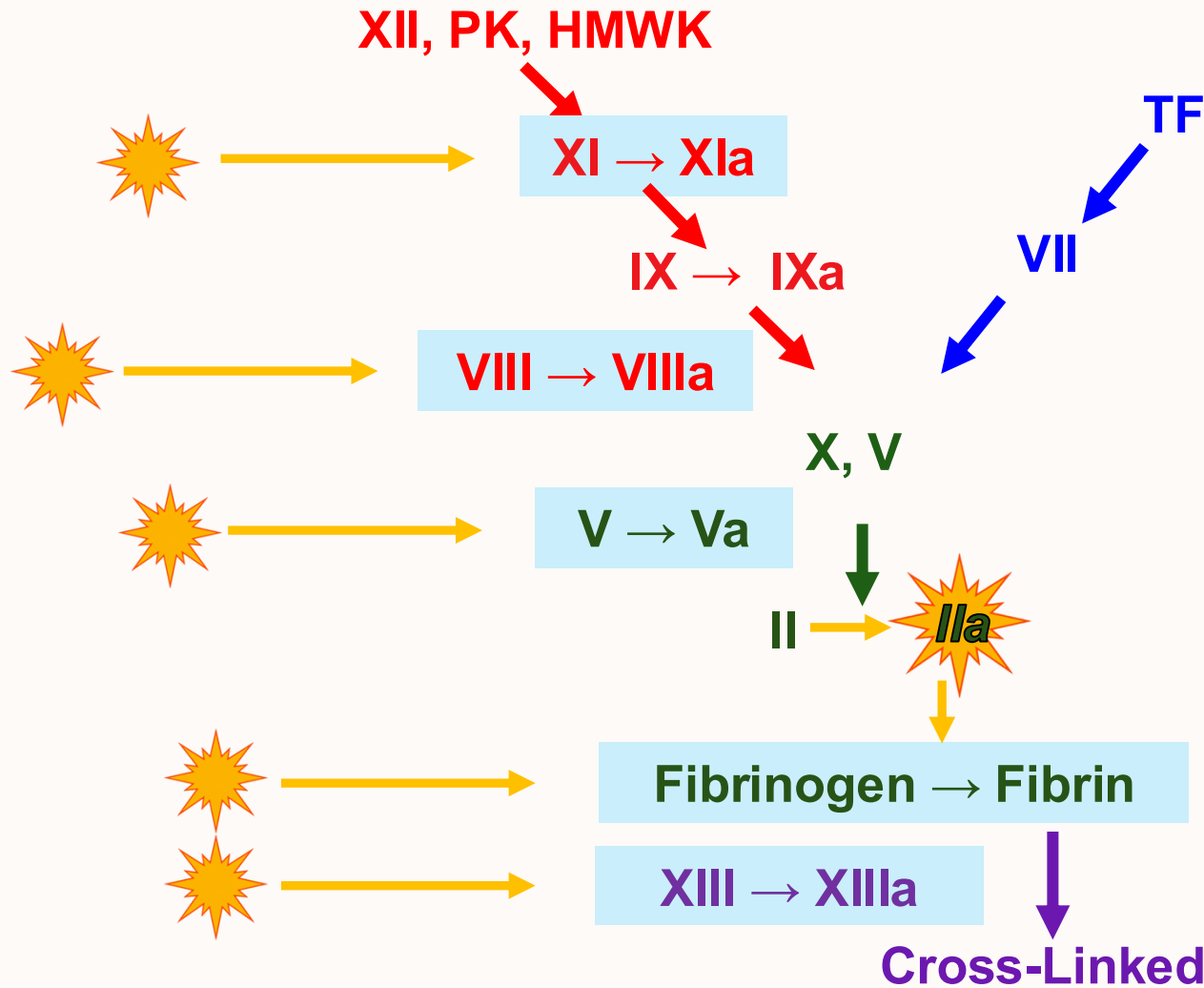


https://commons.wikimedia.org/wiki/File:Most_distant_Gamma-ray_burst.jpg

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.

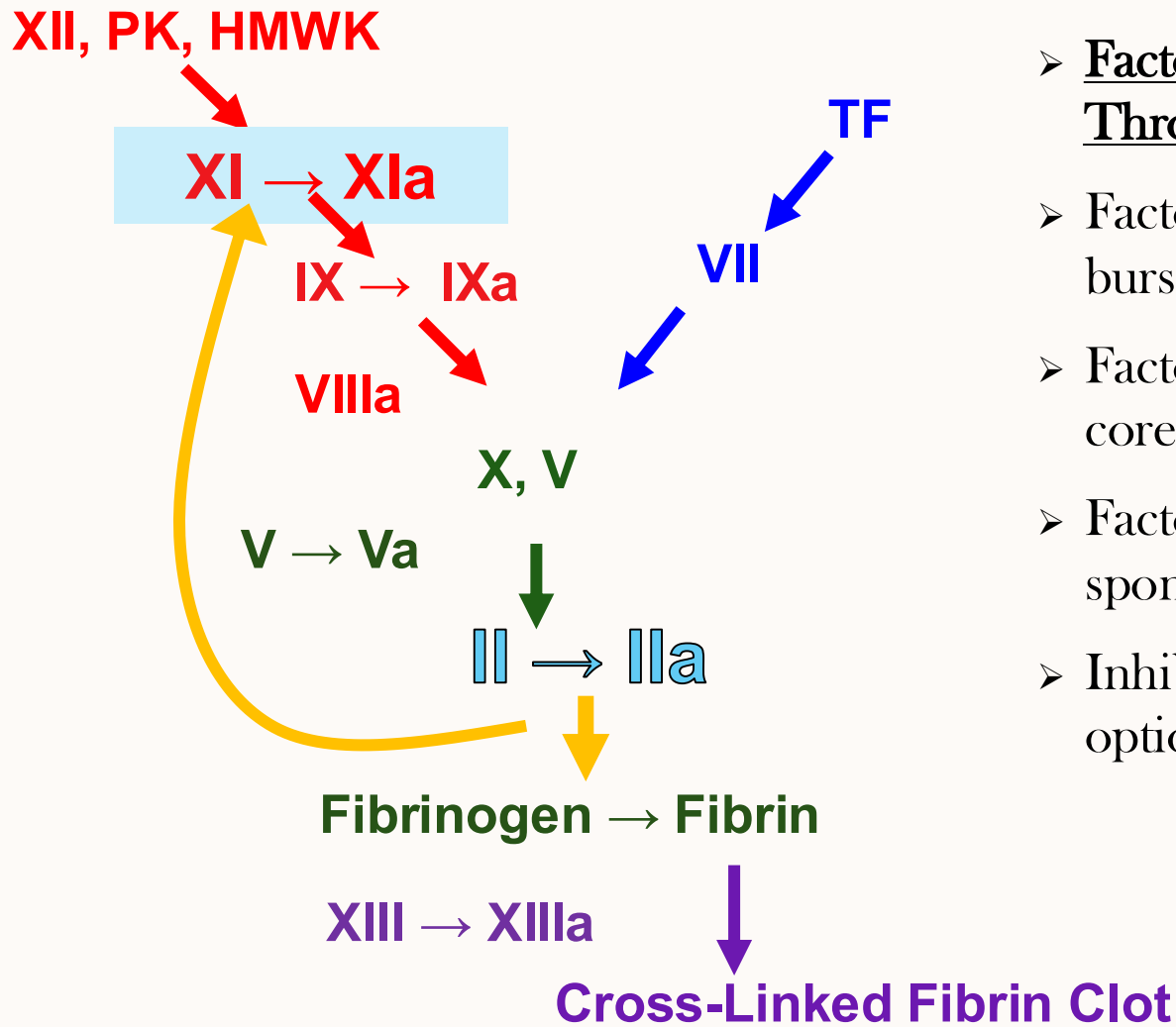
Thrombin Feedback; *Activation of Factors V, VIII, XI, XIII*



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]

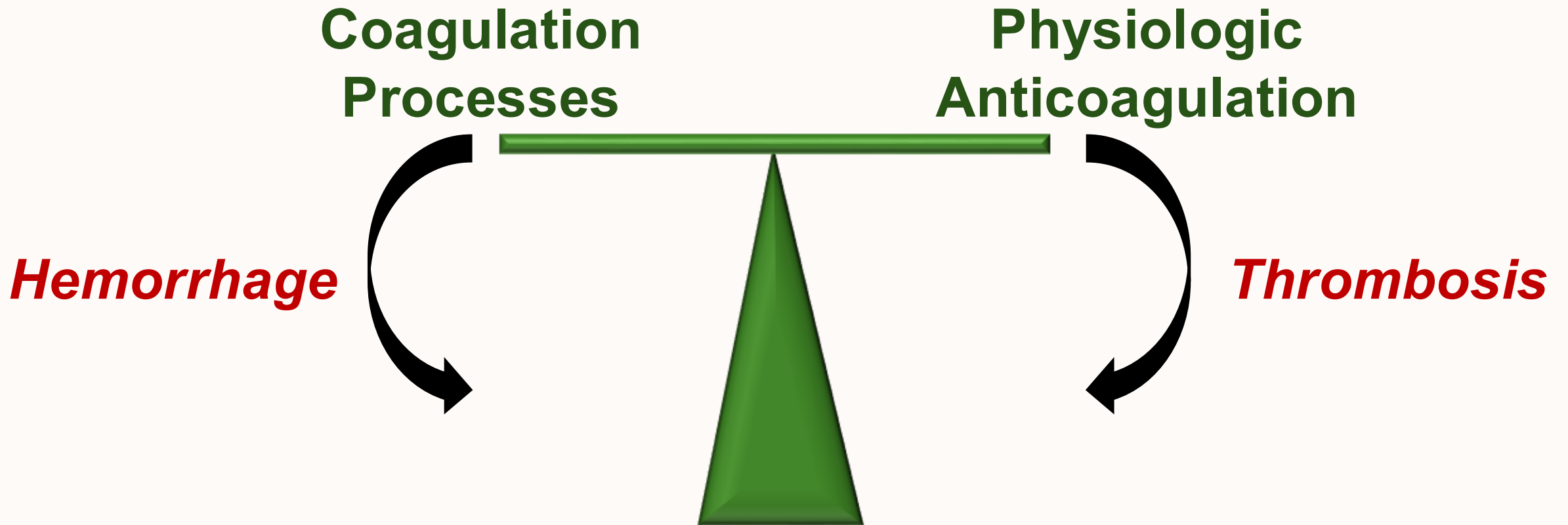
Role of Factor XI



- Factor XI can be activated by XIIa or Thrombin.
- Factor XI is a key component of a the thrombin-burst 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!

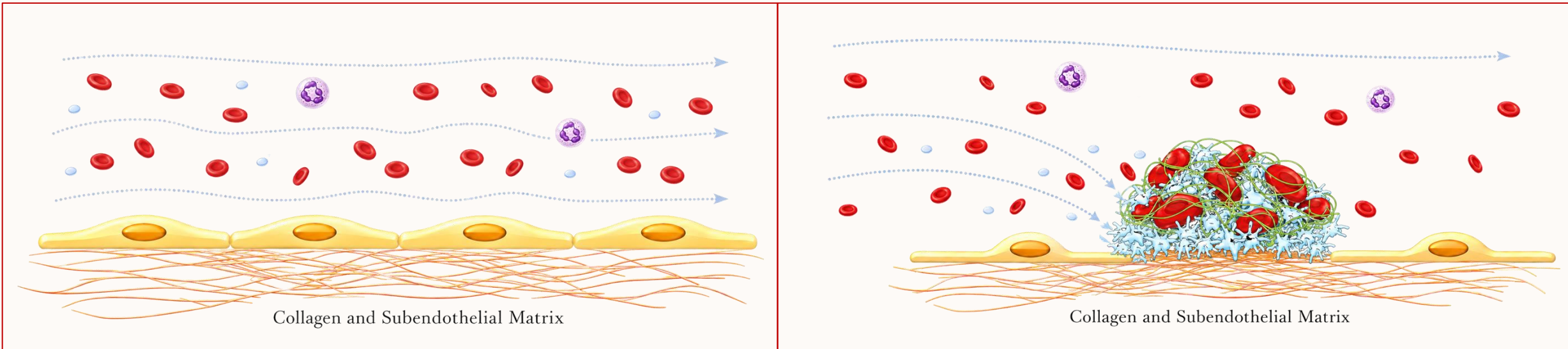
11. The Hemostatic Balance:

Physiologic Anticoagulation Processes



Vascular Endothelial Cells Present Anticoagulant Surface

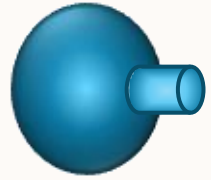
- Vascular endothelial cells present anticoagulant surface.
- Disruption of endothelial surface exposes blood to Collagen and Subendothelial Matrix (procoagulants) leading to activation of coagulation.
- Deficiency of physiologic anticoagulants leads to activation of coagulation.



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 (PGI ₂)	Relaxes smooth muscle and inhibits platelet activation

Antithrombin:
Inactive Conformation



Thrombin



Antithrombin

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

Heparan Sulfate
(Glycosaminoglycan)

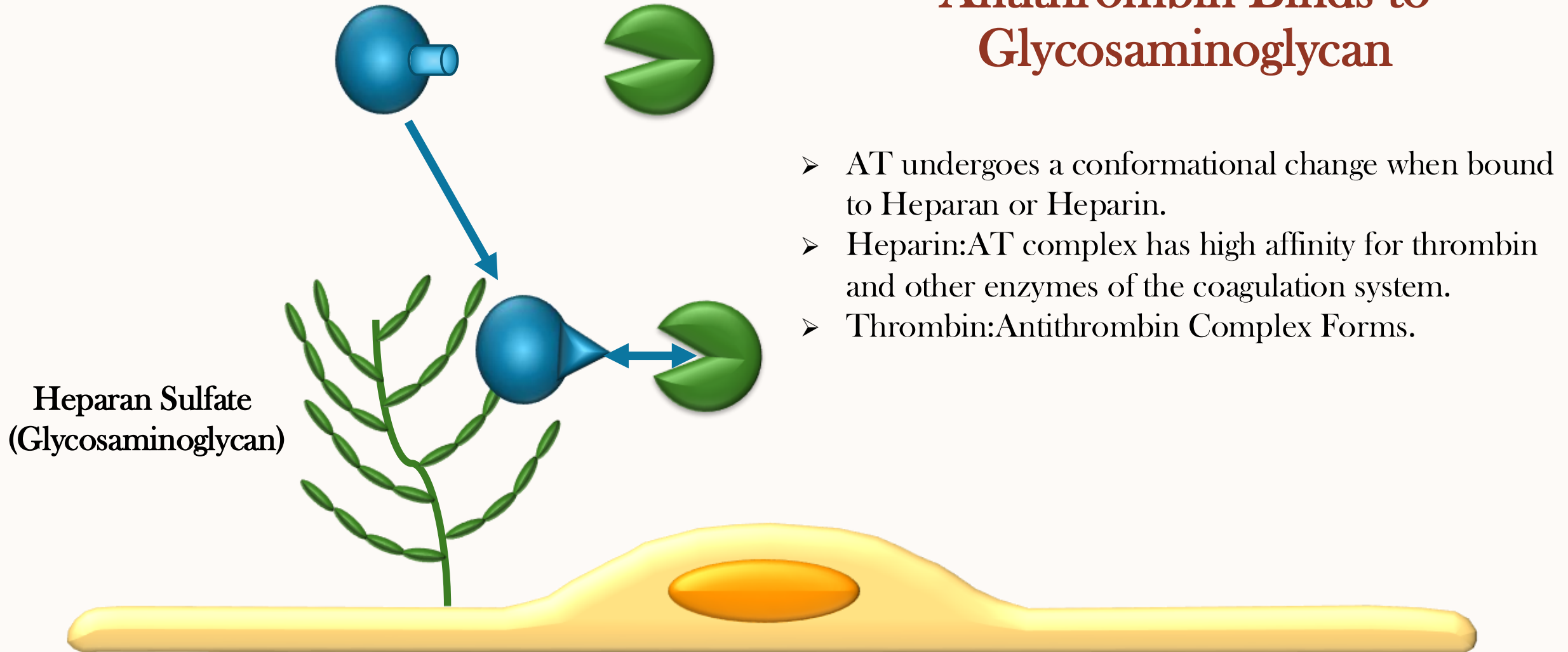


Endothelial Cell

Antithrombin:
Inactive Conformation

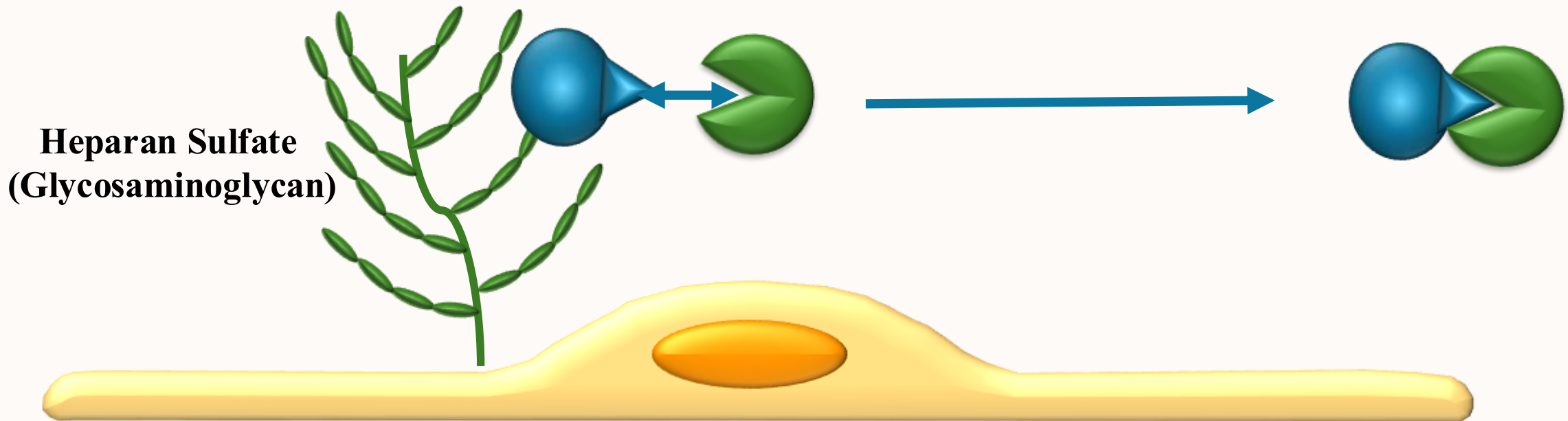
Thrombin

Antithrombin Binds to Glycosaminoglycan



Antithrombin: Glycosaminoglycan

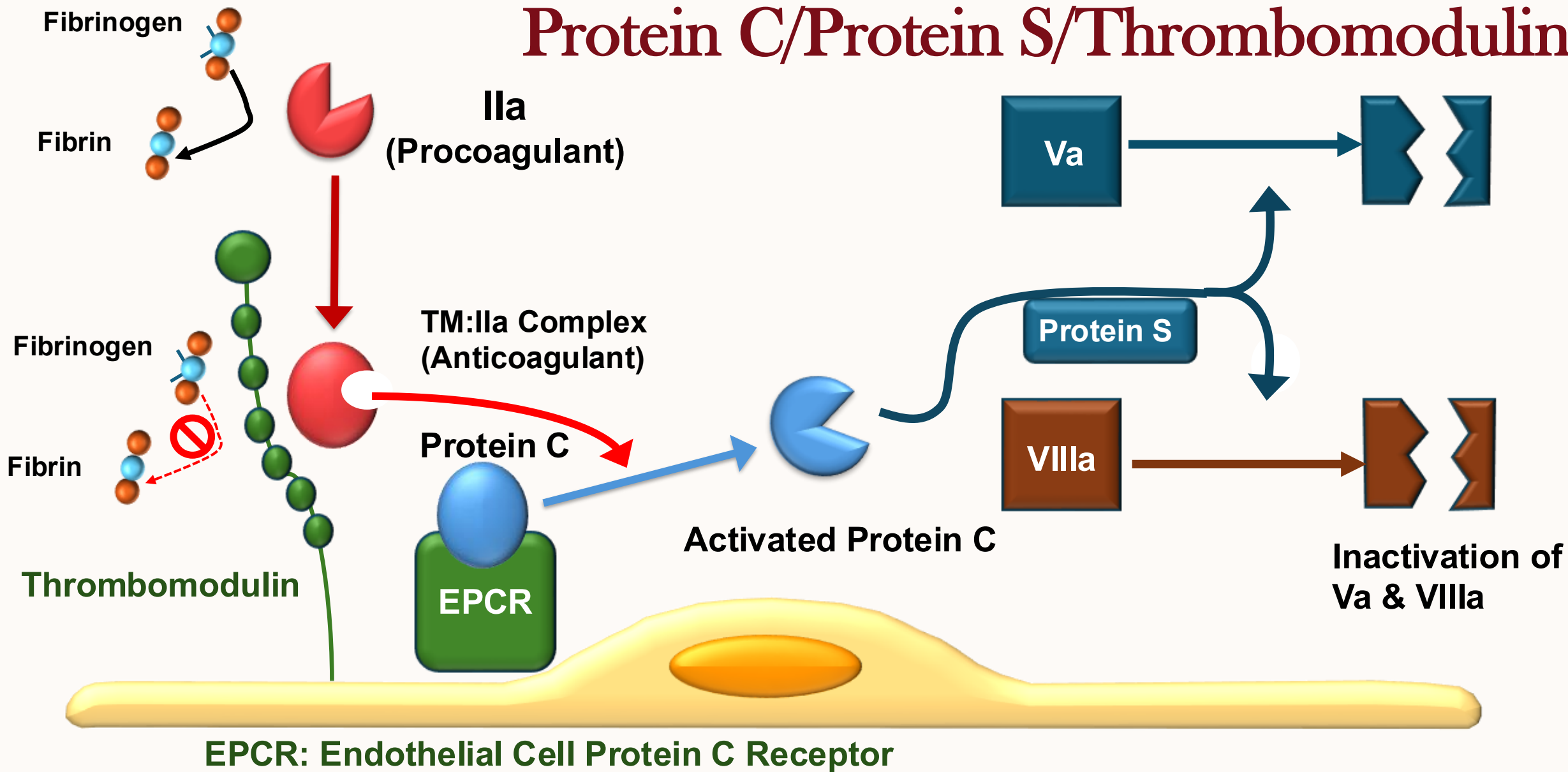
Thrombin:Antithrombin Complex
Dissociates from Glycosaminoglycan and is
cleared in the liver.



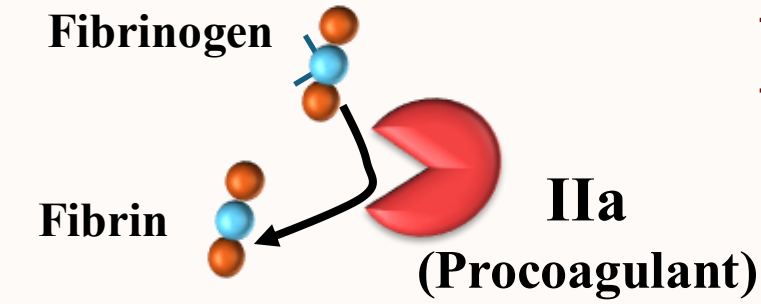
Protein C/Protein S/Thrombomodulin System

- 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.

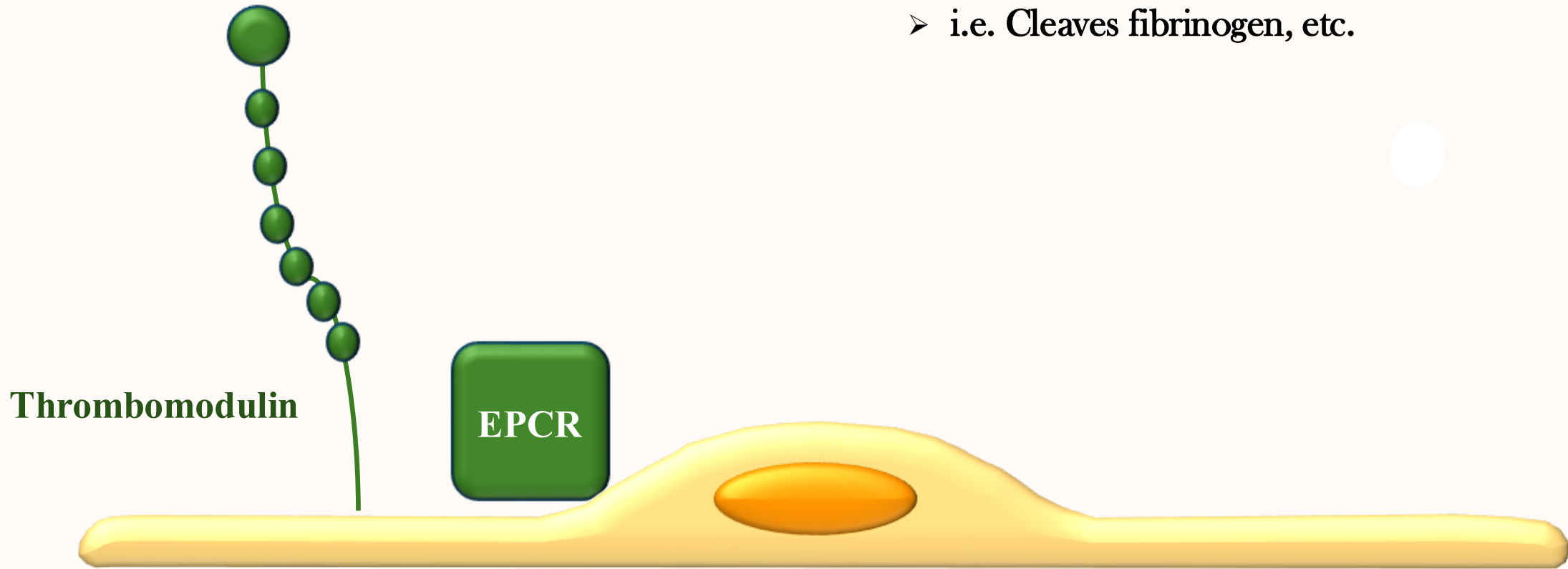
Protein C/Protein S/Thrombomodulin



Protein C/Protein S/Thrombomodulin

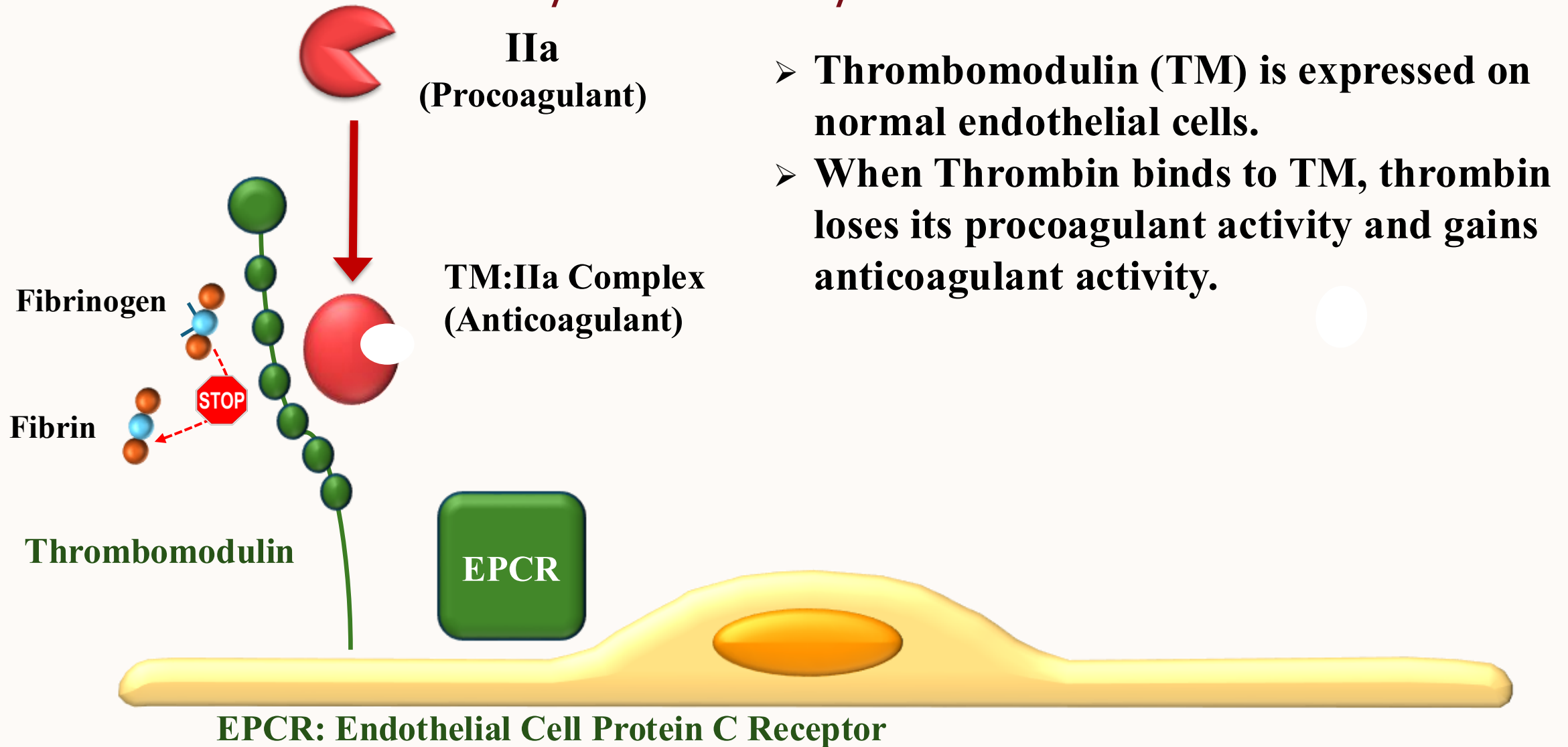


- Thrombin, in fluid phase, is a potent procoagulant.
- i.e. Cleaves fibrinogen, etc.



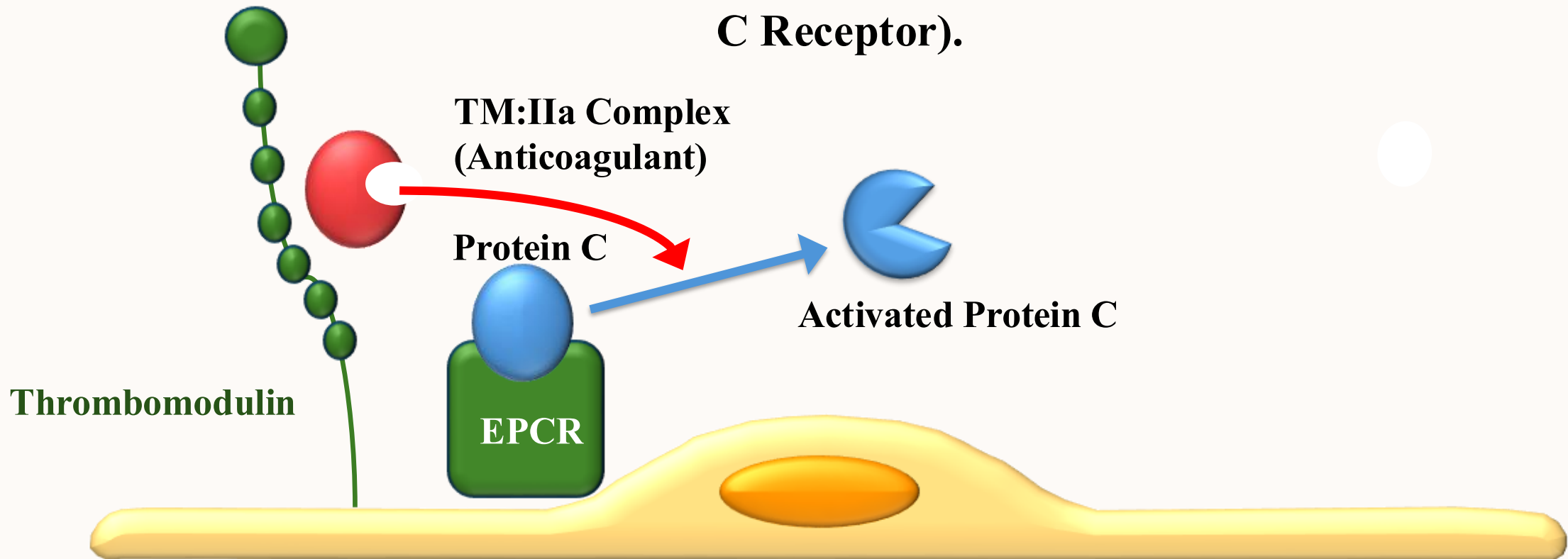
EPCR: Endothelial Cell Protein C Receptor

Protein C/Protein S/Thrombomodulin



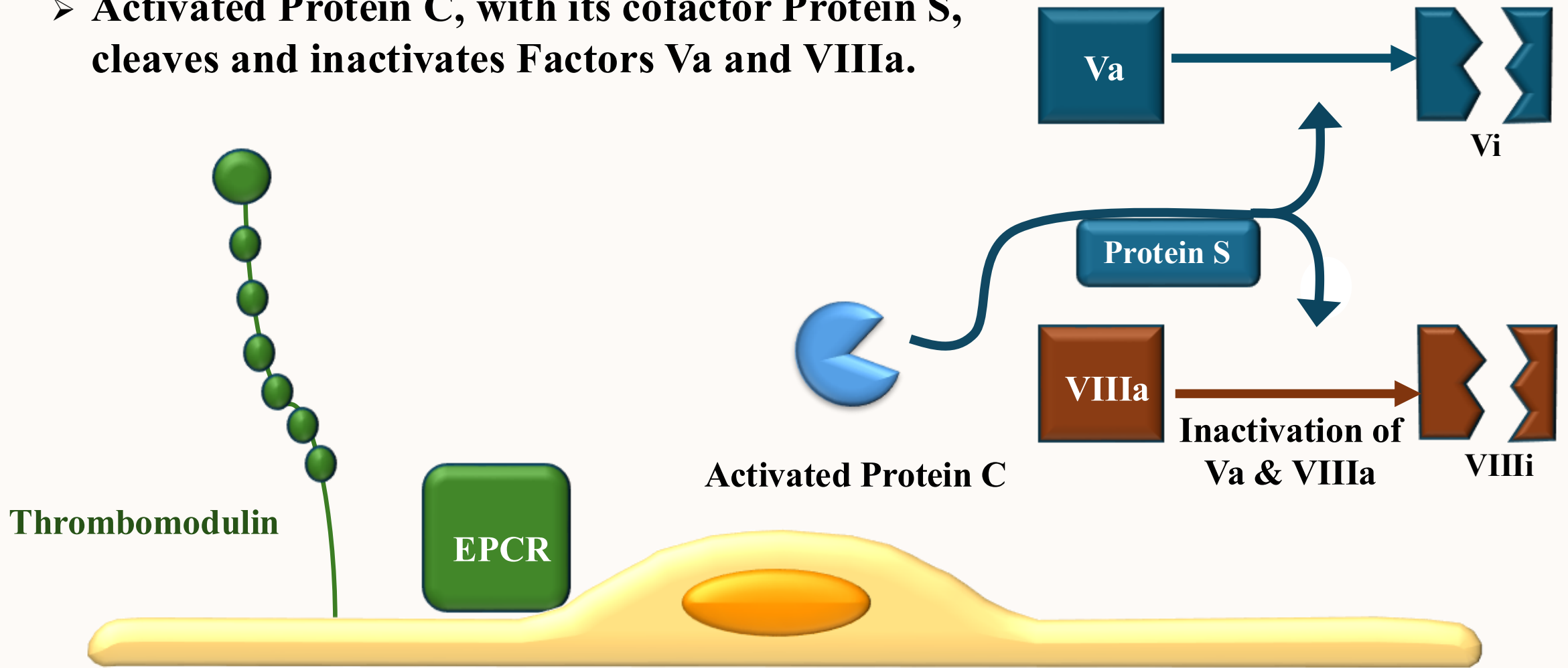
Protein C/Protein S/Thrombomodulin

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



Protein C/Protein S/Thrombomodulin

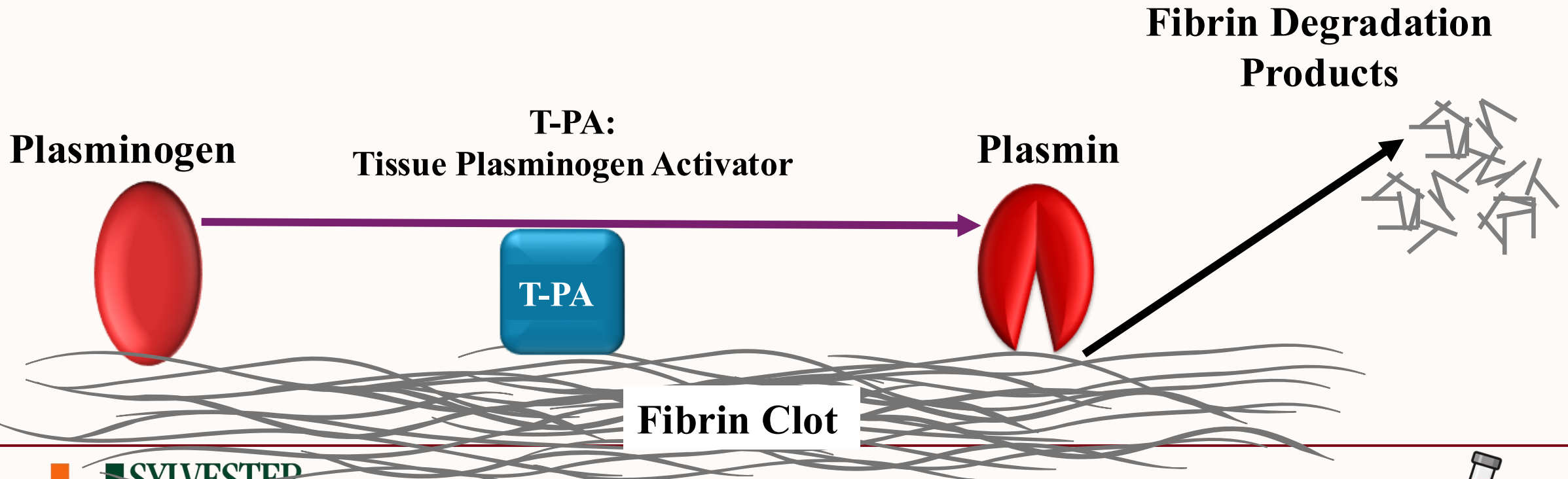
- Activated Protein C, with its cofactor Protein S, cleaves and inactivates Factors Va and VIIIa.



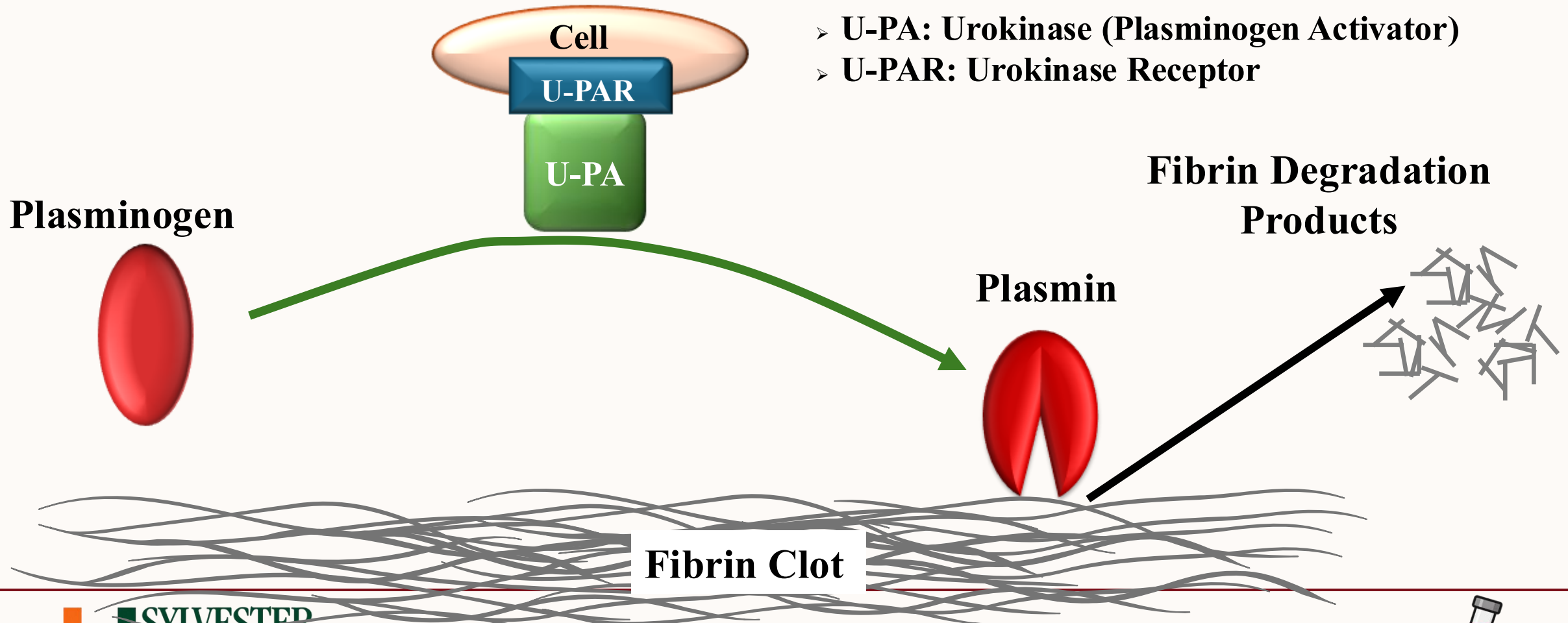
Fibrinolytic Pathway

- 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
 - α 2-Antiplasmin.

Fibrinolytic Pathway: T-PA, Fibrin Clot Based Activation



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



Fibrinolytic Pathway: Inhibitors

